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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Histone 3 mutation in T-ALL validation cohort.

Table SI. Type 3 histone genes.

Table SII. COSMIC version 81 cell lines screened for type 3 histone mutations.

Table SIII. T-cell leukaemia lines screened for type 3 histone mutations.

Table SIV. Internal database screened for histone 3 mutations.

Table SV. TCGA cohort screened for histone 3 mutations.

Table SVI. Validation cohort of 38 primary human T-ALL specimens screened by Sanger sequencing of histone 3 genes.

Table SVII. Primers used to Sanger sequence hotspot residues in histone 3 genes.

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Bortezomib plus EPOCH is effective as frontline treatment in patients with plasmablastic lymphoma

Plasmablastic lymphoma (PBL) is a rare and aggressive CD20-negative lymphoma associated with poor outcomes. Multiple studies have shown median survival times of 12–18 months (Castillo *et al*, 2012; Schommers *et al*, 2013; Morscio *et al*, 2014). Several case reports and small case series have suggested an increased response rate in patients treated with bortezomib alone or in combination, especially the combination of bortezomib and dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin (V-EPOCH) (Castillo *et al*, 2015a; Fedele *et al*, 2016). Due to its rarity, prospective studies exclusively in PBL patients are unlikely to be performed. We evaluated the potential therapeutic value of V-EPOCH in patients with PBL.

We retrospectively reviewed medical records at participating institutions of all patients with a diagnosis of PBL who received frontline V-EPOCH. The lymphomas were required to have plasmablastic morphology, lack CD20 expression and express one plasmacytic marker (CD38, CD138, MUM1/IRF4). This study was approved by institutional review boards at each of the participating centres. This report

includes updated survival data on four previously published cases (Castillo *et al*, 2015a; Fedele *et al*, 2016). Pertinent clinical data were gathered. Pathological samples were reviewed at the respective institutions. Pathological data included expression of CD20, CD38, CD138, Ki67, anaplastic lymphoma kinase (ALK), human herpesvirus 8 (HHV8) latency-associated nuclear antigen (LANA), Epstein–Barr virus (EBV)-encoded RNA (EBER), and *MYC* gene rearrangements. Response to therapy was assessed following the revised response criteria for malignant lymphoma. Overall survival (OS) was estimated as time between diagnosis and last follow-up or death. OS curves were estimated using the Kaplan-Meier method. All calculations and graphs were obtained using STATA (StataCorp, College Station, TX, USA).

Sixteen patients met the inclusion criteria; their clinical characteristics are shown in Table I. Extranodal sites included gastrointestinal tract ($n = 8$), head/neck ($n = 4$), lung/pleura ($n = 4$), bone marrow ($n = 3$), skeletal bone ($n = 3$), testicles ($n = 2$), kidney/adrenals ($n = 2$), and subcutaneous tissue ($n = 1$). Among human immunodeficiency virus (HIV)-infected patients, the median $CD4^+$ count was $0{\cdot}128 \times 10^9$/l (range $0{\cdot}033–0{\cdot}29 \times 10^9$/l). All patients were treated with a curative intent. Bortezomib was administered at 1·3 mg/m^2 SQ or IV weekly or twice weekly with each cycle of EPOCH (etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin). Dose frequency/route of administration of bortezomib was per investigator's choice. Patients with advanced disease received a median of 6 cycles (range 4–6 cycles). The two patients with early stage received 4 cycles of V-EPOCH followed by 30–36 Gy of radiation therapy to the involved area. Complete response was seen in 15 patients (94%) and partial response in 1 (6%). Two patients received autologous stem cell transplantation (ASCT) in first remission. Four patients (31%) experienced

Table I. Patients' clinicopathological characteristics.

Characteristic	Number	Percentage
Age ≥60 years	6/16	38
Male sex	15/16	94
ECOG performance score >1	7/16	44
Increased LDH level	8/16	50
Advanced stage	14/16	88
Extranodal involvement	16/16	100
Low/low intermediate IPI score	5/16	37
High/high intermediate IPI score	10/16	63
HIV infection	6/16	38
CD20 expression	0/16	0
CD38/CD138 expression	16/16	100
HHV8 LANA expression	0/10	0
ALK expression	0/10	0
Ki67 expression ≥80%	14/16	88
EBER ISH positive	9/14	64
MYC rearrangements	8/10	80

ALK, anaplastic lymphoma kinase; EBER ISH, Epstein–Barr virus-encoded RNA *in situ* hybridisation; ECOG, Eastern Central Oncology Group; HHV8 LANA, human herpesvirus 8 latent nuclear antigen; HIV, human immunodeficiency virus; IPI, International Prognostic Index; LDH, lactate dehydrogenase.

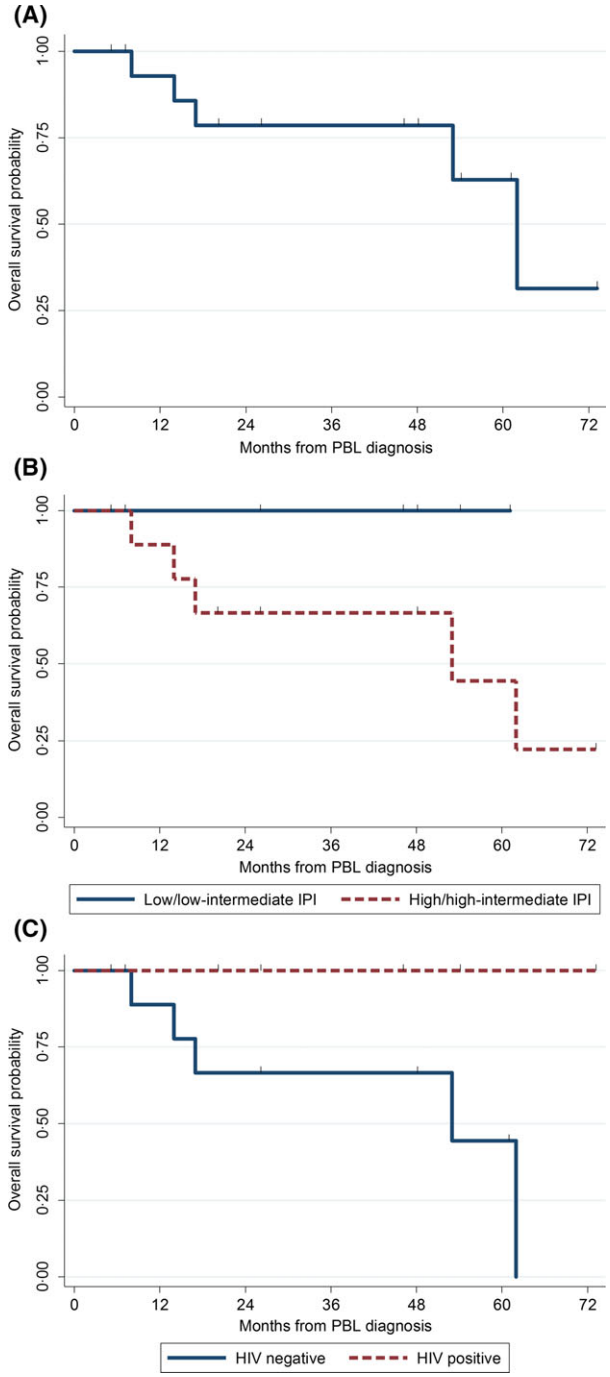

Fig 1. Overall survival estimates for (A) the entire group, (B) per IPI score, and (C) per HIV status. HIV, human immunodeficiency virus; IPI, International Prognostic Index; PBL, plasmablastic lymphoma. [Colour figure can be viewed at wileyonlinelibrary.com]

relapsed disease within 2 years of diagnosis, including one who achieved CR and underwent ASCT. Salvage therapy was provided to only one patient and included daratumumab in combination with ifosfamide, carboplatin and etoposide (ICE) followed by ASCT. The patient achieved CR and is alive at 15 months after salvage therapy. The median follow-up was 48 months [95% confidence interval (CI) 20–61 months], and the median OS was 62 months (95% CI 17-not reached). The 5-year OS rate was 63% (95% CI 24–86%; Fig 1A). There were no deaths in the low International Prognostic Index (IPI) group, and the median OS for patients with high IPI was 53 months (95% CI 8-not reached; log-rank $P = 0{\cdot}10$; Fig 1B). There were no deaths among patients in the HIV-positive group, and the median OS among HIV-negative patients was 53 months (95% CI 8-not reached%; $P = 0{\cdot}06$; Fig 1C). Adverse events (Grade 3 or higher) included thrombocytopenia ($n = 7$), febrile neutropenia ($n = 5$), neuropathy ($n = 3$), infections ($n = 2$), gastrointestinal obstruction ($n = 1$), pleural effusions ($n = 1$), catheter-associated thrombosis ($n = 1$) and atrioventricular block ($n = 1$). Of the 5 deaths, 3 were due to lymphoma progression and 2 due to infections.

Biologically, CD20-negative aggressive lymphomas show plasmacytic differentiation, which can be mediated by the anti-apoptotic effect of nuclear factor-kappa B (NF-kB). Specifically, EBV-related antigens, such as LMP-1, can suppress ATF3 and also inhibit BAX inducing NF-kB activity. MYC dysregulation in PBL allows overcoming the regulatory effects of BCL6 and BLIMP-1 promoting cell cycle dysregulation (Castillo *et al*, 2015b). Bortezomib has been shown to inhibit NF-kB in primary effusion lymphoma (PEL) cell lines (An *et al*, 2004). Furthermore, the combination of doxorubicin and bortezomib was synergistic for inducing PEL cell killing. In a murine xenograft model, bortezomib was associated with downregulation of cell cycle progression, DNA replication and down-regulation of *MYC*-target genes, resulting in longer survival of PEL-bearing mice (Sarosiek *et al*, 2010). EPOCH has been shown to be associated with better outcomes than cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP), especially in double hit and HIV-associated lymphomas (Barta *et al*, 2012; Petrich *et al*, 2014). Our study suggests that V-EPOCH is feasible and effective as frontline treatment in patients with PBL, and is associated with a complete response rate exceeding 90% as well as a 5-year OS rate of 65%.

The patients presented here have been treated at reference centres, which can introduce selection bias. However, some of the patients were older than 60 years, had poor performance status, and many had HIV infection. Despite the encouraging results, HIV-negative and patients with high IPI scores had worse outcomes. Given the observed efficacy, prospective studies are needed to confirm the therapeutic role of V-EPOCH in PBL. However, toxicity has remained an issue with high rates of febrile neutropenia, neuropathy and infectious complications. Therefore, other potent and safe agents are needed. Agents of interest include daratumumab, anti-PD-L1 monoclonal antibodies, and agents that downregulate MYC function.

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Authorship contributions

JJC designed the research and wrote the initial manuscript. JJC and TGG analysed the data. All the authors provided data, and critically read and approved the final manuscript.

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Reducing the need for diagnostic imaging in suspected cases of deep vein thrombosis

The objective diagnosis of deep vein thrombosis (DVT) depends on imaging using compression ultrasound, however, because of cost and the increasing number of negative tests, strategies have been developed which can exclude the diagnosis in some patients without the need for diagnostic imaging. These rely on the use of information from the patients' clinical history and examination (a pre-test probability assessment) and assays to detect D-dimers (Fig 1). Pre-test probability assessment is usually with the Wells score (Wells *et al*, 1997, 2003; Wells, 2007). The original score divided patients into three categories: low (≤0), moderate (1–2) or high (≥3) (Wells *et al*, 1997), but later into two categories: "unlikely" (≤1) and "likely" (≥2) (Wells *et al*, 2003). Imaging is required in those assessed as "likely" (≥2) or high risk (≥3). A negative D-dimer test can be used to forgo imaging in those deemed "unlikely" (≤1) or low/moderate risk (≤2). Clearly, the latter will reduce the number of ultrasounds, as those with a Wells score of 2 have a D-dimer test to determine their need for diagnostic imaging.

The other way of reducing the number of ultrasounds is to increase the D-dimer threshold that defines a negative test. The usual D-dimer threshold is 500 μg/l and strategies to