The role of tumor histogenesis, FDG-PET and short course EPOCH with dose dense rituximab (SC-EPOCH-RR) in HIV-associated diffuse large B-cell lymphoma

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Abstract

This is a phase II study to assess the role of tumor histogenesis (subtype), FDG-PET and SC-EPOCH-RR in newly diagnosed HIV-associated CD20+ diffuse large B-cell lymphoma. Patients received a minimum of 3 and maximum of 6 cycles with one cycle beyond stable radiographic and FDG-PET scans. Overall, 79% of patients received 3 cycles. Combination anti-retroviral therapy was suspended before and resumed after therapy. Thirty-three enrolled patients had a median age of 42 years (range 9-61) and 76% had a high-intermediate or high age-adjusted international prognostic index. At 5 years median follow-up, progression-free and overall survival were 84% and 68%, respectively. There were no treatment related deaths or new opportunistic infections during treatment and patients had sustained CD4 cell count recovery and HIV viral control following treatment. FDG-PET after 2 cycles had an excellent negative but poor positive predictive value. Tumor histogenesis was the only characteristic associated with lymphoma-specific outcome with 95% of Germinal Center B-cell (GCB) versus 44% of non-GCB DLBCL progression-free at 5 years. SC-EPOCH-RR is highly effective and less immunosuppressive with shorter duration therapy compared to standard strategies. However, new therapeutic advances are needed for non-GCB DLBCL which remains the important cause of lymphoma-specific death. This study was registered at http://clinicaltrials.gov as NCT000019253.
Introduction

The survival of acquired immunodeficiency syndrome-related lymphoma (ARL) has significantly improved over the past decade, but it has been mostly attributed to HIV control and not to advances in lymphoma treatment. We tested a strategy based on the da-EPOCH (dose adjusted; etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin) regimen which balanced the competing needs of lymphoma treatment and HIV management. This regimen employed dose adjustment based on the degree of immune suppression and temporarily suspended combination antiretroviral therapy (cART) to obviate untoward drug interactions. da-EPOCH proved to be highly effective with a progression-free (PFS) and overall survival (OS) of 73% and 60%, respectively, at 53 months in ARL, the majority of which were DLBCL. Baseline CD4+ cells $\leq 100/\mu l$ was the only biomarker of decreased survival in a multivariate analysis and patients in remission had significant recovery of immune function and HIV control. Based on these results, da-EPOCH has been identified as a treatment of choice for ARL.

Herein, we report results on a second generation regimen which aimed to improve efficacy and decrease toxicity through the addition of dose dense rituximab to EPOCH. The design was based on the hypothesis that rituximab would significantly enhance the efficacy of chemotherapy, thereby allowing a major reduction in the number of treatment cycles. Interestingly, years after our study commenced, a phase III study of CHOP $\pm$ rituximab concluded that rituximab did not improve the outcome of ARL and was potentially unsafe in immune compromised patients. As we show below, however, our present study does not support these conclusions.
A novel component of the present study was the use of sequential fluorodeoxyglucose positron emission tomography (FDG-PET) to assess early and late responses in HIV-associated DLBCL. Furthermore, this study actively employed interim FDG-PET in the decision to reduce the number of treatment cycles. Our goal was to study for the first time whether DLBCL could be effectively treated with up to fifty percent fewer cycles than a standard course and to assess the role and specificity and sensitivity of FDG-PET in HIV-associated DLBCL.

We also wished to examine the role of tumor biology in the outcome of HIV-associated DLBCL. While studies have assessed histology and CD4 cell count, none have prospectively assessed molecular histogenesis of DLBCL which derive from a germinal center or an activated B-cell (GCB or ABC) and are independently prognostic in HIV negative DLBCL\textsuperscript{11-13}. Importantly, insight into the molecular basis of treatment failure is critical to the development of more effective treatments in HIV-associated DLBCL. Thus, we wished to assess if tumor histogenesis is a major factor in lymphoma-specific survival and if one or both molecular subtypes might benefit from additional novel interventions.

**Methods**

**Patients**

Forty-five patients with untreated CD20+ ARL entered on a study of short course EPOCH and dose dense rituximab at the National Cancer Institute. Thirty-five patients had DLBCL and 10 patients with Burkitt lymphoma will be reported separately. Two
patients with DLBCL were excluded; one received treatment elsewhere and one had primary mediastinal B-cell lymphoma (PMBL), putatively of thymic B-cell origin. Eligible patients were HIV seropositive by Western Blot and had adequate organ function unless due to tumor. Patients with serious infections, pregnancy, breastfeeding or primary central nervous system (CNS) lymphoma were ineligible. Patients were consecutively enrolled between June 2001 and April 2009. The study was Institutional Review Board approved, complied with the Declaration of Helsinki and patients gave written informed consent.

**Evaluation and Treatment**

Evaluation included routine blood tests, imaging (CT body, MRI brain and FDG-PET), bone marrow biopsy and lumbar puncture with cytology and flow cytometry. Serial plasma HIV-1 viral loads (mRNA copies/ml plasma) were measured by the Roche-Amplicor® method and T-lymphocyte subsets were determined by flow cytometry at baseline and end of SC-EPOCH-RR and every 3-6 months thereafter.

The study was designed to administer one cycle beyond no significant radiographic changes for a minimum of 3 and maximum of 6 cycles. cART was suspended before SC-EPOCH-RR and reinstated on day 6 of the last cycle. Response was based on serial CT body and FDG-PET scans beginning at cycle three day 1 and performed after each cycle thereafter until treatment completion (Figure 1). Thus, the number of scans performed was the same as the number of cycles received for each patient. In clinical practice, however, the post-treatment scans could be omitted if the previous scans were
negative. SC-EPOCH-RR was stopped when there was < 25% reduction in bidimensional products compared to the previous interim CT scan and the standardized uptake values (SUV) on FDG-PET decreased > 50% compared to the pre-treatment FDG-PET. This definition was developed to take into account HIV-associated reactive changes that may confound FDG-PET interpretations. Response designation followed the International Workshop criteria\textsuperscript{15}.

SC-EPOCH-RR was administered through a central line on days 1-5 as a 96 hour continuous infusion (CIV) of (mg/m\textsuperscript{2}/day) etoposide (50), doxorubicin (10) and vincristine (0.4 - no cap) and oral prednisone (60) with cyclophosphamide (750 mg/m\textsuperscript{2}) on day 5 as previously described\textsuperscript{16}. Rituximab (375 mg/m\textsuperscript{2}) was administered on day 1 (before CIV began) and on day 5 (after CIV completed and before cyclophosphamide). All patients received filgrastim 300 \( \mu \)g SQ (pediatric dose 5 \( \mu \)g/kg/day-maximum 300 \( \mu \)g/day) from day 6 until absolute neutrophil count (ANC) \( \geq \) 5000 cells/mm\textsuperscript{3} beyond the nadir. Cycles were repeated every 21 days. Cyclophosphamide was reduced 25% for a nadir ANC < 500/mm\textsuperscript{3} or platelets < 25,000/mm\textsuperscript{3} for 2-4 days; and by 50% if the nadir ANC < 500/mm\textsuperscript{3} or platelets < 25,000/mm\textsuperscript{3} for \( \geq \) 5 days based on a twice weekly blood counts. Patients received 12 mg methotrexate intrathecally on days 1 and 5 of cycle 3 and repeated every 3 weeks for a total of 6 doses (i.e. cycles 3-5), irrespective of when SC-EPOCH-RR stopped. Patients with a positive flow cytometry or cytology of cerebrospinal fluid received induction intrathecal or intraventricular methotrexate twice weekly for 2 weeks beyond negative flow cytometry for a minimum of 4 weeks; consolidation weekly for 6 weeks and; maintenance monthly for 6 months. Patients also
received prophylaxis for *Pneumocystis jiroveci* and *mycobacterium avium* if CD4 cells < 100/mm³.

*Immunohistochemical Analyses*

Immunohistochemistry (IHC) was performed on paraffin-embedded tissue. Sections were stained with monoclonal antibodies to Bcl-6 (clone PG-B6p), MUM-1 (clone MUM1p), and CD10 (clone 56C6 from Novocastra, Burlingame, CA). For Bcl-6 and MUM-1, cases were scored as positive if expression occurred in at least 30% of neoplastic cells. CD10 stained uniformly positive or negative in all cases. Classification into GCB or non-GCB (ABC) subtypes was determined by SP using the validated method of Hans et al. *In situ* hybridization analysis for EBV RNA was done on 4-µm-thick formalin-fixed, paraffin-embedded tissue using the INFORM EBV-encoded nontranslated RNA probe (Ventana Medical Systems, AZ). The signal was visualized using the ISH iVIEW Blue Detection kit (Ventana Medical Systems, AZ) with nitroblue tetrazolium/BCIP and a Fast Red nuclear counterstain. All the procedures were done on a BenchMark XT autostainer (Ventana Medical Systems, AZ) according to the manufacturer’s instructions.

*FDG-PET Imaging*

Patients fasted for 6 hours prior to FDG-PET and injected with a nominal amount of FDG based on weight. Patients were scanned on a GE Advance or a GE Discovery ST PET/CT. Images were acquired from mid skull to proximal thighs, performed at a nominal time of 60 minutes post-injection and blindly read by JAC. Images were
interpreted visually as positive when focal areas of uptake were seen that did not correspond to physiologic sites of uptake\textsuperscript{19}.

**Statistical Analysis**

The primary endpoint was to test the hypothesis that the number of chemotherapy cycles could be reduced from a standard of 6 cycles\textsuperscript{7,20}. Based on a mean of 4 cycles, the study would have an 80% power at 0.05 two-sided significance to detect a difference between a mean of 4 and 5 cycles with 1.5 standard deviation in 28 patients. Upon completion of accrual for the primary endpoint, enrollment was increased to more fully address the secondary endpoints of FDG-PET and immune recovery. Assessment of tumor histogenesis and EBV was obtained from standard immunohistochemical panels.

PFS and OS were determined from on-study until death, progression, or last follow-up by the Kaplan-Meier method and statistical significance was determined by a log-rank test. For PFS, deaths unrelated to acute treatment or lymphoma were censored. Death within 30 days of chemotherapy was defined as treatment related. For PFS, Kaplan-Meier curves were developed for all prognostic factor analyses. An exact log-rank test was used to determine the impact of tumor histogenesis (GCB versus non-GCB) on PFS since there were so few failures in one group. For univariate prognostic factors analyses, if patients were grouped into two categories after preliminary evaluation of 3 categories, the subsequent p-value was adjusted by multiplying the unadjusted p-value by two. This would account for the implicit testing which resulted in a decision to place
patients into the two categories with a larger prognostic difference between groups. A Cox proportional hazards model analysis was performed to determine the joint association of factors initially found to have potential association with outcome in the univariate analyses (those with unadjusted p<0.10 from a log-rank test). For PFS, since lactate dehydrogenase (LDH) divided at <226 versus > 266 (above normal) resulted in no failures in one group, the hazard ratio would be infinite, and thus, for PFS, only cell type was available for use in a Cox model. The association between cell type and CD4 cell count was determined using an exact Wilcoxon rank sum test. Other exploratory evaluations between cell type and other dichotomized factors were performed using Fisher’s exact test. All p-values are two-tailed, and except as noted above, are reported without adjustment for multiple comparisons.

Results

Patient Characteristics

Thirty-three patients with untreated DLBCL were enrolled. They had a median age of 42 years and 76% had a high age-adjusted international prognostic index (IPI) (Table 1). Specific adverse prognostic features included poor performance status in 39%, elevated LDH in 61%, and advanced stage disease in 82% of patients. CNS involvement was found in four (12%) patients pre-treatment. Patients had a median CD4 count of 208 with 42% < 100 cells/mm$^3$, and 27% were cART naïve.
**Treatment and Outcome**

Patients received a median of 3 cycles (range 3-5) of SC-EPOCH-RR; 79% received 3, 6% received 4 and 12% received 5 cycles (Figure 1). Complete response (CR) was observed in 30 (91%; 95% Confidence Interval [CI]: 76-98) patients and one patient achieved a partial response (PR). Two patients progressed on therapy and three patients relapsed. Four patients with leptomeningeal involvement by lymphoma achieved durable remissions, although one died of a non-lymphoma related event. At the median potential follow-up of 5 years, the PFS and OS are 84% and 68%, respectively (Figure 2A and 2B). Of 10 deaths on study, five were due to lymphoma and five were in remission. Of these latter deaths, three were from complications of AIDS due to pre-existent *mycobacterium avium complex* infection, and occurred at 5 weeks, 3 months and 30 months after treatment. All three patients received treatment for *mycobacterium avium complex* and were cART resistant with pre- and post- (last available) treatment CD4 cell counts of 0 and 1; 3 and 4; and 12 and 9 cells/mm$^3$, respectively. Two additional patients died at 31 months after treatment; one with progressive motor neuropathy following successful treatment of a secondary and clonally distinct Burkitt lymphoma, and one from exposure associated hypothermia.

Toxicity was evaluable on all 109 cycles of treatment. Hematological toxicity included neutropenia < 500 cells/mm$^3$ on 46% and thrombocytopenia < 50,000/mm$^3$ on 33% of cycles. Febrile neutropenia occurred on 31% of cycles, no new opportunistic infections were observed on treatment and there were no treatment related deaths. Non-hematological toxicity was similar to our report of da-EPOCH$^7$. 


**FDG-PET Scans**

FDG-PET scan after 2 cycles had an excellent negative predictive value and was not significantly different from the post-therapy scan (Table 2). The positive predictive value of FDG-PET scans was quite poor, however, presumably due to a high rate of HIV-associated reactive changes. Hence, FDG-PET had a poor sensitivity and specificity for HIV-associated DLBCL when interpreted using commonly recommended criteria	extsuperscript{19}.

**HIV and CD4 T-cell Dynamics**

A unique aspect of our strategy is suspension of cART during treatment to obviate adverse effects on the lymphoma treatment	extsuperscript{2,7}. We previously demonstrated this approach led to reversible viral load increase and CD4 cell decrement and excellent disease control with da-EPOCH	extsuperscript{7}. The current study aimed to reduce the adverse effects of chemotherapy on immune function through a reduction in cycle number which was achieved in all t patients. The viral load increased a median (range) of 0.47 log	extsubscript{10} (-1.82 to 4.87) and the CD4 count decreased a median (range) of 64 cells/mm	extsuperscript{3} (+271 to -541) among 28 patients without early deaths. Expectedly, cART naïve patients presented with higher viral loads and lower CD4 cell counts compared to those on prior cART (Figure 3A and 3B). The median viral load declined below baseline in both groups by 3 months after treatment and was undetectable in most patients at 12-18 months, and the CD4 cells recovered to baseline by 6-12 months. We compared our current results to those with da-EPOCH and observed a significantly smaller decrement in median CD4 cells/mm	extsuperscript{3} of 64 (range +271 to -541) versus 183 (+135 to -928)(P = 0.03), respectively	extsuperscript{7}. 


**Tumor Biology and Prognostic Models**

GCB and non-GCB DLBCL subtypes comprised 72% and 28% of cases, respectively, and EBV expression was detected in 31% of cases (Table 1). EBV expression was detected somewhat more frequently in the non-GCB (4/7; 57%) compared to GCB (5/19; 26%) (P=0.19) subtype, and in patients with low CD4 cells < 100/mm$^3$ (5/11; 45%) compared to patients with higher counts (4/24; 17%) (P=0.10), though they did not reach statistical significance due to sample size. There was a trend toward a higher median CD4 cell number in patients with GCB (231 cells/mm$^3$) compared to non-GCB (131 cells/mm$^3$) subtype (P=0.15), but no difference in the fraction who had CD4 < versus > 100 cells/mm$^3$ (P=0.38)(Figure 4).

To examine the role of GCB versus non-GCB histogenesis in the outcome of HIV-associated DLBCL, we developed prognostic models based on characteristics in Table 1. In the univariate analysis, only tumor histogenesis was associated with PFS (Figure 2C), whereas tumor histogenesis, EBV and CD4 cell count were all associated with OS (Figures 2D, 2F and 2H). Importantly, no clinical characteristics including IPI had prognostic value. The multivariate Cox model of PFS only showed tumor histogenesis (HR=14.5; 95% CI: 1.6-131; P=0.017), indicating tumor biology drove the lymphoma-specific survival. For OS, histogenesis and immune status were important in the Cox model; CD4 < or > 100 cells/mm$^3$ (HR=27; 95% CI: 2.9-251; P=0.004) and non-GCB versus GCB (HR=7.2; 95% CI: 1.2-42; P=0.028). When HIV viral load was added to this model, it provided further evidence for the importance of HIV control in OS; CD4 < or > 100 cells/mm$^3$ (HR=29.9; 95% CI: 2.6-351; P=0.007), non-GCB versus GCB
(HR=64.3; 95% CI: 2.7-1509; P=0.01), HIV-1 mRNA > versus < 100,000 copies/ml plasma (HR=15.9; 95% CI: 1.1-222; P=0.04).

Discussion

Treatment of ARL presents the dual challenge of achieving tumor control while maintaining immune integrity. Though conventional wisdom argues for maintaining cART during lymphoma treatment to minimize the risks of uncontrolled HIV replication, we have hypothesized that the unpredictable effects of cART on chemotherapy pharmacokinetics and pharmacodynamics and lymphocyte apoptosis may reduce cure\textsuperscript{7,8}. While it is established that structured cART suspension increases AIDS-associated events, a large randomized study showed it required a mean (range) of 16.8 (5.7-42.3) months of cART suspension for the CD4 count to decrease from > 400 to <250 cells/mm\textsuperscript{3}; far in excess of the 1.8 months mean suspension time in the present study\textsuperscript{21}. Furthermore, we previously showed recovery of immune function, HIV viral control and reversion to wild type virus following reinstitution of cART after da-EPOCH chemotherapy\textsuperscript{7}.

We believe our current results provide further evidence for the safety of cART suspension. Indeed, only three patients died in remission from HIV-associated infections and in all cases, the infection(s) were preexistent and the patients were severely immune compromised and cART resistant. Furthermore, only two of these cases were immediately after completing chemotherapy treatment and occurred despite active treatment for their infections. Hence, there is no scientific basis to associate
these deaths with suspension of cART. On the other hand, the results with SC-EPOCH-RR indicates that our treatment strategy provides excellent outcomes.

The present study extends our findings with da-EPOCH through reducing chemotherapy cycles by half with a reduction in cART suspension from 16 to 7 weeks. To achieve this, several modifications were made to da-EPOCH. First, we removed dose adjustment based on initial CD4 cell counts due to the improvement in HIV natural history; thus patients received full dose EPOCH on cycle one with subsequent reductions based on hematological toxicity. We also incorporated dose dense rituximab on day 1 and 5 of each cycle. When we developed SC-EPOCH-RR 9 years ago, we hypothesized that rituximab would be beneficial and that dose dense administration would significantly increase drug exposure; it had been shown that rituximab T½ doubled and Cmax increased 50% between the first and fourth dose on a standard weekly schedule. We also restricted the present study to DLBCL to reduce the confounding effects associated with the biological diversity of ARL.

Using a combination of CT and FDG-PET response criteria, most patients (79%) received 3 cycles of SC-EPOCH-RR. Overall, 91% of patients achieved CR, and 84% are progression-free and 68% are alive at five years. SC-EPOCH-RR had significantly less immune toxicity than da-EPOCH, which was administered for six cycles, and achieved excellent immune reconstitution and HIV control following reinstitution of cART; similar to those findings when chemotherapy is administered with cART. The survival outcomes compare favorably with da-EPOCH where PFS and OS were 73%
and 60%, respectively, at 53 months suggesting dose-dense rituximab may be beneficial, although the study was not designed to address this question. Our results appear to be significantly better than those achieved in a phase III study of CHOP±rituximab, which reported 50% TTP (comparable to PFS in the present study) and OS with R-CHOP at 2.4 years. Importantly, our results do not support that trials’ conclusion that rituximab may be risky as we observed no treatment related deaths. While SC-EPOCH-RR was associated with greater hematological toxicity than da-EPOCH, likely due to higher chemotherapy dose intensity, we did not encounter any treatment related deaths.

The efficacy and safety of EPOCH and rituximab is further supported by preliminary results from the randomized phase II trial of concurrent versus sequential rituximab with da-EPOCH in ARL from the AIDS Malignancy Consortium (AMC) Trial 034. The AMC study, based on da-EPOCH and unpublished results from the present study, aimed to assess if the complete response (CR) rate of da-EPOCH and rituximab was likely to be superior to historical results with CHOP±R; and if da-EPOCH with concurrent versus sequential rituximab was more toxic and/or more effective. Preliminary results found no increase in toxicity between the arms and rejected the null hypothesis of 50% (associated with CHOP±R) in favor of 75% CR for da-EPOCH with concurrent rituximab (P = 0.005; power 0.89). These results provide additional evidence that rituximab does not increase chemotherapy toxicity and that da-EPOCH with concurrent rituximab is likely to be more effective than R-CHOP.
To our knowledge, this is the first study to employ interim FDG-PET in a treatment paradigm to reduce chemotherapy cycles in DLBCL, and provides a unique opportunity to study its utility in HIV-associated lymphomas. This is particularly important because HIV-associated nodal reactive hyperplasia and infections may confound FDG-PET interpretations^{27,28}. To assess the value of FDG-PET, they were blindly interpreted employing commonly accepted criteria^{19}. FDG-PET obtained after 2 cycles and at the end of therapy showed a high negative predictive value of 91% and 87%, respectively, but a poor positive predictive value of 15% and 7%, respectively. It was difficult to differentiate low level FDG-PET inflammatory from active lymphoma. Indeed, 65% of FDG-PET scans were positive after cycle 2 and yet few of these patients relapsed. Though the role of FDG-PET needs further study in ARL, it is likely to remain highly problematic.

Numerous studies have identified IPI and CD4 cell counts as the important predictors of ARL outcome in the post-cART era^{4,6,7}. Indeed, there has been some concern that withholding cART during chemotherapy will promote CD4 cell loss and decrease both HIV and lymphoma-specific survival. While there is ample evidence that ARL pathogenesis is influenced by immune status, there is no scientific evidence or rationale that treatment sensitivity of established lymphomas are dependent on the CD4 cell count^{13}. To help address this issue, we analyzed clinical and biological markers of outcome in our study. Unlike other studies, no clinical prognostic characteristics including IPI were associated with PFS or OS. Only tumor histogenesis was significantly associated with lymphoma-specific outcome with 95% of GCB and 44% of
non-GCB DLBCL progression-free at 5 years. Furthermore, both tumor histogenesis and CD4 cell count were independently associated with survival, reflecting both lymphoma and HIV-specific deaths. These results mirror the importance of tumor histogenesis in the outcome of HIV-negative DLBCL\textsuperscript{11,12,29}. We also examined the relationship between tumor histogenesis, immune status and EBV expression and found an association, albeit a trend, between non-GCB tumor cell type and lower CD4 cell counts and EBV expression, suggesting a role for immune status in pathogenesis. It is important to recognize the potential limitations of IHC determination of tumor histogenesis. As validation for our methods, we previously showed full concordance between IHC and gene expression profiling, the gold standard, in 12 biopsies of DLBCL\textsuperscript{18,30,31}.

Our results show for the first time that tumor biology is the major factor in lymphoma-specific survival in HIV-associated DLBCL. In contrast, a recent publication from the AMC found no relationship between tumor histogenesis or EBV infection and outcome in HIV associated DLBCL, which is contrary to our own results and numerous publications in HIV negative DLBCL\textsuperscript{11,12,18,29,32}. Several factors may explain these negative findings. Firstly, the AMC study was retrospective and included biopsy specimens from two different studies and four different treatment regimens; CHOP±R and EPOCH with concurrent or sequential rituximab. This is likely to confound the results because rituximab and the chemotherapy platform (CHOP versus EPOCH) appear to influence the impact of tumor histogenesis on outcome\textsuperscript{11,12,29}. These results are further limited by the relatively short and variable follow-up in the two studies.
Secondly, the outcome measures did not distinguish lymphoma-specific survival from overall survival and thus may be confounded by a high rate of non-lymphoma-specific deaths. Thirdly, the accuracy of immunohistochemical determination of tumor histogenesis can be highly variable and validation of the laboratory technique against gene expression profiling is an important control\textsuperscript{30,31}.

Understanding the relationship of tumor biology to outcome is important for the identification of molecular targets and improvement of therapy. Our present finding that SC-EPOCH-RR is curative in nearly all cases of GCB DLBCL is similar to our results in HIV negative cases (unpublished observations)\textsuperscript{29,33}. In vitro, sustained exposure of tumor cells to topoisomerase II inhibition by etoposide and low-dose doxorubicin promote the p53-p21 pathway and activates the check-point kinase (Chk2) independently of ATR, pathways that are associated with the GCB Bcl-6 transcription factor\textsuperscript{34,35}. Such studies indicate that prolonged exposure to topoisomerase II inhibition, as achieved with EPOCH, may be particularly effective in GCB DLBCL. In contrast, our results indicate that therapeutic advances are needed in non-GCB DLBCL, similar to findings in HIV negative non-GCB DLBCL\textsuperscript{11,29}. In HIV-negative DLBCL, the majority of cases identified as non-GCB by immunohistochemistry have an ABC DLBCL gene expression profile\textsuperscript{18}. The poor outcome of non-GCB (ABC) DLBCL may be related to the constitutive activation of the NF-kB pathway which has been ascribed to activity of a signaling cascade involving CARD11, BCL-10 and MALT1 leading to activation of IκB kinase\textsuperscript{36-38}. Furthermore, inhibition of NF-kB in ABC DLBCL cell lines is toxic, in keeping with the ability of this pathway to inhibit apoptosis\textsuperscript{36,39}. Clinically, it has been
shown that the combination of EPOCH with the proteasome inhibitor bortezomib, which can inhibit NF-κB through blocking IκBα degradation, had significantly greater benefit in recurrent ABC versus GCB DLBCL; a strategy which may improve the outcome of untreated ABC DLBCL.

In conclusion, most HIV associated DLBCL are curable with three cycles of SC-EPOCH-RR and tumor histogenesis is the most important determinant of lymphoma-specific survival. While FDG-PET is useful when used alongside CT scans to determine when treatment is completed, they should be used with caution in HIV-associated DLBCL. These results suggest that SC-EPOCH-RR is an important advance for HIV-associated DLBCL, although AIDS related deaths and non-GCB DLBCL remain important barriers to overall survival.

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The authors made the following contributions: Kieron Dunleavy-study conduct, analysis and writing; Richard F. Little-study design, conduct; Stefania Pittaluga-study analysis; Nicole Grant-study analysis and data management; Alan S. Wayne-study conduct and
analysis; Jorge A. Carrasquillo-study analysis; Seth M. Steinberg-study design and analysis; Robert Yarchoan-study consultation and sample analysis; Elaine S. Jaffe-study analysis; Wyndham H. Wilson-study design, conduct, analysis and writing.

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References

Table 1. Baseline Characteristics

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<td>Total Patients</td>
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<td>Median Age (Range)</td>
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Table 2. FDG-PET and Outcome

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<td></td>
<td>Cycle 3 Day 1 (N = 31)</td>
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<tr>
<td>Failure</td>
<td>Positive</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
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<td>2</td>
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<tr>
<td>Remission</td>
<td>Positive</td>
<td>17</td>
<td>10</td>
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<tr>
<td></td>
<td>Negative</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Final Scan</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-Treatment (N = 29)</td>
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Predictive Value

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<tr>
<th>Indices</th>
<th>Interim Cycle 3 Day 1</th>
<th>Final Scan Post-Treatment</th>
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<tr>
<td>Sensitivity</td>
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<td>33%</td>
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<tr>
<td>Specificity</td>
<td>37%</td>
<td>54%</td>
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<tr>
<td>+ Predictive Value</td>
<td>15%</td>
<td>7%</td>
</tr>
<tr>
<td>- Predictive Value</td>
<td>91%</td>
<td>87%</td>
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Figure Legends

**Figure 1:** Treatment Paradigm for SC-EPOCH-RR. Thirty-three patients with DLBCL received 2 cycles of EPOCH-RR after which a CT and FDG-PET scan were performed. Response was based on serial CT body and FDG-PET scans beginning at cycle three day 1 and performed after each cycle thereafter until treatment completion. Patients who had “negative” studies, as defined in the methods, received 1 additional cycle (minimum of 3) of therapy. Patients who had a “positive” CT and/or FDG-PET study received additional cycles until they were negative, for a maximum of 6 cycles. Two patients with progressive disease on treatment did not complete their initial therapy.

**Figure 2:** Progression-free and Overall Survival Kaplan-Meier Curves. Progression-free survival (PFS) (A) is 84% and Overall survival (OS) (B) is 68% at the median follow-up of 5 years. PFS (C) and OS (D) for patients with GCB versus non-GCB DLBCL; PFS (E) and OS (F) for EBV negative versus EBV positive DLBCL and; PFS (G) and OS (H) for CD4 count for > 100 versus < 100 cells/mm³ at diagnosis.

**Figure 3:** HIV Viral Load and T-cell Dynamics. (A) Median change in plasma mRNA HIV viral loads in 28 patients without early deaths. Viral loads increased with the peak shown at the end of therapy and declined below baseline at 3 months following completion of therapy and reinstitution of cART. cART naïve (♦) compared to those with prior (■) exposure had slightly higher viral loads at presentation. (B) Median changes in CD4 cells in 28 patients without early deaths. CD4 cells declined to a nadir at end of therapy but recovered to baseline 6-12 months later. cART naïve (♦) compared to those with prior (■) exposure had lower CD4 cells at baseline but equivalent CD4 cells 6-12 months after therapy. Medians with 95% Confidence Intervals calculated by boot strapping are shown.

**Figure 4:** Association of Tumor Histogenesis (cell of origin), EBV Expression, Immune Status (CD4 cell count at diagnosis) and Outcome. Outcome: progression/relapse (Δ) or progression-free (○). EBV Status: positive (red), negative (blue) or unknown (black).
Figure 1

Suspend HAART
EPOCH-RR x 2 cycles
N = 33

CT/PET Negative
N = 26

EPOCH-RR 1 cycle

CT/PET Positive
N = 7

Progressive Disease N = 2

EPOCH-RR 2-3 cycles

CT/PET Negative
N = 5

Resume HAART
Follow-up
Figure 2.

A. Progression-Free Survival (Years)

B. Overall Survival (Years)

C. GCB DLBCL

D. Non-GCB DLBCL

E. EBV Negative DLBCL

F. EBV Positive DLBCL

G. CD4 > 100 cells/m³

H. CD4 < 100 cells/m³
Figure 4.

CD4 Cells/mm$^3$

Cell of Origin

GCB  Non-GCB
The role of tumor histogenesis, FDG-PET, and short course EPOCH with dose-dense rituximab (SC-EPOCH-RR) in HIV-associated diffuse large B-cell lymphoma

Kieron Dunleavy, Richard F. Little, Stefania Pittaluga, Nicole Grant, Alan S. Wayne, Jorge A. Carrasquillo, Seth M. Steinberg, Robert Yarchoan, Elaine S. Jaffe and Wyndham H. Wilson