The tumour microenvironment is immuno-tolerogenic and a principal determinant of patient outcome in EBV-positive diffuse large B-cell lymphoma

Colm Keane1,2 | Joshua Tobin1,2 | Jay Gunawardana1 | Santiyagu Francis1 | Grace Gifford3,4 | Sara Gabrielli3 | Anthony Gill3,4,5 | William Stevenson3,4 | Dipti Talaulikar6,7 | Clare Gould1,2 | Sanjiv Jain6,7 | Simone Birch2,8 | Mark Hertzberg9 | Maher K. Gandhi1,2

1 Mater Research, Translational Research Institute, University of Queensland, Brisbane, Queensland, Australia
2 Princess Alexandra Hospital, Brisbane, Queensland, Australia
3 Kolling Institute of Medical Research, University of Sydney, Sydney, New South Wales, Australia
4 Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, Sydney, New South Wales, Australia
5 NSW Health Pathology, Department of Anatomical Pathology, Royal North Shore Hospital, St Leonards, New South Wales, Australia
6 Canberra Hospital, Canberra, Australian Capital Territory, Australia
7 Australia National University Medical School, Canberra, Australian Capital Territory, Australia
8 Pathology Queensland, Brisbane, Queensland, Australia
9 Prince of Wales Hospital, Sydney, New South Wales, Australia

Correspondence
Colm Keane, Level 4 East, Mater Research Institute, Translational Research Institute, University of Queensland, Brisbane, 4102, Queensland, Australia.
Email: Colm.Keane@mater.uq.edu.au

Funding information
Kasey-Anne Lymphoma Giving Fund; Lord Mayor’s Charitable Foundation; Pathology Queensland Study, Education and Research Committee; Education and Research Committee; Leukaemia Foundation; Bridgestone Award; NHMRC Early Career Fellowship; Haematology Society of Australia and New Zealand New Investigator Award; Cancer Australia; Cancer Cure Australia

Abstract
Objective: Epstein-Barr virus-positive diffuse large B-cell lymphoma (EBV+pos DLBCL) is a recently identified entity. Data regarding outcome to frontline immuno-chemistry are conflicting. Although the prognostic impact of the tumour microenvironment (TME) in EBV−neg DLBCL is well-established, it remains untested whether the TME influences survival in EBV+pos DLBCL. There are no data with new digital gene expression technologies that simultaneously interrogate the virus, B cells and the tumour microenvironment (TME).

Methods: We used the NanoString™ platform in a population-based cohort of 433 patients to establish if the technology could detect EBV in the tumour biopsies and to investigate the influence that EBV has on the complex tumour microenvironment of DLBCL.

Results: Incidence of EBV+pos DLBCL was 6.9% with 5-year survival of 65% vs 82% in EBV−neg DLBCL (P = 0.018). EBV+pos tissues had similar expression of T-cell genes compared to EBV−neg DLBCL but higher levels of the antigen-presenting molecule B2M. This was countered by elevated PD-L1, PD-L2, LAG3 and TIM3 immune checkpoints and a higher CD163/CD68 “M2” macrophage score.

Conclusion: In EBV+pos DLBCL, the TME is immuno-tolerogenic and may explain the poor outcomes seen in this subtype of DLBCL.
1 | INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) not otherwise specified (NOS) without evidence of underlying immunodeficiency and that is positive for Epstein-Barr virus (EBV-\textsuperscript{pos} DLBCL) was recognised as a distinct entity in the early to mid-2000s.\textsuperscript{1-3} In some studies, but not in others, it has been associated with poor outcome with standard “R-CHOP” (rituximab-cyclophosphamide/doxorubicin/vincristine/prednisolone) immuno-chemotherapy.\textsuperscript{4,9} Recent studies indicate EBV-\textsuperscript{pos} DLBCL occurs in both younger and older patients and that age does not influence pathological characteristics.\textsuperscript{4,10}

Epstein-Barr virus represents a foreign antigen against which healthy EBV-specific cytotoxic T-cell (CTL) immunity has been extensively characterised.\textsuperscript{11} This response plays a pivotal role in controlling outgrowth of virus-infected cells. CTLs scan the surface of virus-infected cells to detect EBV peptides bound to MHC molecules and eliminate these cells by direct lysis. Conversely, the presence of EBV within the lymphoma cell acts as a potential target, strongly implicating mechanisms of immune evasion. Indeed, EBV-\textsuperscript{pos} DLBCL cases are associated with alterations in the tumour microenvironment (TME), which, in turn may be modulated by the virus. Biopsies are frequently enriched in histiocytes that show high levels of programmed death ligand 1 (PD-L1) and indoleamine 2,3-dioxygenase.\textsuperscript{12,13} It is postulated that all these factors contribute to a tolerogenic environment that promotes tumour immune escape,\textsuperscript{14} much as iatrogenic immunosuppression does in EBV-\textsuperscript{pos} post-transplant lymphoproliferative disorders.\textsuperscript{15-18}

Interestingly, we have previously shown that the T-cell receptor repertoire is a key determinant of the TME and is associated with outcome.\textsuperscript{19} Furthermore, substantially higher clonal T-cell responses were observed in EBV-\textsuperscript{pos} vs EBV-\textsuperscript{neg} DLBCL.\textsuperscript{19} Although the prognostic impact of the TME in de novo DLBCL is well-established,\textsuperscript{20-24} it remains untested as to whether the TME is associated with differential survival in EBV-\textsuperscript{pos} DLBCL.

Conventionally, EBV-tissue status is determined by EBV-encoded small RNA in situ hybridisation (“EBER-ISH“) testing. Digital multiplex gene expression technologies such as NanoString™ are applicable to formalin-fixed paraffin-embedded (FFPE) tissues, but this approach is yet to be applied to EBV-\textsuperscript{pos} DLBCL. Not only can individual EBER molecules be digitally quantified (“EBER-digital”), but these technologies offer the advantage of simultaneous interrogation of key viral and tumour microenvironment (TME) parameters, all of which are relevant to this unique pathobiological entity.\textsuperscript{25} In this study, we firstly demonstrate that EBER-digital is suitable for detecting EBV-\textsuperscript{pos} DLBCL. Next, the platform was used to concurrently quantify other tumour-related and TME factors with prognostic and/or biological importance, in a large population-based multi-centre series of DLBCL. This indicated that the TME is a principal determinant of survival in EBV-\textsuperscript{pos} DLBCL.

2 | MATERIALS AND METHODS

2.1 | Patients

There were 120 patients with a histological diagnosis of DLBCL (excluding follicular lymphoma IIIB, transformed lymphoma or immunosupression-related lymphoma) presenting between 2003 and 2014 at the Princess Alexandra Hospital (PAH) that had sufficient tissue for EBER-digital testing. Of these, 81 had sufficient tissue for EBER-ISH, and these patients were included in the initial test cohort.

The extension cohort combined the initial test patients with an additional 352 patients with DLBCL, making 433 in total. They were drawn from Canberra Hospital (120) and Royal North Shore Hospital, Sydney (97), patients from the Australasian Leukaemia and Lymphoma Group (ALLG) Discovery Centre (96), and the remaining 39 patients from the PAH. Inclusion criteria differed from the initial test cohort only in that patients required either an EBER-ISH result or sufficient tissue for EBER-digital testing. The additional patients were 309 with EBER-digital and 43 with only EBER-ISH. Of the 433 extension cohort patients, 383 had survival data available; 362 of these were treated with R-CHOP; and 21 patients were treated with alternate regimens. The study was approved by Metro South (Brisbane) Ethics Committee.

2.2 | RNA quantification

RNA was extracted from FFPE tumour biopsies using RecoverAll Total Nucleic Acid Extraction Kit for FFPE (Ambion, Life Technologies) as per manufacturer’s instructions and stored at ~80°C. Genes were quantified using the nCounter platform (NanoString™) as previously outlined.\textsuperscript{22,26} A custom code set was used consisting of selected immune effectors, immune checkpoints, macrophage and antigen-presenting molecules (CD4, CD8, CD137, PD-1, PD-L1, PD-L2, LAG3, TIM3, CD68, CD163, B2M), the EBV-related genes (EBER-1, EBER-2, latent membrane protein-1 [LMP1]), the full Lymph2Cx gene set for cell-of-origin (COO) categorisation and finally BCL6 and CD30. Additionally, in order to quantify sensitivity of nCounter platform for detection of EBV, RNA was extracted from the EBV-positive Burkitt’s lymphoma cell line, Namalwa, and serially diluted and mixed (dilution range 1:1-10\textsuperscript{-9}) with extracted PBMC RNA from an EBV-negative donor. 150 ng of total RNA from both sources was run on a NanoString® PanCancer Immune Panel spiked in with the following EBV-specific genes: EBER-1, EBER-2, EBNA2 and LMP1.
Hybridisations were carried out according to the NanoString™ Gene Expression Assay Manual. From each RNA sample (100-300 ng), 5 μL was mixed with 20 μL of nCounter Reporter probes in hybridisation buffer and 5 μL of nCounter Capture probes for a total reaction volume of 30 μL. The hybridisations incubated at 65°C for approximately 16-20 hours. Raw data were imported and analysed in the NanoString™ data analysis tool nSolver. For normalisation, gene expression data were internally controlled to the mean of the positive control probes to account for inter-assay variability. Gene normalisation was then performed using the geometric mean of four-housekeeper genes to account for factors that affect RNA quality and quantity (PGK1, GAPDH, PGAM1 and OAZ1) as previously published22,24,26 with the exception of COO categorisation, where Lymph2Cx-specific normalisers were used in accordance with guidelines. EBER-ISH was performed as previously described.2,4,8,16,27

Gene Expression Assay Manual. From each RNA sample (100–300 ng), 5 μL (10-fold dilutions) with PBMC from an EBV-negative donor. The undiluted cell line sample and 1:10 dilution samples had high EBER-1 gene expression data were internally controlled to the mean of the positive control probes to account for factors that affect RNA quality and quantity (PGK1, GAPDH, PGAM1 and OAZ1) as previously published22,24,26 with the exception of COO categorisation, where Lymph2Cx-specific normalisers were used in accordance with guidelines. EBER-ISH was performed as previously described.2,4,8,16,27

### TABLE 1 Patient characteristics of the cohort

| Characteristics | EBV-neg (n = 403) | EBV-pos (n = 30) | P Value |
|-----------------|------------------|-----------------|---------|
| Sex(M)          | 177 (54%)        | 15 (65%)        | NS      |
| Age             | 61 (18-89.95)    | 66.7 (38.5-90)  | 0.018   |
| Age > 60        | 215 (58%)        | 21 (72%)        | NS      |
| Stage > 2       | 211 (58%)        | 18 (75%)        | NS      |
| ECOG > 1        | 69 (24%)         | 9 (38%)         | NS      |
| LDH > N         | 179 (59%)        | 12 (48%)        | NS      |
| EN > 1          | 90 (31%)         | 8 (35%)         | NS      |
| IPI (n = 371)   |                  |                 |         |
| 0               | 38 (11%)         | 1 (4%)          | NS      |
| 1,2             | 156 (45%)        | 11 (41%)        |         |
| 3,4,5           | 150 (44%)        | 15 (55%)        |         |
| COO (L2Cx) (n = 307) |           |                 |         |
| GCB             | 174 (61%)        | 16 (69%)        | NS      |
| ABC             | 75 (26%)         | 2 (9%)          | NS      |
| UC              | 35 (13%)         | 5 (22%)         |         |
| Therapy         |                  |                 |         |
| R-CHOP          | 335 (83%)        | 27 (90%)        |         |
| Other therapy (with outcome) | 19 (5%) | 3 (10%) |
| Other (no outcome/trial therapy etc) | 49 (12%) |        |
66.7 years, range 38.5–90, vs 61 years, range 18–90, \( P = 0.018 \)) despite no statistical difference for the IPI-based age cut-off of 60. Of note, 28% of EBV\(^{+}\) tumours occurred in patients ≤60 years, and 2 cases were below the age of 50. Thus, whilst patients with EBV\(^{+}\) DLBCL were significantly older, it does not preclude the possibility of EBV\(^{+}\) DLBCL occurring in younger patients.

### 3.3 | Relationship between EBV\(^{+}\) DLBCL and COO

Tissue was available to establish COO by NanoString\textsuperscript{TM} Lymph2Cx in 307 cases. (284 EBV\(^{−}\) tumours and 23 EBV\(^{+}\) tumours). In EBV\(^{+}\) cases, COO was 61% germinal-centre B cell (GCB), 26% activated B cell (ABC) and 13% unclassified (UC). The COO distribution for EBV\(^{+}\) DLBCL was 69% GCB, 9% ABC and 22% UC, that is similar to EBV\(^{−}\) cases (\( P = NS \), chi-squared test). As expected, EBV\(^{+}\) DLBCL (n = 23) had lower levels of the GCB-associated gene BCL6 than EBV\(^{−}\) (n = 284) cases (median gene count 281 vs 507, respectively, \( P = 0.0009 \), Mann-Whitney test). As expected, when subdivided by COO, GCB DLBCL had higher BCL6 than ABC/UC, but interestingly EBV\(^{+}\) GCB tumours had a lower expression of BCL6 vs EBV\(^{−}\) GCB cases (median gene count 647 (n = 16) vs 982 (n = 174), respectively, \( P = 0.013 \), Mann-Whitney test), whereas no difference was observed for EBV\(^{+}\) ABC/UC tumours vs EBV\(^{−}\) ABC/UC cases (median gene count 310 (n = 7) vs 237 (n = 110), respectively, \( P = 0.09 \)).

Seventy-five cases had both Lymph2Cx and Hans performed: 50 of 75 (67%) samples were concordant between the methods. Thirty-three of 36 classified as GCB by Hans were GCB by Lymph2Cx, with 1 UC and 2 ABC. Hans classification was less accurate with non-GCB classification with only 17/39 concordant. The remaining 22 cases were assigned as GCB by Lymph2Cx. These results are consistent with the previously described performance of the Hans classifier in 3.3.

### 3.4 | EBV\(^{+}\) DLBCL has an adverse impact upon survival independent of IPI and COO

With a median follow-up of 45 months, 362 patients in the extension cohort received R-CHOP immuno-chemotherapy. Of these, 27 (7.4%) had an EBER-digital gene count >500 or were EBER-ISH positive. EBV positivity was associated with poorer outcome, with OS significantly inferior in EBV\(^{+}\) cases compared to EBV\(^{−}\) cases with 5-year OS 65% vs 82%, \( P = 0.018 \) (Figure 1), respectively, and a trend towards inferior 5-year PFS of 55% vs 72%, \( P = 0.09 \). When including all 383 patients with follow-up data irrespective of treatment, EBV\(^{+}\) DLBCL remained very similar with inferior 5-year overall survival of 65% vs 80% for EBV\(^{−}\) cases (\( P = 0.026 \)) and a trend towards poorer 5-year PFS of 56.5% vs 71.8%, \( P = 0.13 \).

There were 307 cases with COO assigned by Lymph2Cx. Patients with tumours classified as GCB had an improved outcome compared to those classified as ABC (5-year OS 84.6% vs 69.7%, \( P = 0.033 \), Figure S1). By multivariate analysis including COO, IPI and EBER-digital, only EBV status (\( P = 0.045 \)) and IPI (\( P = 0.001 \)) but interestingly not COO (\( P = 0.25 \)) were independent predictors of OS. Similarly, in a multivariate model including the 342 patients with EBV status (EBER-digital or EBER-ISH) and IPI data available therapy, both were independent predictors of overall survival (\( P = 0.031 \) and \( P < 0.001 \), respectively). EBV status was not an independent predictor of PFS.

### 3.5 | Epstein-Barr virus associations with host and viral genes

In the 390 cases with EBER-digital, host gene expression results were compared, stratified by EBER-digital status. EBV\(^{+}\) tumours showed strong associations with immune genes. Whilst effector markers such as CD4, CD8 and CD137 did not differ, there were significant differences with regard to immune checkpoint molecules. The expression of immune checkpoints PD-L1 (\( P < 0.0001 \)), PD-L2 (\( P = 0.08 \)), LAG3 (\( P = 0.01 \)) and TIM3 (\( P = 0.05 \)) were all higher in EBV\(^{+}\) cases (Figure 2—gene expression and Figure S2—immunohistochemistry), but PD-1 expression did not differ. CD163 gene counts were higher in EBV\(^{+}\) DLBCL than EBV\(^{−}\) DLBCL, median 700 (range 67–3281) vs 280 (range 1–6192) gene counts, respectively, \( P = 0.008 \). The ratio of CD163/CD68 (a more specific M2 signature) was also significantly associated with EBV\(^{+}\) disease (\( P = 0.005 \)).

Effective antigen presentation by the malignant B cell is required for effective T cell-mediated elimination. The B2M molecule is vital for the recognition of antigen by cytotoxic T cells as part of the MHC class I structure. Interestingly, we found that B2M gene expression by NanoString\textsuperscript{TM} was significantly higher in EBV-pos cases.
Consistent with previous IHC-based CD30 observations, CD30 was strongly associated with EBV-pos tumours with a ~4-fold increase (P < 0.0001). LMP1, an EBV-related oncogene with immunomodulatory properties, showed significant correlations with CD163 (r = 0.61, P = 0.003) and the M2 signature (r = 0.6, P = 0.003) as well as PD-L1 (r = 0.49, P = 0.014).

### 3.6 Impact of EBV-pos DLBCL of prognosis could potentially be influenced M2 macrophages

We then tested whether host gene expression might in part explain the adverse outcomes observed in our small cohort of 23 EBV-pos DLBCL EBER-digital cases treated with R-CHOP immuno-chemotherapy. The CD163/CD68 M2 ratio (cut-off ratio CD163/CD68 = 0.75) divided patients into two distinct prognostic groups for 5-year PFS (P = 0.004) and 5-year OS (P = 0.01) with high levels of M2 associated with inferior outcome (Figure 3). Results should be interpreted with caution given small numbers in our cohort. This M2 ratio was also predictive of poor outcome in EBV-neg cases, but a higher level of M2 infiltration was found in significantly more EBV-pos DLBCL cases (56% vs. 34%, P = 0.038, Fisher’s test).

### 4 DISCUSSION

In a large population-based Australian cohort, the incidence of EBV-pos DLBCL was 6.9%. Outcome was inferior to EBV-neg DLBCL after treatment with first-line immuno-chemotherapy. By digital multiplex gene expression, EBV-pos DLBCL had a distinct TME, with elevated immune checkpoint expression. The CD163/CD68 "M2" ratio segregated EBV-pos DLBCL into groups with highly contrasting survival outcomes to R-CHOP, indicating that the TME is a principal determinant of survival. The differences in the TME between patients have not been accounted for in previous studies and may explain why the inferior survival in patients with EBV-pos DLBCL treated by R-CHOP has been demonstrated in some studies but not confirmed in all. Application of NanoString™ enabled genes reflective of the TME to be simultaneously interrogated along with EBV-tissue status. Intratumoral T-cell infiltration has previously been shown to be prognostic in DLBCL treated with R-CHOP. However, we observed that both CD4 and CD8 were equivalent between EBV-pos and EBV-neg tumours. Against this, PD-L1, PD-L2, LAG3 and TIM3 immune checkpoint expression levels were elevated in EBV-pos relative to EBV-neg biopsies. This is in keeping with our previous observations that EBV is also associated with up-regulated LAG3 in classical Hodgkin lymphoma (cHL). LMP1 is a key viral oncogene with established immunomodulatory abilities, and its levels correlated with PD-L1—consistent with its known ability to induce PD-L1 in other EBV-neg lymphomas. Importantly, the antigen-presenting molecule B2M was present at higher levels in EBV-pos disease. These data are consistent with antigen presentation typically being intact in EBV-pos disease and that viral-induced immune evasion occurs at least in part via an immune-tolerogenic TME.

We demonstrated significantly higher levels of M2-type macrophages compared to M1-type macrophages in the tumour in EBV-pos DLBCL vs EBV-neg DLBC using the ratio of CD163:CD68 to indicate the intratumoral level of this macrophage subset. Results should be interpreted with caution given small numbers in our cohort. M2 immunosuppressive macrophages are associated with inferior outcome in...
many cancers including DLBCL. In CHL, we have previously demonstrated that EBV levels correlate with CD163. Similarly, in EBV-positive DLBCL LMP1 correlated with M2. Next, we tested to see whether M2 could stratify outcome within EBV-positive DLBCL. Patients with low levels of M2 macrophages had improved outcome compared to those with high levels of M2 macrophages. However, numbers are small and must be interpreted with caution. These results require validation in larger patient groups before definitive conclusions can be drawn.

Rates of EBV-positive DLBCL appear to vary geographically. However, many centres apply EBER-ISH in a targeted fashion, such as elderly patients or those with specific histological features. Results of large population-based series that have formally examined the impact of EBV-tissue status in patients with de novo DLBCL treated with frontline R-CHOP immuno-chemotherapy have been conflicting. It remains unclear to what extent these differences reflect ethnic variations between studies conducted in either predominantly Asian or Caucasian population, and further large-scale studies in new geographic localities are required. However, addition of rituximab to CHOP (“R-CHOP”) improves response and survival. The incidence of EBV-positive disease in Australia (6.9%) lies between rates observed in Europe and North American of 2%-5% and that seen in South-East Asia and South America where 4%-15% of newly diagnosed DLBCL can be EBV-positive. One explanation is that this reflects the country’s evolving demographics, including increasing immigration from South-East Asia. The ethnicity of patients in this study was not obtained (to our knowledge, ethnicity has not been specifically been included in prior studies of EBV-positive DLBCL), and these data should be collected in future studies of EBV-positive DLBCL. Our findings also confirm recent descriptions of the disease in younger age groups with approximately a quarter of our cohort under the age of 60 and 2 cases occurring in patients <50 years of age. However, it remains a disease of predominantly older patients.

Although the Hans classifier demonstrates that EBV-positive DLBCL occurs in both GCB and non-GCB cases, many studies show enrichment in non-GCB tumours. In contrast, in one of the largest studies to date there was no significant difference in the frequency of non-GCB- to GCB-typed patients. In another study in which the majority of COO was performed by gene expression, with the remaining performed by Hans, the proportions of GCB vs ABC were almost identical. Due to its inherent subjectivity and variability in scoring, the concordance between IHC and gene expression is modest and the prognostic value of the Hans method has been challenged. To our knowledge, the present study is the first to use the Lymph2Cx COO classifier in EBV-positive DLBCL. We observe that the rate of GCB in EBV-positive DLBCL biopsies was higher than previously described using the Hans classifier and that there was no enrichment of one subtype of COO over another. Consistent with reported BCL6 IHC findings, EBV-positive DLBCL had lower levels of the Hans/GCB-related gene BCL6 than EBV-negative cases. However, this difference was confined to the EBV-positive GCB DLBCL subset only. These findings appear to be consistent with evidence suggesting EBV induces an atypical GCB reaction associated with persistent infection in a latent form, and to the known ability of EBV-miR-BART9 and BART17-5p to down-regulate BCL6 expression. It is unclear what impact (if any) this might cause upon discrepancies in COO classification by IHC and gene expression in EBV-positive DLBCL. It is also possible that the Lymph2Cx assay is not as accurate in subsets of DLBCL cases with unique tumour microenvironments as occurs in EBV-positive DLBCL given the assay performed specifically on tumour cells only. Additional studies are required before firm conclusions can be made.

In summary, we demonstrate that whilst the effector immune response in EBV DLBCL appears intact, it is counterbalanced by high levels of immune suppression in the TME with both elevated immune checkpoints and very high levels of tumour-associated macrophages that appear to impact survival. Future clinical trials that focus on targeting these pathways may lead to improved outcomes for this poor prognosis subgroup of DLBCL.

ACKNOWLEDGEMENTS

This study was supported by the Kasey-Anne Lymphoma Giving Fund—a giving account of the Lord Mayor’s Charitable Foundation—and the Pathology Queensland Study, Education and Research Committee. CK is supported by a Leukaemia Foundation Bridgestone Award, an NHMRC Early Career Fellowship, a Princess Alexandra Hospital Award, a Haematology Society of Australia and New Zealand new investigator grant, and Cancer Australia and Cancer Cure Australia; MKG is supported by the Leukaemia Foundation. Use of samples from the ACT Haematology Research Tissue Bank is acknowledged.
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(20):2375-2390.

2. Kuze T, Nakamura N, Hashimoto Y, Sasaki Y, Abe M. The characteristics of Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma: comparison between EBV(+)- and EBV(-) cases in Japanese population. Jpn J Cancer Res. 2009;91(12):1233-1240.

3. Ok CY, Papathomas TG, Medeiros LJ, Young KH. EBV-positive diffuse large B-cell lymphoma of the elderly. Blood. 2013;122(3):328-340.

4. 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(16):13933-13945.

5. 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(3):548-555.

6. Tracy SI, Habermann TM, Feldman AL, et al. Outcomes among North American patients with diffuse large B-cell lymphoma are independent of tumor Epstein-Barr virus positivity or immunosuppression. Haematologica. 2018;103(2):297-303.

7. 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(9):2338-2349.

8. Ahn J-S, Yang D-H, 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(9):774-779.

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(9):1170-1175.

10. Lu T-X, Liang J-H, Miao YJ, et al. Epstein-Barr virus-positive diffuse large B-cell lymphoma predict poor outcome, regardless of the age. Sci Rep. 2015;5:12168.

11. Hislop AD, Taylor GS, Sauce D, Richardson AB. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. Annu Rev Immunol. 2007;25:587-617.

12. 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(7):863-872.

13. Chen BJ, Chapuy B, Ouyang J, et al. PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. Clin Cancer Res. 2013;19(13):3462-3473.

14. Nicholas NS, Apollonio B, Ramsay AG. Tumor microenvironment (TME)-driven immune suppression in B-cell malignancy. Biochim Biophys Acta. 2016;1863(3):471-482.

15. Gandhi MK. Epstein-Barr virus-associated lymphomas. Expert Rev Anti Infect Ther. 2006;4(1):77-82.

16. Jones K, Nourse JP, Morrison L, et al. Expansion of EBNA1-specific effector T cells in posttransplantation lymphoproliferative disorders. Blood. 2010;116(13):2425-2422.

17. Nourse JP, Jones K, Gandhi MK. Epstein-Barr Virus-related post-transplant lymphoproliferative disorders: pathogenetic insights for targeted therapy. Am J Transplant. 2011;11(5):888-895.

18. Shannon-Lowe C, Rickinson AB, Bell AI. Epstein-Barr virus-associated lymphomas. Philos Trans R Soc Lond B Biol Sci. 2017;372(1732):20160271.

19. Keane C, Gould J, Jones K, et al. The T-cell receptor repertoire influences the Tumor microenvironment and is associated with survival in aggressive B-cell lymphoma. Clin Cancer Res. 2017;23(7):1820-1828.

20. Fowler NH, Cheah CY, Gascoyne RD, et al. Role of the tumor microenvironment in mature B-cell lymphoid malignancies. Haematologica. 2016;101(5):531-540.

21. Keane C, Gill D, Vari F, Cross D, Griffiths L, Gandhi M. CD4(+) tumor infiltrating lymphocytes are prognostic and independent of R-IPI in patients with DLBCL receiving R-CHOP chemo-immunotherapy. Am J Hematol. 2013;88(4):273-276.

22. Keane C, Vari F, Hertzberg M, et al. Ratios of T-cell immune effectors and checkpoint molecules as prognostic biomarkers in diffuse large B-cell lymphoma: a population-based study. Lancet Haematol. 2015;2(10):e445-455.

23. Scott DW, Gascoyne RD. The tumour microenvironment in B cell lymphomas. Nat Rev Cancer. 2014;14(8):517-534.

24. Vari F, Arpon D, Keane C, et al. Immune evasion via PD-1/PD-L1 on NK-cells and monocyte/macrophages is more prominent in Hodgkin lymphoma than DLBCL. Blood. 2018;131(16):1809-1819.

25. Scott DW, Wright GW, Williams PM, et al. Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue. Blood. 2014;123(8):1214-1217.

26. Jones K, Wockner L, Brennan RM, et al. The impact of HLA class I and EBV latency-II antigen-specific CD8(+) T cells on the pathogenesis of EBV(+)-Hodgkin lymphoma. Clin Exp Immunol. 2016;183(2):206-220.

27. 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(3):352-357.

28. Nourse JP, Crooks P, Keane C, et al. Expression profiling of Epstein-Barr virus-encoded microRNAs from paraffin-embedded formalin-fixed primary Epstein-Barr virus-positive B-cell lymphoma samples. J Virol Methods. 2012;184(1–2):46-54.

29. Yoon N, Ahn S, Yong Yoo H, Jin Kim S, Seog Kim W, Hyeh KY. Cell-of-origin of diffuse large B-cell lymphomas determined by the Lymph2Cx assay: better prognostic indicator than Hans algorithm. Oncotarget. 2017;8(13):22014-22022.

30. Gandhi MK, Lambley E, Duraliswamy J, et al. Expression of LAG-3 by tumor-infiltrating lymphocytes is coincident with the suppression of latent membrane antigen-specific CD8+ T-cell function in Hodgkin lymphoma patients. Blood. 2006;108(7):2280-2289.

31. Green MR, Rodig S, Juszczynski P, et al. Constitutive AP-1 activity and EBV infection induce PD-L1 in Hodgkin lymphomas and post-transplant lymphoproliferative disorders: implications for targeted therapy. Clin Cancer Res. 2012;18(6):1611-1618.

32. Lee SP, Constandinou CM, Thomas WA, et al. Antigen-presenting phenotype of Hodgkin Reed-Sternberg cells: analysis of the HLA class I processing pathway and the effects of interleukin-10 on Epstein-Barr virus-specific cytotoxic T-cell recognition. Blood. 1998;92(3):1020-1030.

33. Murray PG, Constandinou CM, Crocker J, Young LS, Ambinder RF. Analysis of major histocompatibility complex class I, TAP expression, and LMP2 epitope sequence in Epstein-Barr virus-positive Hodgkin's disease. Blood. 1998;92(7):2477-2483.

34. Jones K, Vari F, Keane C, et al. Serum CD163 and TARC as disease response biomarkers in classical Hodgkin lymphoma. Clin Cancer Res. 2013;19(3):731-742.

35. 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(17):5124-5132.

36. Beltran BE, Quiñones P, Morales D, et al. Response and survival benefit with chemoimmunotherapy in Epstein-Barr virus-positive diffuse large B-cell lymphoma. Hematol Oncol. 2018;36(1):93-97.

37. Hofschier A, Ponciano A, Bonzheim I, et al. Geographic variation in the prevalence of Epstein-Barr virus-positive diffuse large B-cell

ORCID

Colm Keane https://orcid.org/0000-0002-9009-9934
lymphoma of the elderly: a comparative analysis of a Mexican and a German population. Mod Pathol. 2011;24(8):1046-1054.

38. 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(3):972-978.

39. Wright G, Tan B, Rosenwald A, Hurt EH, Wiestner A, Staudt LM. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proc Natl Acad Sci U S A. 2003;100(17):9991-9996.

40. Castillo JJ, Beltran BE, Song M-K, et al. The Hans algorithm is not prognostic in patients with diffuse large B-cell lymphoma treated with R-CHOP. Leuk Res. 2012;36(4):413-417.

41. Thorley-Lawson DA, Gross A. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. N Engl J Med. 2004;350(13):1328-1337.

42. Martín-Pérez D, Vargiu P, Montes-Moreno S, et al. Epstein-Barr virus microRNAs repress BCL6 expression in diffuse large B-cell lymphoma. Leukemia. 2012;26(1):180-183.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Keane C, Tobin J, Gunawardana J, et al. The tumour microenvironment is immuno-tolerogenic and a principal determinant of patient outcome in EBV-positive diffuse large B-cell lymphoma. Eur J Haematol. 2019:103:200–207. https://doi.org/10.1111/ejh.13274