Rituximab plus concurrent infusional EPOCH chemotherapy is highly effective in HIV-associated, B-cell non-Hodgkin's lymphoma


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Keywords: Chemotherapy; non-Hodgkin’s lymphoma; HIV infection; Rituximab.
ABSTRACT

Rituximab plus intravenous (IV) bolus chemotherapy is a standard treatment for immunocompetent patients with B-cell non-Hodgkin’s lymphoma (NHL). Some studies have suggested that rituximab is associated with excessive toxicity in HIV-associated NHL, and that infusional chemotherapy may be more effective. We performed a randomized phase II trial of rituximab (375 mg/m²) given either concurrently prior to each infusional EPOCH chemotherapy cycle, or sequentially (weekly for 6 weeks) following completion of all chemotherapy in patients with HIV-associated NHL. EPOCH consisted of a 96-hour IV infusion of etoposide, doxorubicin, and vincristine plus oral prednisone followed by IV bolus cyclophosphamide given every 21 days for 4-6 cycles. In the concurrent arm, 35 of 48 evaluable patients (73%; 95% confidence intervals [CI] 58%, 85%) had a CR. In the sequential arm, 29 of 53 evaluable patients (55%; 95% CI 41%, 68%) had a CR. The prespecified primary efficacy endpoint was met for the concurrent arm only. Toxicity was comparable in the two arms, although patients with a baseline CD4 count < 50/uL had a high infectious death rate in the concurrent arm. We conclude that concurrent rituximab plus infusional EPOCH is an effective regimen for HIV-associated lymphoma which merits further evaluation. This study is registered at http://clinicaltrials.gov as NCT00049036.
INTRODUCTION

Rituximab is a chimeric monoclonal antibody that is directed against CD20, a B-cell antigen expressed by normal B lymphocytes and also by approximately 85% of non-Hodgkin’s lymphoma.1 The combination of rituximab plus chemotherapy is now considered standard therapy for diffuse, large B-cell lymphoma (DLBCL) based upon several trials which have consistently demonstrated improved complete response (CR), failure free survival, and overall survival in older2,3 and younger4 immunocompetent patients with DLBCL who received rituximab plus chemotherapy compared with chemotherapy alone. In several trials, the standard chemotherapy regimen consisted of intravenous bolus cyclophosphamide, doxorubicin, and vincristine plus oral prednisone (CHOP), or CHOP-like regimens. At least one report also suggests benefit when rituximab is combined with more intensive cytotoxic regimens for aggressive B-cell lymphoma.5

For patients with lymphoma associated with human immunodeficiency virus (HIV) infection, however, the benefits of rituximab are less certain. HIV-associated lymphoma tends to present with advanced stage disease, aggressive histology, and compromised performance status, immune function, and hematopoietic reserve; these factors contribute to a poorer prognosis compared to B-cell lymphoma in immunocompetent individuals.6,7 In a previous trial performed by the AIDS Malignancy Consortium, the addition of rituximab to CHOP chemotherapy (R-CHOP) was associated with a significantly higher risk of treatment associated death when compared with CHOP alone (14% vs. 2%; P = .035), and resulted in comparable CR rates (58% vs. 47%; P = 0.147) and survival.8 This raised concerns about the safety and efficacy of R-CHOP in patients with HIV-associated lymphoma.9

Infusional administration of cytotoxic therapy has also been explored as a potential strategy in patients with poor-risk lymphoma10-13, including HIV-associated lymphoma when
used as first-line therapy. For example, Little et al evaluated a 96 hour continuous intravenous infusional regimen called EPOCH in 39 patients with HIV-associated lymphoma; 29 patients (74%) achieved a CR, and after a median follow-up of 53 months, the median progression-free survival rate was 73% and overall survival rate was 60%.\textsuperscript{14} Other studies evaluating a 96 hour infusion of cyclophosphamide, doxorubicin, and etoposide in HIV-associated lymphoma produced similar results\textsuperscript{15}, with outcome improved for patients treated in the HAART era.\textsuperscript{16} In addition, the safety and efficacy of rituximab plus infusional chemotherapy\textsuperscript{17} or CHOP chemotherapy\textsuperscript{18} \textsuperscript{19} in patients with HIV-associated lymphoma has also been demonstrated in other studies, suggesting that HIV-infected patients may derive similar benefit from rituximab.\textsuperscript{20}

Based upon these considerations, the AIDS Malignancy Consortium (AMC) initiated a randomized phase II trial evaluating the safety and efficacy of infusional EPOCH chemotherapy given either concurrently with rituximab every 3 weeks for up to 6 cycles, or EPOCH given alone every 3 weeks for up to 6 cycles sequentially prior to initiating weekly rituximab for up to 6 doses. This design would facilitate the evaluation of each experimental treatment arm independently compared with historical data. The trial was designed to distinguish between a CR rate of 50% expected for standard therapy and 75%, which was defined \textit{a priori} as promising. This design also permitted a direct comparison of the toxicity in each treatment arm.

**METHODS**

**Eligibility Criteria.** Eligibility criteria included previously untreated histologically or cytologically documented aggressive CD20-positive B-cell non-Hodgkin’s lymphoma, including diffuse large B-cell lymphoma, Burkitt’s/Burkitt’s-like lymphoma, or other aggressive lymphomas associated with HIV infection. Other criteria included stage II-IV disease (or stage I disease with an elevated serum lactate dehydrogenase [LDH]), Eastern Cooperative Oncology
Group (ECOG) performance status of 0-2, age 18 years or older, and adequate organ function similar to a previous AMC lymphoma trial; exclusion criteria were also similar to the previous trial. All patients were also required to be able to provide written informed consent.

**Rituximab, EPOCH, Supportive Care, and Clinical Evaluation.** Details regarding treatment program are summarized in Table 1. Response was defined by the International Response Criteria for Non-Hodgkin's Lymphoma (which utilizes anatomical but not functional imaging). Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0). Response was evaluated after every 2 cycles of EPOCH therapy (with computerized tomography of the chest, abdomen, and pelvis) and continued for 2 cycles beyond achieving a CR (for a minimum of 4 and maximum of 6 cycles), including after completion of R-EPOCH in the concurrent arm, and after completion of EPOCH alone and by rituximab alone in the sequential arm. All patients were required to have bone marrow biopsy and lumbar puncture for cerebrospinal fluid cytologic exam at baseline. A repeat bone marrow biopsy was required if the original study demonstrated lymphomatous marrow involvement, and if the physical exam and imaging studies were consistent with a complete response.

A 24-hour supply of vincristine, doxorubicin, and etoposide were administered in 500 ml of 0.9% sodium chloride and delivered by a portable infusion pump given by a central venous catheter. Rituximab (375 mg/m²) was given either before each EPOCH course in the concurrent arm (arm A), or sequentially following the completion of all EPOCH on a weekly basis for 6 consecutive weeks (arm B) beginning about 3 weeks after the last EPOCH cycle when there was satisfactory recovery from toxicity. Rituximab (Rituxan®, Genentech, Inc., San Francisco, CA) was provided by the National Cancer Institute for all patients. EPOCH treatment cycles were repeated every 3 weeks if the neutrophil count was at least 1000/uL, platelets at least 50,000/uL,
and the patient had recovered from non-hematologic toxicity. EPOCH dose modification was performed as previously described, and as summarized in Table 1. Central nervous system (CNS) prophylaxis was given at the discretion of the treating physician, and was recommended for individuals with small non-cleaved cell histology, bone marrow involvement, paranasal sinus involvement, testicular involvement, or epidural disease. The recommended CNS prophylaxis regimen included intrathecal injection of either cytarabine (50 mg) or methotrexate (12 mg) weekly for four consecutive weeks during cycle 1 of EPOCH. Patients with a positive baseline CSF cytology were permitted to remain on study and receive an intrathecal chemotherapy regimen of the physician’s choice.

Supportive care used is summarized in Table 1, and included antibacterial prophylaxis for those in the concurrent arm (using a fluoroquinolone of the investigator’s choice on days 8-15 or longer of each cycle). Patients in the sequential arm were also required to use quinolone prophylaxis if there were high risk features for infection, including a baseline CD4 count of < 100/uL or decreasing below that level during therapy, a nadir neutrophil count of < 500/uL in a previous or current cycle, or a nadir neutrophil count of < 1000/uL associated with fever of at least 38.0° Celsius occurring in a previous cycle. A complete blood count was obtained prior to beginning each cycle, and on or about days 8 and 15 of EPOCH therapy in order to document adequate recovery of the neutrophil count to at least 1000/uL before the quinolone was discontinued.

The use of antiretroviral therapy was left to the choice of the treating physician. For patients who were never previously treated with antiretroviral agents, or who were not receiving them within 4 weeks of registration, it was recommended that concurrent antiretroviral therapy be withheld until completion of EPOCH chemotherapy. For those who were taking
antiretrovirals at the time of registration and were on a stable, well tolerated regimen that was producing adequate viral suppression, it was recommended that patients remain on antiretroviral therapy.

**Randomization, Statistical Considerations and Analysis, and Protocol Amendment.**

Randomization was performed centrally by the AMC Statistical Office. Stratification factors included CD4 count (< 100/uL vs. ≥ 100/uL), age-adjusted International Prognostic Index (IPI 0-1 vs. 2-3 adverse risk factors), and planned concurrent antiretroviral therapy (yes vs. no). The primary endpoint was CR rate, including unconfirmed and confirmed CR as defined by the International Response Criteria (which utilizes anatomical but not functional imaging).21 The primary objective was to distinguish between a CR rate of 75% with experimental therapy compared with 50% typically expected with standard therapy for HIV-associated lymphoma. Because neither of the regimens evaluated in this trial were considered standard therapy, both treatment arms were regarded as experimental. For each treatment arm, a sample size of 31 eligible and evaluable patients was planned based on testing the null hypothesis that the CR rate was 50% against the alternative that it is 75% at the two-sided 0.10 significance level with power of 0.90. Assuming a 10% ineligible or unevaluable for response, it was projected that 35 patients in each arm were required. All analyses were performed using Statistical Analysis System (SAS) software. An early stopping rule was also employed that was designed to interrupt accrual if the incidence of treatment associated deaths exceeded 10% for either arm.

After accrual of 35 patients, an error in the protocol document was found that resulted in omission of the cyclophosphamide dose escalation. The study was suspended to accrual in March 2004, the protocol corrected, and all patients were informed. The study reopened in May 2004 after the amendment to assure that approximately 70 total patients (35 per arm) would be
enrolled after protocol correction to include cyclophosphamide dose escalation as originally
described for the EPOCH regimen by Little et al.\textsuperscript{14} The study completed its accrual goal in April
2006. The amended analysis plan indicated that the primary efficacy analysis for CR rate would
be based upon patients treated after the amendment, and that efficacy and toxicity would also be
analyzed for the entire study population. Progression free survival (PFS), time to progression
(TTP), and overall survival were estimated using the method of Kaplan and Meier. PFS was
defined as the time between registration and either relapse or progression of lymphoma or death
from any cause. TTP was defined as time to progression or relapse of lymphoma, with deaths
from other causes censored.

\textit{Informed Consent and Regulatory Approval.} The study was reviewed and approved by
the Cancer Evaluation Therapy Program of the National Cancer Institute, and by the institutional
review board at each participating institution (Clinical Trials.gov identifier NCT00049036). All
patients provided written informed consent in accordance with the Declaration of Helsinki.

\textbf{RESULTS}

\textit{Patient Characteristics}

A total of 110 patients were enrolled at 20 sites between December 2002 and April 2006,
of whom 106 were treated and included in this analysis (see \textbf{Figure 1} Consort Diagram); 35
treated patients were enrolled prior to the amendment and 71 were enrolled after the amendment.
The characteristics of the 106 treated patients are shown in \textbf{Table 2}, including the characteristics
of the pre-amendment, post-amendment, and total population in each treatment arm. The
populations were generally comparable with respect to gender, median age, concurrent
antiretroviral therapy, histology (as determined by the local institutional pathologist), and
number of age-adjusted International Prognostic Index (IPI) risk factors. The populations were
also comparable with regard to race and ethnicity. With regard to ethnicity, 30 patients (28.3%) were Hispanic (and the remaining were non-Hispanic); with regard to race, 69 were white (65.1%), 28 were black (26.4%), 4 were native Pacific islanders (3.8%), 3 were Asian (2.8%), and 2 were characterized as other (1.9%). However, significantly more patients in the post-amendment cohorts including both arms received concurrent antiretroviral therapy (77% vs. 57%, p=0.041). In addition, patients in the concurrent arm had a numerically (but not significantly) lower median CD4 count in the post-amendment compared with the pre-amendment cohort (median 153/uL vs. 295/uL), a trend that was evident in the sequential arm (median 194/uL vs. 212/uL). However, for the total population, median CD4 count was generally similar in the concurrent and sequential arms (median 181/uL vs. 194/uL).

**Treatment Administered**

A total of 587 EPOCH cycles were given to all 106 patients, including 233 in the concurrent arm (median of 5, range 1-6 cycles per patient) and 254 in the sequential arm (median of 6, range 1-6 per patient). Some dose reduction of EPOCH was required for 15 patients (29%) treated in arm A and 17 patients (31%) treated in arm B. The median relative dose intensity was comparable in arms A and B, averaging respectively 100% and 99% for cyclophosphamide (relative to the planned cyclophosphamide dose in cycle 1), 100% and 99% for etoposide, 98% and 98% for doxorubicin, and 97% and 92% for vincristine. Cyclophosphamide was not given in the second or subsequent cycles (due to protocol specified dose reduction stipulated in Table 1) in 15 patients (29 treatment cycles) in the concurrent arm, and 12 patients (26 treatment cycles) in the sequential arm. All patients in the concurrent arm received rituximab (median of 5 doses, range 1-7 doses), whereas only 28 patients in the concurrent arm (51%) received
rituximab (median of 6 doses, range 2-6 doses for those who received it). Of the 27 patients in the sequential arm who did not receive rituximab, reasons included disease progression (N=8), death (N=4), patient withdrawal or non-compliance (N=4), physician discretion (N=4), adverse event (N=1), or other unspecified reasons (N=6, of whom 2 had a PR and 4 had a CR).

For those treated after the amendment, a total of 329 cycles were given to 71 patients, including 156 in the concurrent arm (median of 5, range 1-6 cycles per patient) and 173 in the sequential arm (median of 6, range 1-6 per patient). Some dose reduction of EPOCH was required for 10 patients (29%) treated in arm A and 15 patients (41%) treated in arm B. For the post-amendment population, the median relative dose intensity was also comparable in arms A and B, averaging respectively 118% and 102% for cyclophosphamide (relative to the planned cyclophosphamide dose in cycle 1), 100% and 98% for etoposide, 100% and 96% for doxorubicin, and 97% and 88% for vincristine. For all patients treated after the amendment, the protocol stipulated escalation in the cyclophosphamide dose if the neutrophil nadir were above 500/uL and platelet nadir were above 25,000/uL. Patients treated before the amendment were generally not dose escalated, although some of the patients were escalated after sites were notified. After the protocol amendment, 16 of 34 patients (47%) in the concurrent arm and 18 of 37 patients (49%) in the sequential arm had cyclophosphamide dose escalation.

**Response Data**

Response data are shown in Table 3 for the pre-amendment, post-amendment, and total populations. Five patients were considered unevaluable for response in the entire study population (Figure 1) because either response data were unknown (N=2 in arm A), physician withdrawal from therapy (N=1 in arm A), patient withdrawal (N=1 in arm B), or patient
noncompliance (N=1 in arm B). In the concurrent arm, 48 of 51 treated patients were evaluable for response (3 were unevaluable for response after amendment). In the sequential arm, 53 of 55 treated patients were evaluable for response (2 were unevaluable for response before amendment).

The primary efficacy analysis was based upon CR rate in the post-amendment population. CR occurred in 21 of 31 evaluable patients (68%; 95% confidence intervals [CI] 49%, 83%) in the concurrent arm, and in 18 of 37 patients (49%; 95% CI 32%, 66%) in the sequential arm. Therefore, the null hypothesis that the CR rate is 50% was rejected in favor of the alternative that is 75% for the concurrent arm, but not the sequential arm; in other words, the concurrent arm met the prespecified efficacy endpoint, whereas the sequential arm did not. The CR rates tended to be lower in the post-amendment compared with the pre-amendment cohorts in both the concurrent arm (68% vs. 82%) and sequential arm (49% vs. 69%), which may reflect inclusion of patients with more advanced immunosuppression in the post-amendment cohort as previously described in the patient characteristic section.

For the secondary efficacy analysis in the entire population, CR occurred in 35 of 48 evaluable patients (73%; 95% CI 58%, 85%) in the concurrent arm; an additional 7 patients had a partial response (PR), yielding an overall response rate of 88%. In the sequential arm, 29 of 53 evaluable patients (55%; 95% CI 41%, 68%) had a CR; an additional 12 patients had a PR, yielding an overall response rate of 77%. For the entire population, the null hypothesis that the CR rate is 50% was rejected in favor of the alternative that is 75% for the concurrent arm (post hoc p=0.005, power 0.89) but not the sequential arm (post hoc p=0.394). Therefore, the results were similar for the secondary efficacy analysis in the entire population as for the primary efficacy analysis in the post-amendment cohort. The results were also similar if an intention to
treated analysis including all treated patients was performed for the entire population, with CR rates being 69% in the concurrent arm (95% CI 54%, 81%) and 53% in the sequential arm (95% CI 39%, 66%). Recurrences occurred in 11 of 64 patients (17%) with CR and 10 of 19 patients (53%) with PR in both arms.

**Response by Histology and Results of Central Pathology Review**

When the response rate was analyzed in all treated patients who had DLBCL, CR occurred in 25 of 35 patients (71%, 95% CI 54%, 85%) in the concurrent arm and 20 of 44 patients in the sequential arm (46%; 95% CI 30%, 61%). For those who had Burkitt’s like lymphoma and other aggressive subtypes, CR occurred in 10 of 16 patients in the concurrent arm (63%; 95% CI 35%, 85%) and 9 of 11 patients in the sequential arm (82%, 95% CI 48%, 98%).

Central review of pathologic specimens was performed by two hematopathologists (A.C. and E.C.) for 58 cases. There was complete concordance between local and central diagnosis in 65%. Only 1 of 12 tumors (8%) classified as Burkitt’s/Burkitt-like by the local pathologist was classified as DLBCL centrally, and only 2 of 43 cases (5%) classified as DLCBL locally were classified as Burkitt’s/Burkitt’s-like centrally. The majority of cases without exact concordance were not precisely classified centrally due to insufficient material for review.

**Adverse Events**

The incidence of grade 3-4 adverse events occurring in at least 5% of patients are shown in Table 4 for the entire population. The overall toxicity profile was comparable in the two treatment arms. The most common grade 3-4 events in both treatment arms included neutropenia in 42%, infection in 28%, anemia in 16%, thrombocytopenia in 12%, febrile neutropenia in 12%,...
mucositis in 5%, and neuropathy in 2%. The toxicity profile was similar in the post-amendment population (data not shown). Grade 3-4 adverse events leading to premature discontinuation of therapy occurred in 3 patients (6%) in arm A (including neutropenia, fever, and hypotension in one patient each), and 3 patients (5%) in arm B (including infection in 1 patient and hepatitis in 2 patients).

Treatment-associated deaths were defined as deaths occurring during or within 30 days of treatment from causes other than lymphoma that were judged to be possibly or probably associated with therapy. Treatment-associated deaths did not occur at a rate which triggered study interruption. There were 5 treatment-associated deaths (9.8%) in the concurrent arm and 4 (7.3%) in the sequential arm. Causes of death (and baseline CD4 count) in the concurrent arm included cryptosporidiosis (32/uL), wasting (2/uL), sepsis (75/uL), multi-system failure (42/uL), and JC virus (153/uL); the median time between treatment initiation and death was 2 months (range 1-5 months). Causes of death in the sequential arm included sepsis in 3 patients (94/uL, 194/uL, 194/uL) and HIV complications (662/uL) in 1 patient; the median time between treatment initiation and death was 1 month (range 1-4 months). Although the treatment-associated death rate was comparable in the two treatment arms, they were numerically (although not significantly) more common in severely immunosuppressed patients in the concurrent arm, occurring in 3 of 8 patients (38%) with a baseline CD4 count of less than 50/uL in the concurrent arm compared with 0 of 7 in the sequential arm (Fisher’s exact 2-sided p =0.2)

**Progression-Free Survival, Overall Survival, and Time to Progression**

After a median follow-up of 30.0 months (range 0-68 months) in all treated patients, 17 patients (33%) died in the concurrent arm and 19 (35%) died in the sequential arm. Lymphoma was the
cause of death in 9 patients (18%) in the concurrent arm and 10 (16%) in the sequential arm. The 1 year and 2 year progression-free survival (PFS) rates in the concurrent arm were 78% (95% CI 67%, 90%) and 66% (95% CI 53%, 79%), respectively, and in the sequential arm were 66% (95% CI 54%, 79%) and 63% (95% CI, 50%, 76%), respectively (Figure 2a). Two year overall survival rates were 70% (95% CI 57%, 83%) in the concurrent and 67% (95% CI 54%, 80%) in the sequential arms, respectively (Figure 2b). The two year TTP rates in the concurrent and sequential arms were 75.4% (95% CI 63.2%, 87.5%) and 71.3% (95% CI 58.5%, 84.0%), respectively, indicating that about 70-75% of surviving patients were without evidence of progressive lymphoma at 2 years (Figure 2c). Results were similar in the post-amendment population. The 2 year PFS rates in the concurrent and sequential arms were 64% (95% CI 48%, 80%) and 60% (95% CI 44%, 75%), respectively. Two year overall survival rates were 63% (95% CI 47%, 80%) and 66% (95% CI 51%, 82%), respectively.

DISCUSSION

The incidence of B-cell non-Hodgkin's lymphoma is increased in HIV-infected individuals. Since the routine use of "highly active antiretroviral therapy" (HAART) in clinical practice beginning in 1996, there have been significant reductions in HIV-associated morbidity and mortality. Although the incidence of HIV-associated lymphoma has also declined in the HAART era and its prognosis has improved compared with the dismal prognosis in pre-HAART era, it remains a common complication of HIV infection that is cured in only 30-50% with standard anti-lymphoma therapy.

Our trial was designed to determine the efficacy of infusional EPOCH chemotherapy used alone (followed sequentially by rituximab) and infusional EPOCH given concurrently with
rituximab in patients with HIV-associated B-cell non-Hodgkin’s lymphoma. CR rate was chosen as the primary efficacy endpoint because it is a well accepted short term surrogate known to be associated with long term cure, and standard response criteria and response definitions were used. We used what some have referred to as a “selection” or “pick the winner” randomized phase II trial design, wherein each arm is regarded as experimental, and the efficacy of each arm is evaluated independently and compared to historical data with standard therapy.\textsuperscript{27} We sought to determine whether either experimental arm was associated with a CR rate of at least 75%, substantially higher than the approximately 50% CR rate typically observed for standard regimens, including CHOP or R-CHOP therapy in a previous AMC trial (AMC 010) performed in the HAART era.\textsuperscript{8} Although this design does not allow a direct efficacy comparison between the two arms, it does facilitate a direct comparison of toxicity in the two arms.

First, we found that concurrent rituximab plus infusional EPOCH chemotherapy resulted in a sufficiently high CR rate required to reject that null hypothesis of no difference compared with standard therapy; in other words, the CR rate was significantly higher than would be expected with standard therapy for this arm. Second, the CR rate observed with EPOCH alone (followed sequentially by rituximab) was consistent with CR rates typically observed with standard regimens. Third, although progression-free and overall survival rates were similar for the two arms, the trial was not adequately powered to detect differences for these endpoints. In addition, some evidence suggests benefit for rituximab when given sequentially following chemotherapy, thereby confounding any evaluation of progression-free or overall survival.\textsuperscript{3} However, the 1year progression-free survival rates observed for both the concurrent arm (78%; 95% CI 67%, 90%) and sequential arm (66%; 95% CI 54%, 79%) in this trial compare favorably with those observed with either CHOP alone (50%; 95% CI 39%, 61%) or R-CHOP (48%; 95%
CI 32%, 64%) in the AMC 010 trial, suggesting that infusional EPOCH may have contributed to this improvement compared with historical data. Fourth, the toxicity profile of infusional EPOCH was comparable whether given alone, or concurrently with rituximab. Although the treatment associated death rate in the concurrent arm was 10%, it was similar to the 7% rate in the sequential arm. Moreover, the infectious death rate for concurrent R-EPOCH may be minimized by administering it only in patients with a CD4 count of at least 50/uL.

The results of this trial seem to contradict the results of a prior study conducted by the AMC (AMC010) which found a significantly higher death rate in the concurrent rituximab-CHOP arm compared with CHOP alone. There are several potential explanations for this difference. First, in the prior trial, patients received concurrent rituximab plus CHOP followed by monthly rituximab infusions for 3 months, which may have contributed to late infectious deaths that occurred in about 50% of all patients with treatment-associated deaths. Second, patients in the current trial received quinolone prophylaxis, which was not used in the previous trial. Third, patients in the current trial treated with concurrent R-EPOCH had a substantially higher median CD4 count than the R-CHOP arm of the prior AMC trial (median 194/uL vs. 128/uL). However, despite the overall acceptable safety profile associated with concurrent R-EPOCH, patients with a very low CD4 count may be better served by treating with chemotherapy alone, followed by rituximab if there is evidence of good response.

A potential concern about this trial was the failure to consistently dose escalate cyclophosphamide as described in the original reports of EPOCH in HIV-associated lymphoma and immunocompetent patients with lymphoma, a factor that may have contributed to a lower CR rate than expected in the sequential arm of our trial. Although this dose escalation may very well be an important factor which contributes to the efficacy of EPOCH in an
immunocompetent population or in Burkitt’s or Burkitt-like lymphoma, we believe that the absence of consistent dose escalation did not compromise the efficacy of the sequential arm of our trial for several reasons. First, the trial was amended to assure that a sufficient number of patients received cyclophosphamide dose escalation in the post-amendment population. Second, the CR rates were if anything lower (although not significantly so) in the post-amendment population, including the concurrent arm (67% post vs. 82% pre) and sequential arm (49% post vs. 69% pre). Third, other studies involving intensification of cyclophosphamide and other agents have not proven to be superior to CHOP in HIV-associated lymphoma. Therefore, we are confident that a therapeutic strategy of EPOCH alone including cyclophosphamide dose escalation as originally described is not likely to be associated with the 75% CR rate which we were targeting. In addition, the subtle differences amongst the pre and post-amendment populations support our approach of also evaluating efficacy in the total population, and not just in the post-amendment population alone, because of the known adverse impact of severe immunosuppression on response and prognosis in HIV-associated lymphoma. This also points out the importance of including a concurrent control arm in future trials evaluating novel treatment approaches in HIV-associated lymphoma when a standard potentially curative treatment option is available.

This trial included patients with both DLBCL and Burkitt’s/Burkitt-like lymphoma, with DLBCL accounting for about 74% of the patients treated. Although R-CHOP or R-CHOP-like regimens are commonly used for DLBCL, more intensive regimens are typically recommended for Burkitt’s/Burkitt-like lymphomas. For DLBCL, the CR rate appeared to be particularly encouraging for concurrent R-EPOCH arm (71%, 95% CI 54%, 85%), but not for the sequential arm (46%; 95% CI 30%, 61%). For Burkitt’/Burkitt-like/other lymphomas, CR rates were
encouraging for both the concurrent arm (63%; 95% CI 35%, 85%) and sequential arm (82%, 95% CI 48%, 98%), although the number of patients evaluated was relatively small. Our findings suggest that concurrent R-EPOCH may be a particularly effective regimen for HIV-associated DLBCL, and that an EPOCH-containing regimen may be considered a reasonable alternative for patients with HIV-associated Burkitt’s/Burkitt-like lymphoma who may not be suitable candidates for more intensive regimens. Given the importance of cyclophosphamide dose in Burkitt’s/Burkitt-like lymphoma, additional studies in Burkitt’s/Burkitt-like lymphoma of rituximab plus EPOCH including cyclophosphamide dose escalation are warranted.

It has recently been shown that among immunocompetent patients, DLBCL is a heterogeneous disease that may be further characterized into germinal center (GC) and non-germinal center (non-GC) subtypes that have differing prognoses which arrive by distinct genetic pathways. In addition, certain markers have been associated with prognosis, including proliferation index (measured by expression of Ki67) and others. We have recently reported that in a subgroup of 82 patients with DLBCL derived from this trial and in a previous trial including CHOP (used alone or in combination with rituximab), there was no significant difference in survival, CD4 count, or EBV expression among patients with the GC and non-GC subtypes. The only predictive immunohistochemical marker was found to be Ki67, wherein a higher proliferation index was associated with improved survival. These results suggest that patients with DLBCL and high proliferation index, a characteristic that is usually associated with Burkitt’s/Burkitt-like lymphoma, may be adequately treated with R-EPOCH therapy.

Our findings are also consistent with the results of other trials evaluating rituximab plus infusional chemotherapy in aggressive grade lymphoma, including EPOCH in immunocompetent patients, or infusional cyclophosphamide, doxorubicin, and etoposide (CDE) in HIV-positive
patients. The latter study included 74 patients with HIV-associated B-cell lymphoma; the CR rate was 70%, lethal treatment-associated toxicity rate was 6%, and the estimated two-year progression free survival was 59%, similar to our study. Given that rituximab is known to sensitize lymphoma cells to cytotoxic agents in vitro and has a long-half life of approximately 7 days, infusional administration of cytotoxic therapy may offer greater potential for therapeutic synergy than the same agents given by a conventional IV bolus route. The failure to administer rituximab in about 50% of those in the sequential arm provides further evidence supporting concurrent delivery with EPOCH. These findings support the design of a phase III trial comparing concurrent R-EPOCH with R-CHOP in immunocompetent patients with diffuse, large B-cell lymphoma (NCT00118209), which will provides level 1 evidence supporting infusional EPOCH should the trial demonstrate improved efficacy for this arm. In the interim, this report provides level 2 evidence supporting the use of concurrent R-EPOCH in patients with HIV-associated lymphoma, especially DLBCL.
**Acknowledgement:** Supported by a grant from the Department of Health and Human Services (U01 CA121947; Ronald Mitsuyasu, MD, Chair). The authors fondly acknowledge and remember Dr. William Harrington, who made an important contribution to this work, and who died unexpectedly on Jan 29, 2009. Dr. Harrington was an outstanding scientist, clinician, scholar, and friend who will be missed by his colleagues in the AIDS Malignancy Consortium.

**Author contributions:** The manuscript was written by J.A.S. and was approved by all co-authors. The clinical protocol was written by J.A.S., J.Y.L., and L.D.K. The data and statistical analysis were performed by J.Y.L., and administrative support and oversight was provide by R.M. Pathologic review of tumor specimens was performed by E.C. and A.C. Individuals who contributed subjects to the trial included J.A.S., L.D.K., A.M.L., J.C.R., R.F.A., D.A., A.N., D.H.H., J.V.R., B.J.D., S.C.R., M.H.S., L.L., L.R., E.C., and A.C.. The authors have no financial disclosures or conflicts of interest to report.
References:


<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose &amp; Route</th>
<th>Duration</th>
<th>Schedule</th>
<th>Dose Modifications for Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>375 mg/m2 IV infusion</td>
<td>Over 2-6 hours</td>
<td>Prior to each EPOCH cycle (arm A) or weekly x 6 weeks after EPOCH completed (arm B)</td>
<td>Skin/mucositis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Discontinue if attributed to rituximab</td>
</tr>
<tr>
<td>EPOCH</td>
<td>Etoposide 50 mg/m2/day</td>
<td>4 days (96 hours)</td>
<td>Every 3 weeks</td>
<td>Hematolotogic toxicity+</td>
</tr>
<tr>
<td></td>
<td>By continuous IV infusion</td>
<td></td>
<td></td>
<td>• Reduce by 25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Renal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Reduce to 25% if creatinine clearance &lt; 40 ml/min</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>10 mg/m2/day</td>
<td>4 days (96 hours)</td>
<td>Every 3 weeks</td>
<td>Hematologic toxicity+</td>
</tr>
<tr>
<td></td>
<td>By continuous IV infusion</td>
<td></td>
<td></td>
<td>• Reduce by 25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hepatic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Reduce to 25% if direct bilirubin &gt; 2.5 mg/dl</td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.4 mg/m2/day IV infusion</td>
<td>4 days (96 hours)</td>
<td>Every 3 weeks</td>
<td>Neurotoxicity:</td>
</tr>
<tr>
<td></td>
<td>By continuous IV infusion</td>
<td></td>
<td></td>
<td>• Obstipation or unable to walk on heels – reduce to 0.3 mg/m2/day</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Difficulty ambulating – discontinue</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Renal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Reduce to 25% if direct bilirubin &gt; 2.5 mg/dl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Renal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Reduce to 25% if creatinine clearance &lt; 40 ml/min</td>
</tr>
<tr>
<td>Prednisone</td>
<td>60 mg/m2/day orally</td>
<td>5 days</td>
<td>Every 3 weeks</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Cycle 1: 187 mg/m2 IV if CD 4&lt; 100/uL 375 mg/m2 IV if CD4 &gt; 100/uL</td>
<td>60 minute infusion</td>
<td>Every 3 weeks after completion of etoposide, doxorubicin, vincristine infusion</td>
<td>Cycle 2-6:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Escalate dose in increments of 187 mg/m2/cycle if neutrophil nadir above 500/uL and platelet nadir above 25,000/uL in preceding cycle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Reduce dose in increments of 187 mg/m2 if the nadir neutrophil count was less than 500/uL or platelet nadir less than 25,000/uL in preceding cycle</td>
</tr>
<tr>
<td>Supportive Care</td>
<td>Filgrastim (or pegfilgrastim) 5 mcg/kg subcutaneous injection (or pegfilgrastim 6 mg)</td>
<td>Daily until neutrophil recovery (or</td>
<td>Begin on day 6 after EPOCH completed</td>
<td></td>
</tr>
</tbody>
</table>
about 24 hours after EPOCH completed once for pegfilgrastim)

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dose/Administration</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim-</td>
<td>160-800 mg PO</td>
<td>Continuous Thrice weekly (eg,</td>
</tr>
<tr>
<td>sulfamethoxazole</td>
<td></td>
<td>Monday, Wednesday, Friday)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>100 mg PO</td>
<td>Continuous Daily</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>500 mg PO BID (or</td>
<td>At least 8 days each cycle</td>
</tr>
<tr>
<td></td>
<td>alternative quinolone and dose at discretion of physician)</td>
<td>Begin on days 8 of each cycle, continue until day 15 or post-nadir neutrophil count ≥ 1000/μL</td>
</tr>
</tbody>
</table>

+ Hematologic toxicity: toxicity calling for dose reduction of doxorubicin and etoposide included a neutrophil nadir less than 500/μL for at least 3 days or platelets less than 25,000/μL for at least 3 days if this occurs during a cycle in which no cyclophosphamide was given; otherwise, doxorubicin/etoposide were not reduced.
Table 2. Clinical characteristics of the entire population by treatment arm

<table>
<thead>
<tr>
<th></th>
<th>Pre-Amendment</th>
<th>Post-Amendment</th>
<th>All Patients</th>
<th>Pre-Amendment</th>
<th>Post-Amendment</th>
<th>All Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. treated</td>
<td>17</td>
<td>34</td>
<td>51</td>
<td>18</td>
<td>37</td>
<td>55</td>
</tr>
<tr>
<td>Male gender</td>
<td>14 (82%)</td>
<td>29 (85%)</td>
<td>43 (84%)</td>
<td>16 (89%)</td>
<td>32 (86%)</td>
<td>48 (87%)</td>
</tr>
<tr>
<td>Median age</td>
<td>45 years</td>
<td>44 years</td>
<td>44 years</td>
<td>44 years</td>
<td>43 years</td>
<td>43 years</td>
</tr>
<tr>
<td>CD 4 count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>295/uL</td>
<td>153/uL</td>
<td>181/uL</td>
<td>212/uL</td>
<td>194/uL</td>
<td>194/uL</td>
</tr>
<tr>
<td>&lt; 100/uL</td>
<td>4 (24%)</td>
<td>12 (35%)</td>
<td>16 (31%)</td>
<td>5 (28%)</td>
<td>12 (32%)</td>
<td>17 (31%)</td>
</tr>
<tr>
<td>Concurrent ARV</td>
<td>10 (59%)</td>
<td>26 (76%)</td>
<td>36 (71%)</td>
<td>10 (56%)</td>
<td>29 (78%)</td>
<td>39 (71%)</td>
</tr>
<tr>
<td>Local histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse large cell</td>
<td>12 (71%)</td>
<td>23 (68%)</td>
<td>35 (69%)</td>
<td>14 (78%)</td>
<td>30 (81%)</td>
<td>44 (80%)</td>
</tr>
<tr>
<td>Burkitt’s/Burkitt’s like or other +</td>
<td>5 (29%)</td>
<td>11 (32%)</td>
<td>16 (31%)</td>
<td>4 (22%)</td>
<td>7 (19%)</td>
<td>11 (20%)</td>
</tr>
<tr>
<td>Stage III-IV</td>
<td>14 (82%)</td>
<td>29 (85%)</td>
<td>43 (84%)</td>
<td>13 (72%)</td>
<td>28 (76%)</td>
<td>41 (75%)</td>
</tr>
<tr>
<td>Elevated LDH</td>
<td>11 (65%)</td>
<td>25 (73%)</td>
<td>36 (71%)</td>
<td>11 (61%)</td>
<td>25 (68%)</td>
<td>36 (65%)</td>
</tr>
<tr>
<td>ECOG PS 2</td>
<td>6 (35%)</td>
<td>8 (24%)</td>
<td>14 (27%)</td>
<td>2 (11%)</td>
<td>9 (24%)</td>
<td>11 (20%)</td>
</tr>
<tr>
<td>Age-Adjusted IPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 or 1 Risk Factors</td>
<td>6 (35%)</td>
<td>10 (29%)</td>
<td>16 (31%)</td>
<td>6 (33%)</td>
<td>14 (38%)</td>
<td>20 (36%)</td>
</tr>
<tr>
<td>2 or 3 Risk Factors</td>
<td>11 (65%)</td>
<td>24 (71%)</td>
<td>35 (69%)</td>
<td>12 (67%)</td>
<td>23 (62%)</td>
<td>35 (64%)</td>
</tr>
</tbody>
</table>

Abbreviations: ARV – antiretroviral therapy; ECOG PS – Eastern Cooperative Oncology Group performance status; IPI – International Prognostic Index (adverse risk factors included stage III-IV disease, elevated serum lactate dehydrogenase above normal, and Eastern Cooperative Oncology performance status 2); + for the Burkitt’s/Burkitt’s like/other category, this included 15 of 16 patents with Burkitt’s or Burkitt’s-like lymphoma in arm A, and 8 of 11 in arm B; other cases included malignant lymphoma, not otherwise specified.
Table 3. Response data by treatment arm

<table>
<thead>
<tr>
<th></th>
<th>Arm A</th>
<th>Arm B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concurrent R-EPOCH</td>
<td>Sequential EPOCH→R</td>
</tr>
<tr>
<td>Pre-amendment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Treated/Evaluable</td>
<td>17/17</td>
<td>18/16</td>
</tr>
<tr>
<td>CR or CR u</td>
<td>14 (82%)</td>
<td>11 (69%)</td>
</tr>
<tr>
<td>95% Confidence Intervals</td>
<td>57%, 96%</td>
<td>41%, 89%</td>
</tr>
<tr>
<td>CR + PR</td>
<td>16 (94%)</td>
<td>13 (82%)</td>
</tr>
<tr>
<td>Post-amendment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Treated/Evaluable</td>
<td>34/31</td>
<td>37/37</td>
</tr>
<tr>
<td>CR or CR u</td>
<td>21 (68%)</td>
<td>18 (49%)</td>
</tr>
<tr>
<td>95% Confidence Intervals</td>
<td>49%, 83%</td>
<td>32%, 66%</td>
</tr>
<tr>
<td>CR + PR</td>
<td>26 (84%)</td>
<td>28 (76%)</td>
</tr>
<tr>
<td>All Patients +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Treated/Evaluable</td>
<td>51/48</td>
<td>55/53</td>
</tr>
<tr>
<td>CR or CR u</td>
<td>35 (73%)</td>
<td>29 (55%)</td>
</tr>
<tr>
<td>95% Confidence Intervals</td>
<td>58%, 85%</td>
<td>41%, 68%</td>
</tr>
<tr>
<td>CR + PR</td>
<td>42 (88%)</td>
<td>41 (77%)</td>
</tr>
</tbody>
</table>

+In an intention to treat analysis in all treated patients, the CR rates 69% in the concurrent arm (95% CI 54%, 81%) and 53% in the sequential arm (95% CI 39%, 66%)
Table 4. Grade 3-4 adverse events occurring in at least 5% of patients for the entire population by treatment arm

<table>
<thead>
<tr>
<th>Grade 3-4 Adverse Events</th>
<th>Arm A Concurrent R-EPOCH</th>
<th>Arm B Sequential EPOCH⇒R</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>51</td>
<td>55</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>22 (43%)</td>
<td>22 (40%)</td>
</tr>
<tr>
<td>Anemia</td>
<td>10 (22%)</td>
<td>7 (13%)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>4 (8%)</td>
<td>9 (16%)</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>8 (16%)</td>
<td>8 (15%)</td>
</tr>
<tr>
<td>Infection</td>
<td>14 (27%)</td>
<td>16 (29%)</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>0</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Mucositis</td>
<td>1 (2%)</td>
<td>4 (7%)</td>
</tr>
</tbody>
</table>
Legends

Figure 1. Consort diagram.

Figure 2a. Progression-free survival for the entire population by treatment arm (N=106)

Figure 2b. Overall survival for the entire population by treatment arm (N=106)

Figure 2c. Time to progression for the entire population by treatment arm (N=106)
Assessed for Eligibility, Registered and Randomized (n=110)

Enrollment

Arm A: Concurrent rituximab + EPOCH
Allocated to intervention (n=54)
Received allocated intervention (n=51)
Did not receive allocated intervention (n=3)
- Patient withdrawal (N=3)
Lost to follow-up (n=2)
- No response information (n=2)
Discontinued intervention (n=1)
- Physician discretion (n=1)

Arm B: Sequential EPOCH → rituximab
Allocated to intervention (n=56)
Received allocated intervention (n=55)
Did not receive allocated intervention (n=1)
- Patient withdrawal (N=1)
Lost to follow-up (n=0)
Discontinued intervention (n=2)
- Patient withdrawal (n=1)
- Patient non-compliance (n=1)

All Patients
Analyzed (n=51)
Analyzed for response (n=48)
Excluded from response analysis (n=3)
- No response information (n=2)
- Physician discretion (n=1)

Post Amendment Population
Analyzed (n=34)
Analyzed for response (n=31)
Excluded from response analysis (n=3)
- No response information (n=2)
- Physician discretion (n=1)

All Patients
Analyzed (n=55)
Analyzed for response (n=53)
Excluded from response analysis (n=2)
- Patient withdrawal (n=1)
- Patient non-compliance (n=1)

Post Amendment Population
Analyzed (n=37)
Analyzed for response (n=37)
Figure 2a
Progression-Free Survival

Months

Group

Arm A: Concurrent

Arm B: Sequential
Figure 2b

Overall Survival

Cumulative % Survival vs. Months

Group
- Arm A: Concurrent
- Arm B: Sequential
Figure 2c

Time to Progression

Cumulative % Progression-free

Months

Group

Arm A: Concurrent

Arm B: Sequential
Rituximab plus concurrent infusional EPOCH chemotherapy is highly effective in HIV-associated, B-cell non-Hodgkin’s lymphoma