Abbreviated Title: *EPOCH-R^2 in PEL*
NIH Protocol #: 16C0171
Version Date: 06/06/2023
NCT Number: NCT02911142

Title: Phase I/II Study of Lenalidomide Combined with Modified DA-EPOCH and Rituximab (EPOCH-R^2) in Primary Effusion Lymphoma or KSHV-Associated Large Cell Lymphoma

NCI Principal Investigator: Kathryn Lurain, MD
HIV/AIDS Malignancy Branch
Center for Cancer Research
National Cancer Institute
10 Center Drive, Room 6N110
Bethesda, MD 20892-1020
Tel: 301-250-5156
Email: Kathryn.Lurain@nih.gov

Drug Name:	Lenalidomide	Etoposide, Vincristine, Prednisone, Doxorubicin, Cyclophosphamide	Rituximab (including biosimilar)	Filgrastim (including biosimilars)
IND Number:	131663			
Sponsor:	Center for Cancer Research			
Manufacturer:	Celgene Corporation*	Generic	Multiple	Multiple
Supplier	Celgene Corporation	NIH CC pharmacy	NIH CC Pharmacy	NIH CC Pharmacy

* On November 20, 2019, Celgene was acquired by Bristol-Myers Squibb (BMS) and Celgene is a subsidiary of BMS.To reflect this development, as of Amendment D, we retain the name Celgene within the protocol as the CRADA still reflects Celgene as the manufacturer. .

PRÉCIS

Background

- Kaposi sarcoma herpesvirus (KSHV)-associated primary effusion lymphoma (PEL) is an aggressive B cell neoplasm with clinicopathologic and molecular profiles distinct from other AIDS-related lymphomas.
- There are no prospective studies on these rare lymphomas. Clinical experience is limited; however, reported prognosis is poor, with median survival estimated at less than 6 months using conventional CHOP-like chemotherapy.
- Novel treatment is urgently needed for KSHV-associated lymphomas, and the therapeutic approach must take into account concurrent KSHV-associated malignancies which are commonly seen in this patient population
- Lenalidomide, an immune-modulatory derivative of thalidomide (IMiD drug) has *in vitro* direct antitumor effect in KSHV-lymphomas as well as immune modulatory and anti-angiogenic effects that may be beneficial in treating PEL
- Rituximab, an anti-CD20 monoclonal antibody, has recently been shown to be an active agent in the management of KSHV-MCD. Although PEL is a CD20-negative tumor, advances in the understanding the biology of KSHV-infection of B-cells, the pathobiology of IL-6 syndromes in KSHV-MCD and KSHV-NHL, and clinical experience using rituximab in the treatment of KSHV-MCD, support use of rituximab in the treatment of PEL, especially in patients with concurrent KSHV-MCD.
- Modified dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (DA)-EPOCH is an anthracycline-based regimen that allows for personalization of dose-intensity showing that inclusion of etoposide and infusional administration decreases tumor cell resistance.
- The use of DA-EPOCH in combination with rituximab for the treatment of HIV-associated diffuse large B-cell lymphoma or Burkitt lymphoma has been shown to be safe and effective.
- Given the central role of controlling HIV viremia with combination antiretroviral therapy (cART) in the management of KSHV-associated malignancies, as well as the likely contribution of uncontrolled HIV viremia to PEL pathogenesis, cART will be employed as an important part of the treatment regimen.

Objectives

Phase I

- Evaluate safety and tolerability of lenalidomide in combination with DA-EPOCH-R and determine the maximum tolerated dose and/or recommended phase II dose of this regimen.

Phase II

- Evaluate overall survival in treatment-naïve participants with KSHV-positive aggressive B cell lymphomas treated with lenalidomide in combination with DA-EPOCH and rituximab (DA-EPOCH-R^2).

Eligibility

- Adult participants ≥ 18 years with pathology confirmed any KSHV-positive aggressive B cell lymphomas, such as primary effusion lymphoma, and KSHV-associated large cell lymphoma
- Lymphoma that is measurable or assessable
- Any HIV status
- Hematologic and biochemical parameters within pre-specified limits at screening
- Willing to use effective birth control, as defined in the full protocol
- Neither pregnant nor breast feeding
- Excluded if other serious co-morbid condition that would prohibit administration of planned chemotherapeutic intervention is present

Design

- This is a phase I/ II study of lenalidomide in combination rituximab and modified DA-EPOCH (EPOCH-R^2) in participants with KSHV-positive aggressive B cell lymphomas.
- Phase I of the study will evaluate lenalidomide 25 mg days 1-10 in combination with modified DA-EPOCH-R to determine safety and tolerability. Dose de-escalation doses of lenalidomide are 20 mg and 15 mg.
- Participants with HIV will generally be prescribed cART.
- In phase I, with up to 3 dose levels, 6-18 participants will be accrued (3-6 participants per level).
- In the phase II portion of the study, 15 evaluable participants will be enrolled over 48-60 months and 12 months follow-up after the last participant has enrolled, a 1-tailed 0.10 alpha level test would have 80% power to determine if OS curve would demonstrate a 1-year OS consistent with 45% or better and ruling out 20% or worse.

TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- **Phase I**

  Evaluate the safety and tolerability of lenalidomide in combination with modified dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab (DA-EPOCH-R^2) and determine the maximum tolerated dose and/or recommended phase II dose of lenalidomide in this regimen.

- **Phase II**

  Evaluate overall survival in treatment-naïve participants with KSHV-positive aggressive B cell lymphomas treated with with lenalidomide in combination with DA-EPOCH-R^2.

1.1.2 Secondary Objectives

- Evaluate pharmacokinetics of lenalidomide in blood, effusion and CSF
- Evaluate response rates and progression-free survival, and event-free survival for primary effusion lymphoma treated with DA-EPOCH-R^2
- Evaluate the effect of the regimen on concurrent Kaposi sarcoma, KSHV-associated multicentric Castleman disease, and KSHV-associated inflammatory cytokine syndrome

1.1.3 Exploratory Objectives

- Evaluate the effect of lenalidomide and rituximab on PBMC-associated KSHV and EBV viral load and serum cytokines
- Evaluate lymphocyte subset reconstitution and peripheral blood KSHV and EBV viral load after modified DA-EPOCH-R^2

- In HIV infected participants, evaluate the effect of lenalidomide alone and in combination with EPOCH-R on HIV latency reversal and cellular measures of HIV
- Evaluate the pharmacokinetics of tenofovir and tenofovir-diphosphate in plasma and PBMCs, respectively

1.2 BACKGROUND AND RATIONALE

1.2.1 Rationale for Proposed Regimen

1.2.1.1 Rationale for lenalidomide dosing

Proposed dose of lenalidomide is based on safety from the study of lenalidomide in combination with CHOP for the treatment of diffuse large B-cell lymphoma[1],[2] (See Rationale for EPOCH-R² below). The regimen is designed to maximize IRF4 downregulation and anti-tumor effect when given in combination with cytotoxic chemotherapy. Phase I will evaluate the safety and tolerability of modified DA-EPOCH-R when given in combination with lenalidomide on days 1-10. The regimen is named "EPOCH-R²" to reflect the components of the regimen: etoposide (E), prednisone (P), oncovin (vincristine) (O), cyclophosphamide (C), hydroxyldaunorubicin (doxorubin) (H), rituximab (R) and revlimid (lenalidomide) (R).

1.2.1.2 Rationale for use of modified dose-adjusted EPOCH as a platform for treating PEL

Although PEL has a viral etiology, it is a clonal aggressive B-cell neoplasm. In addition to the work done to study the contribution of viral genes in the pathogenesis of PEL, evaluation of B-cell immunoglobulin heavy chain demonstrates that these PEL cells are clonal[3], and in some cases that have been well characterized, have complex cytogenetic abnormalities and several regions of recurrent focal genomic amplification.[4, 5] Dose adjusted (DA)-EPOCH is an anthracycline-based regimen based on pre-clinical models showing that inclusion of etoposide and infusional administration decreased tumor cell resistance. DA-EPOCH is a pharmacodynamic adaptive regimen that allows for personalization of dose-intensity.[6] The use of DA-EPOCH in the treatment of HIV-infected patients with diffuse large B-cell lymphoma or Burkitt lymphoma was pioneered by Wyndham Wilson and colleagues in collaboration with the HAMB, providing experience with the safety and toxicity profile in patients with HIV[7, 8]. Once-daily prednisone, and limitation of cyclophosphamide escalation to 750 mg/m² are based on the short course EPOCH-R² regimen evaluated in HIV-associated diffuse large B-cell lymphoma.

1.2.1.3 Rationale for the inclusion of rituximab in treating PEL:

Rituximab is an anti-CD20 monoclonal antibody effective in a number of B-cell lymphomas. It has recently been shown to be an active agent in the management of KSHV-MCD. Although PEL is a CD20-negative tumor, advances in understanding the biology of KSHV-infection of B-cells[9], the pathobiology of IL-6 syndromes in KSHV-MCD and KSHV-NHL, and clinical experience using rituximab in the treatment of KSHV-MCD support a role for use of rituximab in the treatment of KSHV-NHL, especially in patients with concurrent KSHV-MCD. Patients with symptomatic KSHV-MCD can have severe inflammatory symptoms associated with detectable circulating viral IL-6,[10] marked increases in human IL-6 and IL-10, and high levels of circulating KSHV-infected PBMCs.[11]

Pathologic features of KSHV-MCD include regressed germinal centers and expansion of the marginal zone with KSHV-infected plasmablasts and CD20+ KSHV-uninfected B-cells. Treatment with rituximab-based therapy leads to normalization of adenopathy, decreases in IL-6

and IL-10, and decreases in circulating PBMC-associated KSHV (which are thought to largely represent KSHV-infected B-cells in most cases) in patients with MCD[12]. Interestingly, rituximab is effective in KSHV-MCD despite the fact that the KSHV-infected plasmablasts are almost always CD20-negative by immunohistochemistry. The same cytokine and virologic profile can be noted in HIV/KSHV co-infected patients with similar clinical presentations but no pathologic evidence of KSHV-MCD, and this KSHV-associated inflammatory cytokine syndrome was associated with progressive, chemotherapy resistant KS and high mortality in a small case series.[13]

Clinically, patients with PEL often present with inflammatory syndromes and dramatic increases in circulating levels of hIL-6 and IL-10, with levels comparable to those seen in MCD. Interestingly, thrombocytopenia and hypoalbuminemia, common laboratory abnormalities in patients with KSHV-associated IL6 inflammatory cytokine syndromes, have been associated with poor clinical outcomes in patients with PEL, suggesting IL-6 associated syndromes may contribute to mortality in KSHV-MCD.[14] **We hypothesize that these overlapping symptoms may share a common pathophysiology, even in the absence of pathologically confirmed MCD; and that they are at least partially driven by KSHV-infected B-cells, associated IL-6, IL-10, and v-IL-6 excess**. In a pilot study, the laboratory of Dr. Whitby has shown that it is possible that KSHV infection of B-cells may affect the pleural fluid inflammatory environment. To determine whether the high viral load noted in the PEL effusions was driven by the PEL cells, we fractionated cells from one subject and measured viral load in $CD138^+$ cells, $CD19^+$ cells and $CD14^+$ cells. Viral load was high in all three cell types, suggesting that KSHV infected B cells and monocytes contribute the high viral load observed in total effusion cells and may play a role in the pathology of pleural effusions. CD19+ and CD138+ cells can be isolated from effusions from patients with PEL using magnetic bead separation. The KSHV gene expression profile appears to differ greatly between these cell populations, with the CD19+ subset expressing a broader range of KSHV-encoded genes. (**Figure 7**)

1.2.1.4 Rationale for the use of EPOCH-R² in PEL

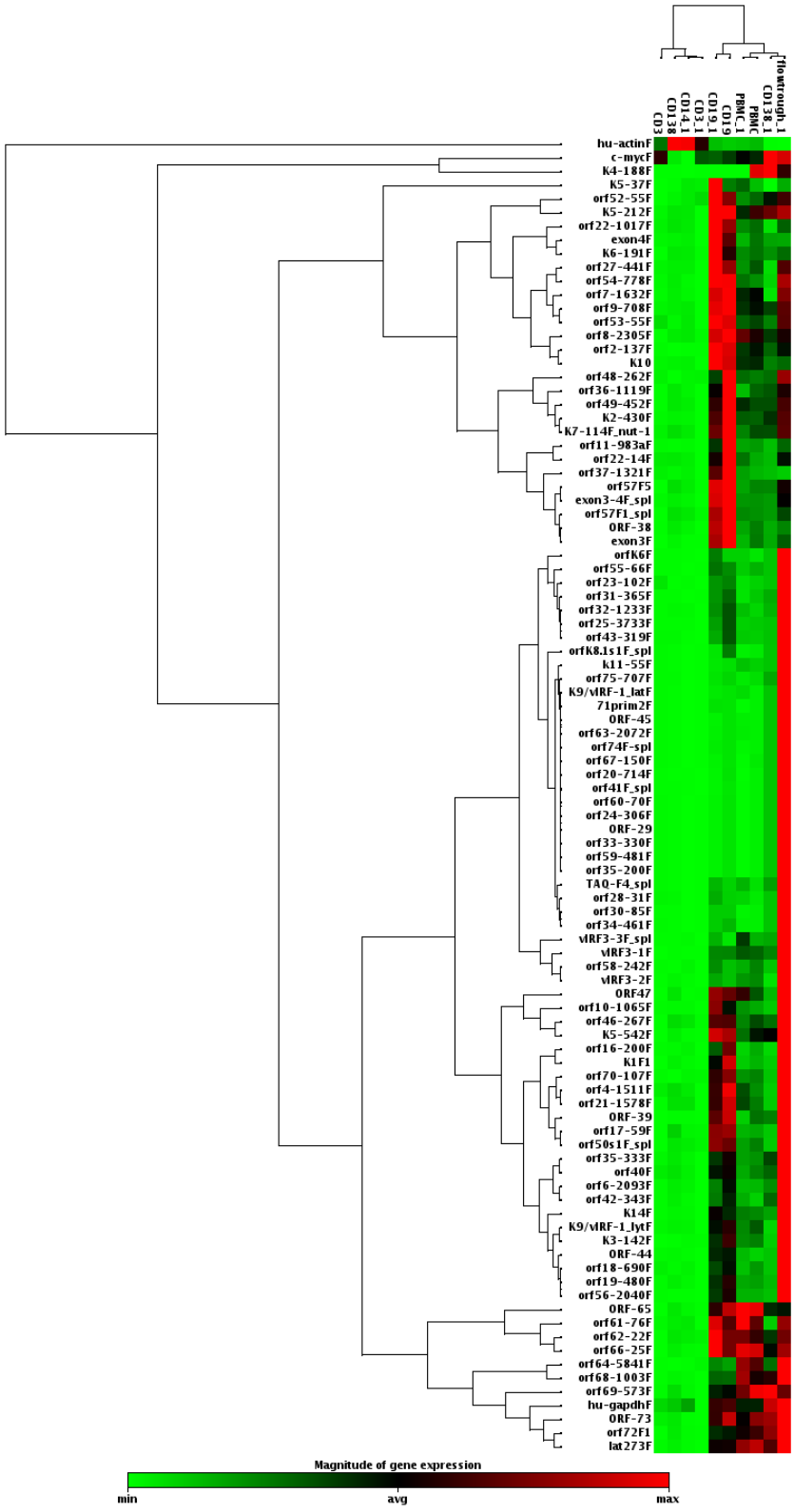
In a phase 1 study, Nowakowski et al. showed that lenalidomide and R-CHOP can be safely combined to treat patients with newly diagnosed, untreated aggressive B-cell lymphomas. Patients received oral lenalidomide on days 1-10 with standard dose R-CHOP every 21 days with pegfilgrastim support on day 2 and aspirin prophylaxis. The recommended dose for the phase II study was 25 mg on days 1-10 after no dose limiting toxicities were found.

Nowakowski et al. enrolled 64 patients in the phase 2 study with newly-diagnosed, untreated diffuse large B-cell lymphoma with performance status 0-2 and median age of 65[15]. Notably, patients with HIV were excluded from the study. Patients were treated on the same treatment schedule and dose of lenalidomide as that used in the phase 1 trial. The trial participants' tumors were classified as germinal center B-cell (GCB) versus non-germinal center B-cell (non-GCB) subtypes and compared to control patients from the Lymphoma Database treated with conventional R-CHOP. Of the 60 evaluable patients, the overall response rate was 98% with 80% achieving a complete response. The median follow-up in surviving patients was 23.5 months with an event-free survival of 70% at 12 months and 59% at 24 months with no difference based upon tumor classification. In the control R-CHOP patients, 24-month progression free survival and overall survival were 28% versus 64% and 46% and 78% in non-GCB DLBCL versus GCB DLBCL.

Importantly, the addition of lenalidomide did not impact the dose intensity of R-CHOP in either the phase I or phase II studies.

In another phase 2 trial conducted by Vitolo et al. in elderly patients with untreated diffuse large B-cell lymphoma, lenalidomide was combined with R-CHOP but at a dose of 15 mg on days 1-14 of six 21-day cycles. Ninety-two percent of patients achieved a response with 86% achieving a complete response. No grade 4 non-hematologic adverse events were reported. This study also found similar outcomes for non-GCB and GCB disease. The authors of this study attributed the difference in dosage between their study and that used by Nowakowski et al. to patient characteristics, including patient age.

Figure 1: KSHV gene expression in mononuclear cell subsets from pleural fluid in a patient with PEL



As with KSHV-MCD, we hypothesize that CD20+ B-cell depletion with rituximab may modulate KSHV-lytic syndromes, may significantly contribute to control of IL-6 associated deleterious inflammatory syndromes, and may modulate IL-10 and v-IL-6. In addition to the fact that some patients with KSHV-NHL have concurrent KSHV-MCD, a condition that is known to benefit from the use of rituximab, modulation of proliferation of benign KSHV-infected B-cells and associated cytokine abnormalities may also have an antiproliferative effect on the lymphoma itself. *In vitro* studies of PEL have demonstrated that vIL-6 and IL-10 promote cell proliferation,[16] and thus, controlling the inflammatory milieu (i.e. non-lymphoma cell sources of IL-10 and vIL-6) appears to be important in the treatment of KSHV-NHL. In fact, *in vitro* inhibition of IL-6 and vIL-6 proliferative signaling through gp130 can be blocked by inhibiting STAT3 signaling or through pharmacologic JAK2 inhibition, which leads to downregulation of survivin and activation of apoptosis in PEL cell lines[17]. Also, although PEL is generally considered a CD20 negative neoplasm based on immunohistochemistry and mRNA, it remains possible that rituximab has direct anti-tumor effects on PEL cells, as CD20 expression has been noted occasionally in this mature B-cell neoplasm[18], and even low level expression of CD20 may render cells susceptible to rituximab.

1.2.1.5 Rationale for combination anti-retroviral therapy in HIV-infected patients

Anti-retroviral therapy is essential in the management of two KSHV-associated malignancies, KS and MCD. Also, effective cART can prevent the development of KS in patients with HIV [19, 20]·[21] and cART has been shown to be an important component in the effective treatment of HIV-associated KS. Benefits include enhanced immunologic control of KS and possibly other effects as well, such as reduction in enhancement of KSHV infection.[22] [23] Introduction of cART has led to dramatic improvements in overall survival[24] in patients with HIV-associated KS, and a percentage for HIV-associated KS can be treated with cART alone. Maintenance of cART in the treatment of KSHV-MCD is standard of care. While the effect of cART on PEL has never been studied clinically, uncontrolled HIV viremia may contribute to PEL pathogenesis, and has a deleterious effect on concurrent KSHV-associated malignancies.

Many manifestations of KSHV-associated malignancies are due to cytokine dysregulation, the pathophysiology of which depends on interactions between KSHV infected cells, inflammatory cytokines, angiogenic growth factors, and the HIV-1 transactivating protein, tat.[25] Tat is an HIV regulatory protein that is essential for HIV viral replication; however, HIV-1 tat is also secreted from activated T-cells infected with HIV[26]. Preclinical studies support several mechanisms by which HIV-1 tat may interact with KSHV to promote KS tumorigenesis. Tat acts as a growth factor for spindle cell cultures derived from AIDS- KS lesions, and is synergistic with bFGF in promoting angioproliferative lesions in mice.[27, 28] HIV-1 tat has a basic domain that is similar to that of other angiogenic growth factors such as bFGF and VEGF-A, and through specific interactions with heparin and VEGFR2, HIV-1 tat may serve as an angiogenic growth factor[29-31]. HIV tat also promotes adhesion of AIDS-KS spindle cells and normal vascular cells through interaction between a RGD motif in the carboxy terminal of tat and $\alpha_V\beta_3$ and $\alpha_V\beta_1$ integrins.[26, 28] Adhesion and migration of PEL cells is also enhanced by HIV-tat.[32] Lastly, HIV-1 tat promotes transmission of free KSHV into human endothelial cells through interactions with heparin and integrins that serve to increase KSHV contact with target cells.[33] Given the central role of controlling HIV viremia with cART in the management of KSHV-associated malignancies, as well as the likely contribution of uncontrolled HIV viremia to PEL pathogenesis, cART will be employed as an important part of the treatment regimen.

1.2.2 Background

Primary effusion lymphoma (PEL) is a rare Kaposi sarcoma herpesvirus (KSHV)-associated aggressive mature B-cell lymphoma[34] with an historically poor prognosis. It was first noted as an unusual pleomorphic neoplasm among patients with AIDS that was not classifiable due to unusual morphology and lack of lineage-specific markers. Three cases of unusual genotypic B-cell neoplasms that lacked B-cell lineage-restricted antigens were first described in patients with AIDS in 1989. These tumors expressed leukocyte common antigen, as well as activation markers, but lacked B-cell or T-cell restricted surface antigens; however, they were notable for EBV expression and clonal rearrangement of Ig heavy chain and kappa light chain genes.[35] After the discovery of KSHV in 1994[36], an evaluation of 193 lymphomas, including 42 patients with AIDS, found that KSHV viral sequences were found in eight body cavity lymphomas in patients with AIDS, but none of the other lymphomas evaluated.[18] PEL was proposed as a distinct clinicopathologic entity in 1996.[37] It has since been found that besides PEL, KSHV is the etiologic agent of a second proposed B-cell lymphoma, large cell lymphoma arising in the setting of KSHV-associated multicentric Castleman disease (MCD), or KSHV-associated large cell lymphoma[38, 39]. KSHV-associated large cell lymphoma remains a provisional entity, and it is unknown whether the natural history is different than that of PEL. While PEL is most common in HIV-infected individuals, it has also been described in elderly and other immuno-suppressed individuals.[40-43] PEL may also have extracavitary presentations that precede the development of the characteristic body cavity effusion lymphoma.[18, 42] There are no prospective studies on this rare lymphoma, and clinical experience is limited. Based on retrospective analysis, prognosis for patients with PEL or large cell lymphoma arising in the setting of KSHV-MCD is dismal, with median survival estimated at 10 months in the most recent published series.[14, 44, 45] Importantly, PEL is often associated with other KSHV-associated malignancies, Kaposi sarcoma and KSHV-MCD.[14]

Immunophenotypically, PEL resembles a post-germinal center neoplasm, generally expressing activation markers[37], CD138 and IRF4[46, 47]. In approximately 80% of cases, the PEL cells are co-infected with EBV, and can be κ or λ restricted. Although morphologically similar, large cell lymphoma arising in KSHV-MCD is a proposed distinct EBV negative and lambda-restricted lymphoma. Unlike PEL, large cell lymphoma arising in KSHV-MCD expresses IgM, but does not show evidence of somatic hypermutation and is CD138 negative, suggesting its histogenic origin is a KSHV-infected naïve B-cell.[48, 49] The KSHV-infected plasmablasts detected in KSHV-MCD have some similar features, and express plasma cell transcription factors such as IRF4[50]. However, these KSHV-MCD plasmablasts are polyclonal, and are thought to be a premalignant precursor to large cell lymphoma arising in KSHV-MCD.

While the immunophenotype suggests a post-germinal center origin, gene expression profiling (GEP) of human genes in PEL suggests that the cells are not memory B-cells, and demonstrate an expression profile distinct from other aggressive B-cell lymphomas. While PEL has features of both multiple myeloma and lymphoblastoid cell lines, it is distinct from both and most similar to B-cells arrested prior to terminal differentiation into antibody secreting plasma cells[51-53]. While IRF4 and BLIMP1 are expressed in PEL, unlike other plasma cell malignancies, XBP1 is mainly expressed in its unspliced form in PEL cell lines, which largely are latently infected with KSHV. Indeed, in PEL, splicing of XBP1, which can be induced by hypoxia, is associated with lytic activation of KSHV due to an XBP-1 response element in the KSHV transactivating gene, ORF50/RTA[53-55]. Work from the Yarchoan lab suggests spliced XBP1 also has several binding

elements (XBE) in the promoter of vIL6, and can also directly upregulate vIL-6, suggesting a potential direct mechanism of vIL-6 upregulation that does not require lytic activation.

Many human genes, most uniquely upregulated in PEL, are remarkable for their involvement in inflammation, adhesion, and invasion. The unique biology of PEL appears partially due to dysregulation of inflammatory cytokines and growth factors. In PEL, there is upregulation of VEGF and VEGF-receptors[56-58]. VEGF is an important autocrine and paracrine growth factor that is upregulated by redundant viral and cellular mechanisms in PEL.[52, 59] Importantly, VEGF, otherwise known as vascular permeability factor, is detected at high levels in PEL effusions, and largely contributes to the maintenance of KSHV-associated effusions.[57, 60-62] *In vitro* studies suggest that the hypoxic condition of effusions contributes to the pathophysiology of PEL through upregulation of VEGF, as well as induction of lytic activation of PEL cells and expression of KSHV encoded lytic proteins such as viral IL-6 (vIL-6).[63] VEGF appears important in allowing the development and maintenance of effusions as well as recruitment of inflammatory cells that in turn may further support PEL cells' proliferation through secretion of cytokines such as IL-10 and vIL-6.[16] Furthermore, PEL cells up regulate the VEGF receptor, VEGF-R1.[56, 64] In addition to its role in the maintenance of vascular permeability, through interaction with VEGF-R1, VEGF may also affect chemotactic activity in PEL.[56, 64, 65] In a PEL mouse xenograph model, anti-VEGF antibody prevented the development of PEL effusions, raising the possibility that anti-VEGF therapy may inhibit homing of PEL to effusion spaces. SELPG, a membrane-associated selectin binding glycoprotein important for leukocyte migration is upregulated due to gene amplification in some cases[5, 52], and may contribute some of the unusual properties and anatomical distribution of PEL. Additional potentially targetable genes that are upregulated include VDR and CD30.

Lenalidomide Mechanism of Action and Preclinical Studies in PEL

Immunomodulatory derivatives of thalidomide (IMiDs), including pomalidomide (CC-4047) and lenalidomide (CC-5013) are structural derivatives of thalidomide, itself a glutamic acid derivative.

Recent studies have identified cereblon as a direct protein target of thalidomide[66], as well as lenalidomide and pomalidomide[67, 68]. Cereblon (CRBN) is an E3 ubiquitin ligase, whose ubiquitinase function is regulated by IMiDs. Modulation of CRBN by lenalidomide causes selective degradation of transcription factors IKZF1 and IKZF3 in multiple myeloma cells, suggesting a novel mechanism of action in this plasma cell malignancy[69, 70].

Direct anti-tumor effects of IMiDs in other IRF-4 associated tumors - multiple myeloma[71] and activated B-cell subtype of diffuse large B-cell lymphoma (ABC-DLBCL) [72] are mediated through modulation of cereblon. IRF4 expression is downregulated by inhibition of *CRBN* or *IKZF3* by shRNA, as well as by lenalidomide[67, 70]. Pomalidomide downregulates IRF4 in multiple myeloma cell lines[68, 73] and ABC-DLBCL. Additionally, in ABC-DLBCL, lenalidomide derepresses IRF7 leading to a type I interferon response, and downregulates NF-κB signaling[72, 74]. Given these direct effects of IMiD in other IRF4 associated tumors, we evaluated lenalidomide alone, and in combination with cytotoxic chemotherapeutic agents in PEL cell lines (below). On-going studies in the Yarchoan lab will continue to dissect other effects of lenalidomide on other KSHV-encoded and cellular genes in KSHV-infected cells.

The immunomodulatory activity of lenalidomide includes inhibition of pro-inflammatory cytokine and chemokine production by peripheral blood mononuclear cells (PBMCs) Key effects include inhibition of TNF-α, IL-1β, IL-6, monocyte chemoattractant protein-1 (MCP-1), and macrophage

inflammatory protein-1α (MIP-1α)[75, 76] [77]. Lenalidomide effects on angiogenesis have been demonstrated in *in vitro* models of endothelial sprout formation and vessel migration and other systems[78-80]. In addition to direct anti-tumor activity, these anti-inflammatory and anti-angiogenic properties of lenalidomide may have a desirable effect on the tumor microenvironment in the treatment of KSHV-associated lymphomas, as demonstrated in mouse models of other B-cell lymphomas[79]. Lenalidomide was approved by the FDA for the treatment of multiple myeloma in 2006 and mantle cell lymphoma in 2013.

Preclinical Studies of Lenalidomide in PEL

Two PEL cell lines, BCBL1 and BC3, as well as the KSHV-negative Burkitt Lymphoma cell line CA46 were expose to a range of doses of lenalidomide for 72 hours. Cell viability was measured using an ATP assay. After 72 hours, lenalidomide showed a negative effect on viability in BCBL1 and BC3 cell lines, but not CA46 viability.

Figure 2. Effect of lenalidomide on the viability of BC-3, BCBL-1, JSC-1 and CA-46 cells: Dose-response curve for lenalidomide on BC-3, BCBL-1, JSC-1 and CA-46 cells. The cells were plated at 150,000 cells per ml and then treated with drug (DMSO control and 1 nM to 100 µM with 10 fold increments) for 72 hours followed by analysis for cell viability. Cell viability was determined using the CellTiter Glo assay to measure total ATP and then normalized to control.

B

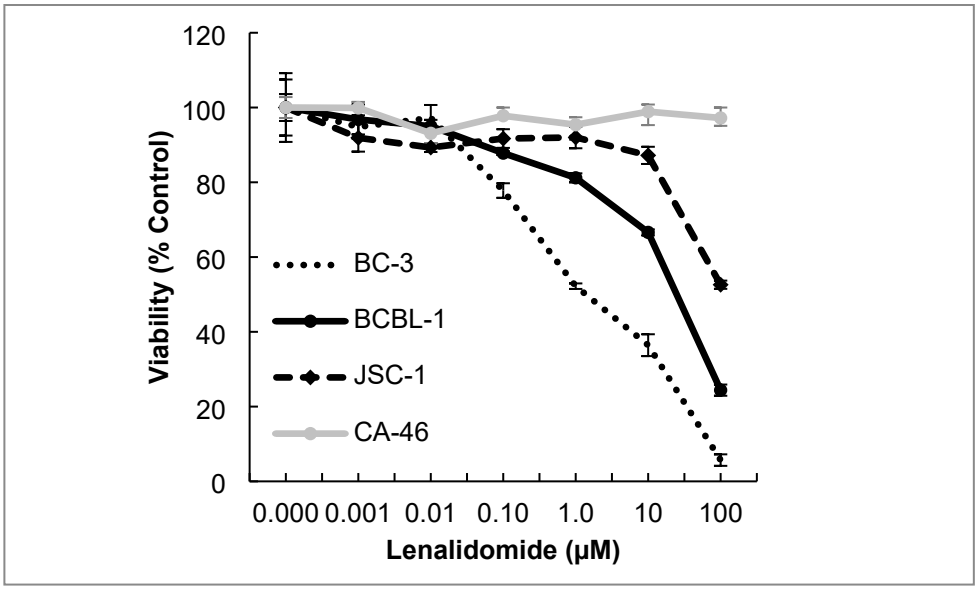


Figure 3. PEL viability after 72 hours of varying doses of Pomalidomide in combination with doxorubicin 10nM.

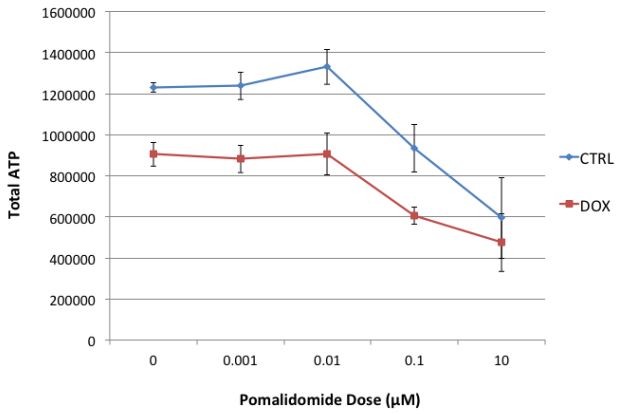
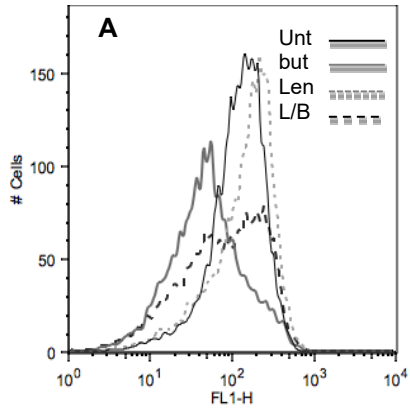


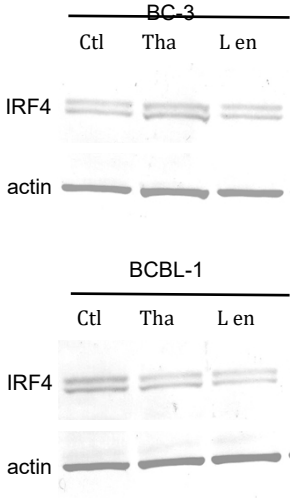
Figure 4. Inhibition of the KSHV lytic-induced down-regulation of MHC-I by lenalidomide. (A) MHC-1 expression by FACS in BCBL-1 cells treated with vehicle control (DMSO), butyrate (0.3 mM), lenalidomide (10 µM or lenalidomide and butyrate (0.3 mM). (B) MHC-1 median fluorescence values for each of the treatments shown in A-B. Results are representative of 3 separate experiments.



B	MHC-I Median Fluorescence Values - BCBL-1 Cells							
Butyrate (0.3 mM)	- Butyrate				+ Butyrate			
IMiD (10 µM)	Ctl		Len		Ctl		Len	
Median Fluorescence	131		168		49		86	

BC-3 JSC-1

Figure 5. Effect of Lenalidomide on IRF4 in PEL cell lines. At 10 µM Len decreases IRF4 levels in BC-3 Cells and BCBL-1 Cells.



Nuclear extracts of BCBL1 and BC3 cells showed a reduction of IRF4 after treatment with lenalidomide 10 µM for 48 hours.

Natural History Observations of Patients with PEL

Comparison of patients with PEL with those who have KSHV-MCD and those with other HIV-associated lymphomas suggest that PEL has clinical features overlapping with KSHV-MCD and are unique among HIV-associated lymphomas. Recent studies by our group have shown that PEL patients with no pathologic evidence of KSHV-MCD frequently have MCD-like symptoms and laboratory abnormalities and meet criteria for KSHV-associated inflammatory cytokine syndrome (KICS)[13, 81].

We evaluated clinical records of 14 patients with PEL diagnosed 2000-12, treated in the HIV and AIDS Malignancy Branch Clinic, and evaluated for clinical, and laboratory abnormalities using our criteria for KSHV-Associated Inflammatory Cytokine Syndrome (KICS; NCT01419561, see Section **6.4.3**)

Also, we evaluated:

- Serum inflammatory cytokines, (IFN-gamma, TNF-alpha, IL-1beta, IL-6, IL-8, IL-10, IL12p70; Mesoscale Discovery, Gaithersburg, MD)
- PBMC-associated KSHV viral load
- Platelets
- Hemoglobin
- Albumin
- Sodium

KSHV-NHL patients were compared to patients with:

- 19 Symptomatic KSHV-MCD patients without KSHV-NHL
- 28 Controls with other HIV-associated lymphomas

Comparisons used exact two-tailed Wilcoxon rank sum tests, $p<0.005$ was considered statistically significant, $p<0.05$ strong trends.

The study cohort included 14 patients with HIV and KSHV-NHL.

- 13 Men, 1 Woman
- Median age 43 yr (Range 26, 60 yr);
- 4 African American, 2 African, 4 Hispanic, 4 white.
- PEL (12, 5 with extracavitary manifestations)
- Large cell lymphoma in KSHV-MCD (2, both had effusions)
- Pathologically confirmed KSHV-MCD (5), cutaneous KS (10).
- Median CD4 126 cells/uL (15,1072),
- HIV viral load <100 copies/mL (7).
- All 10 KSHV-NHL with complete CRP and KSHV data either met criteria for KICS or had a history of KSHV-MCD; med CRP 48.8 g/dL (4.6, 114).

19 KSHV-MCD controls:

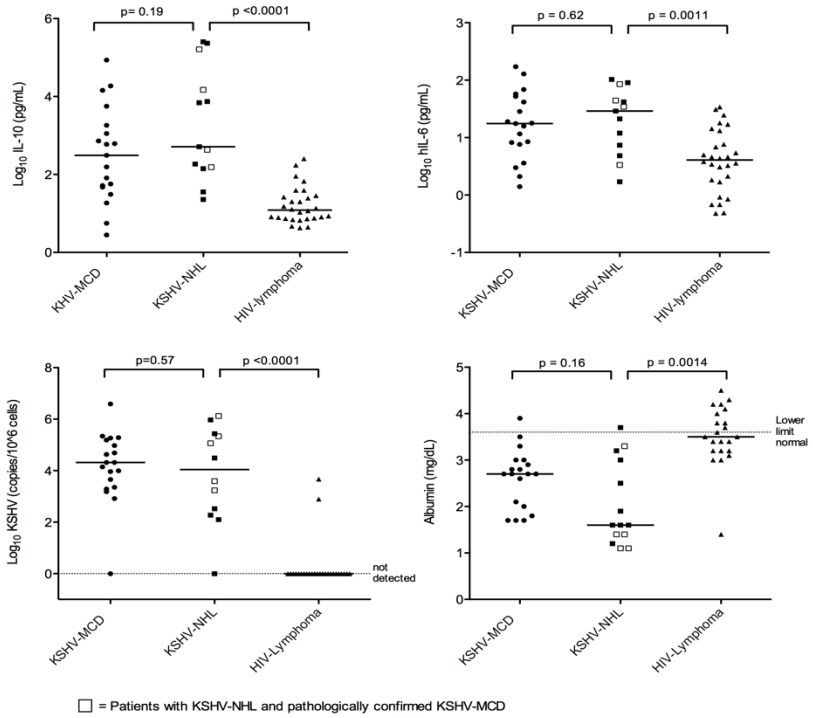
- 17 men, 2 women
- Median age 43 (29, 54)

28 HIV-associated lymphoma controls:

- 23 men, 5 women
- Median age 37 (21, 60)

Results of clinical laboratory, inflammatory cytokines, and KSHV viral load comparisons are noted in **Figure 5**.

Figure 6. Inflammatory cytokines, KSHV viral load and albumin in symptomatic KSHV-NHL compared to KSHV-MCD and other HIV-lymphomas

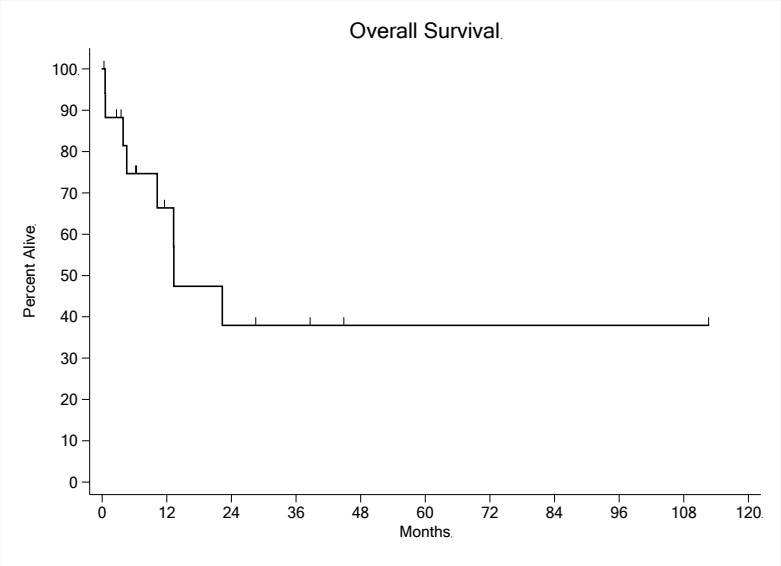


These findings also suggest that in the era of antiretroviral therapy, KSHV-NHL in HIV occurs at a broader range of CD4 counts than previously appreciated, often in the setting of suppressed HIV. Significant overlap exists between KSHV-associated lymphoproliferative disease and KSHV-NHL. Essentially all KSHV-NHL patients seen by our group in the NCI presented with inflammatory symptoms, elevated CRP, hypoalbuminemia, and cytopenias, and either had KSHV-MCD or met our working criteria for KICS. While the very high percentage of patients with PEL and no history of MCD met criteria for KICS, this may have in part reflected the fact that the HAMB research team was recruiting for a protocol to study KICS. Nonetheless, IL-6 related syndromes appear common in KSHV-NHLs. Elevated KSHV-infected PBMCs and associated KSHV modulation of host immune response leading to marked elevations in IL10 and IL6 likely contribute to symptoms and KSHV-NHL pathogenesis. Indeed, this data suggests that the clinical abnormalities and poor performance status of patients with KSHV-NHL is largely due to the lymphoma and KSHV-associated cytokine disturbances, and not underlying HIV infection. Evaluation of curative-intent regimens for KSHV-NHL should incorporate strategies to target these unique immunologic and virologic abnormalities. Additionally monitoring biomarkers of KSHV-associated inflammation will be important in evaluating responses to therapy.

HAMB Experience Treating PEL with Immune Modulated, Modified Dose Adjusted EPOCH combined with cART

Retrospective analysis of OS in 19 HIV-infected PEL patients diagnosed 2000-13 and treated with modified DA-EPOCH (etoposide, prednisone, doxorubicin, vincristine, cyclophosphamide) and combination antiretroviral therapy. Additional therapies: rituximab (15, 80%), bevacizumab (9, 47%), and/or methotrexate (3, 16%).Med age 44 years (22-69). 42% had HIV viral load (VL) <100 copies/mL. Med $CD4^{+}120$ cells/uL. All had anemia (med hemoglobin 9.3 g/dL, 7.4-13), low albumin (med 2.1 g/dL, 1.1-3.4), elevated C-reactive protein (med 52 g/dL, 8-164) and detectable KSHV viral load (med 29,727 copies/10^6 cells, 40-1.3 million). With 3.3 years med potential follow up, 3-year OS was 42.8%. Predictors for substantially inferior OS in univariate analysis were elevated interferon-γ (IFN γ) (p=0.01), IL-10 (p=0.02), IL-6 (p=0.03), IgE (p=0.01), and KSHV VL (0.09). Modified DA-EPOCH resulted in 3-year OS of 43%. Inflammatory profiles associated with dysregulated KSHV-lytic activation (IL-10, IL-6, IgE) and macrophage activation (IFNg, IL-6, IL-10 and ferritin) suggest abnormalities in innate and acquired immunity are common in PEL and contribute to prognosis. Evaluation of pathogenesis-targeted therapies for PEL is warranted. Overall survival for this retrospective cohort is noted in **Figure 6.**

Figure 7. Retrospective Analysis of Overall Survival in HIV-infected patients with KSHV-NHL treated with modified DA-EPOCH based regimens



Clinical Experience Using IMiDs in the treatment of KS

Our group first became interested in using thalidomide, and later lenalidomide, in treating KS because of their potent anti-angiogenic and immunomodulatory properties.

There are completed and ongoing studies evaluating IMiDs in KS. HAMB evaluated thalidomide for the treatment of HIV-associated KS. The response rate was 47% in assessable subjects in this phase II study, although the dosing in this study was not tolerable in a number of patients due to neurotoxicity, somnolence, and depression[82]. Immunomodulatory derivatives of thalidomide (IMiDs), including pomalidomide (CC-4047) and lenalidomide (CC-5013) are currently under

investigation in the HAMB and in the extramural AIDS Malignancy Consortium respectively for the treatment of KS due their ability to enhance CD4- and CD8-positive T cell co-stimulation, inhibit angiogenesis, and inhibit pro-inflammatory cytokine and chemokine (i.e. IL-1β, IL-6, monocyte chemoattractant protein-1) production.

We have completed a Phase I/II study of pomalidomide 5 mg daily, days 1-21 of 28-day cycles in patients with KS (any HIV status). Twenty-two patients were treated, including 15 (68%) HIV-infected patients. All patients were treated with 5 mg as no dose limiting toxicities occurred at that level. Over 156 cycles, grade 3/4 adverse events possibly attributable to therapy were: neutropenia (23 cycles, 10 patients); infection (1 cycle) and edema (1 cycle). Sixteen patients responded (73%, 95% CI 50-89%); 9 of 15 HIV-infected (60%, 95% CI 32-84%) and all 7 HIV-uninfected (100%, 95% CI 59-100%). The median time to response was 4 weeks (4-36).

Pomalidomide PK on this study showed $T_{½}$ 7.1±2.5 hours, C_{MAX} 61.2±29.3 ng/mL, AUC_{LAST} 624.4 ± 448.4, consistent with prior estimates, and with no accumulation and no difference by HIV status or ART type.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Any KSHV-positive aggressive B cell lymphomas, such as primary effusion lymphoma (PEL), and KSHV-associated large cell lymphoma that is pathologically confirmed by the NCI Laboratory of Pathology

2.1.1.2 Measurable or assessable lymphoma, as defined in Section **6.4.1.1**.

2.1.1.3 Any HIV status

2.1.1.4 Age 18 years or greater. Because no dosing or adverse event data are currently available on the use of lenalidomide in combination with EPOCH-R in participants <18 years of age, children are excluded from this study, but may be eligible for future pediatric trials.

2.1.1.5 ECOG performance status 0-4 (see **Appendix A:Performance Status** Criteria).

2.1.1.6 Females of childbearing potential (FCBP) as defined in Appendix C: Lenalidomide Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods must have a negative serum or urine pregnancy test with a sensitivity of at least 25 mIU/mL within 14 days prior to and again within 1 day before starting lenalidomide and must either commit to continued abstinence from heterosexual intercourse or begin TWO acceptable methods of birth control, one highly effective method and one additional effective method AT THE SAME TIME, at least 28 days before she starts taking lenalidomide. Females of reproductive potential must adhere to the scheduled pregnancy testing as required in the Revlimid REMS®™ program. Men must agree to use a latex condom during sexual contact with a FCBP even if they have had a vasectomy. All subjects must be counseled at a minimum of every 28 days about pregnancy precautions and risks of fetal exposure. See **Appendix C: Lenalidomide Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods, Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control**

2.1.1.7 All study participants must agree to be registered into the mandatory REVLIMID REMS®™ program, and be willing and able to comply with the requirements of the REVLIMID REMS®™ program.

2.1.1.8 Able to take aspirin 81mg orally daily or if intolerant of aspirin, able to take a substitute thromboprophylaxis such as low molecular weight heparin.

2.1.1.9 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Use of other systemic anticancer treatments or agents within the past 2 weeks. The use of rituximab for the treatment of KSHV-associated MCD or KICS or the use of steroids are allowed within 2 weeks prior to start of treatment.

2.1.2.2 Phase I or II participants who have received prior dose-adjusted EPOCH for treatment for PEL or KSHV-associated large cell lymphoma

2.1.2.3 Phase II participants who have received any prior curative-intent therapy for PEL or KSHV-associated large cell lymphoma. Participants who have received prior treatment as a bridge to curative-intent therapy will be considered per PI discretion.

2.1.2.4 Parenchymal brain involvement with lymphoma

2.1.2.5 History of malignant tumors other than KS or KSHV-associated MCD, unless:

- In complete remission for ≥1 year from the time response was first documented or
- Completely resected basal cell carcinoma or
- In situ squamous cell carcinoma of the cervix or anus

2.1.2.6 Inadequate renal function, defined as calculated or estimated creatinine clearance < 60 mL/min unless lymphoma, KSHV-MCD, or KICS- related (See **Appendix B: Calculation of Creatinine Clearance** for calculation of creatinine clearance)

2.1.2.7 Inadequate hepatic function

2.1.2.7.1 Bilirubin (total) > 1.5 times the upper limit of normal; AST and/or ALT > 3 times the upper limit of normal; EXCEPTIONS:

- Total bilirubin ≥ 5 mg/dL in participants with Gilbert's syndrome as defined by >80% unconjugated
- Total bilirubin ≥ 7.5 with direct fraction > 0.7 if participant is receiving a protease inhibitor at the time of initial evaluation
- Hepatic dysfunction attributed to lymphoma, KSHV-MCD, or KICS

2.1.2.8 ANC <1000/mm^3 and platelets < 75,000/mm^3 unless lymphoma, KSHV-MCD, or KICS-related.

2.1.2.9 CTCAEv5.0 Grade 3-4 neuropathy

2.1.2.10 Ejection fraction less than 40% by echocardiography

2.1.2.11 Known drug-related, inherited, or acquired procoagulant disorder including prothrombin gene mutation 20210, antithrombin III deficiency, protein C deficiency, protein S deficiency and antiphospholipid syndrome but *not* including heterozygosity for the Factor

V Leiden mutation or the presence of a lupus anticoagulant in the absence of other criteria for the antiphospholipid syndrome.

2.1.2.12 History of hypersensitivity reactions attributed to thalidomide, lenalidomide, or pomalidomide, including prior development of erythema nodosum if characterized by a desquamating rash while taking thalidomide, lenalidomide, or pomalidomide.

2.1.2.13 Breast feeding (if lactating, must agree not to breast feed while taking lenalidomide). Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with lenalidomide, breastfeeding should be discontinued if the mother is treated with lenalidomide.

2.1.2.14 Uncontrolled severe intercurrent illness including, but not limited to: bacterial, fungal, or life-threatening viral infection; symptomatic congestive heart failure; unstable angina pectoris; cardiac arrhythmia; or psychiatric illness/social situations that would limit compliance with study requirements. Participants with severe intercurrent illnesses attributed to lymphoma, KSHV-MCD, or KICS may be eligible per PI's or designee's discretion.

2.1.2.15 Any condition, including laboratory abnormalities, which in the opinion of the Principal Investigator or Lead Associate Investigator, would prohibit administration of planned chemotherapeutic intervention, places the subject at unacceptable risk if they were to participate in the study or confounds the ability to interpret data from the study

2.1.2.16 Pregnant women are excluded from this study because lenalidomide is a Category X agent with the potential for teratogenic or abortifacient effects. These potential risks may also apply to other agents used in this study. (See Section **2.1.1.6**)

2.2 RECRUITMENT STRATEGIES

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms.

2.3 SCREENING EVALUATION

2.3.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.

A waiver of consent for these activities has been requested in Section **12.6.2**.

2.3.2 Screening activities performed after a consent for screening has been signed

Screening evaluation testing/procedures are conducted under the separate screening protocol, 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols).

Potential subjects will be evaluated by a HAMB physician investigator for protocol eligibility.

Screening tests to be done within 3 weeks before enrollment except for certain laboratories and imaging.

Screening evaluation will include a complete medical history and physical examination including documentation of the presence and extent of any KS lesions. See Section **3.4**.

2.3.3 Clinical Examination

2.3.3.1 Complete history and physical examination including performance status and vital signs

2.3.4 Clinical Laboratory Data

(Studies performed in Clinical Center Department of Laboratory Medicine)

2.3.4.1 CBC with differential

2.3.4.2 Acute care panel (Sodium, Potassium, Chloride, CO2, Creatinine, Glucose, and Urea Nitrogen); Mineral panel (Phosphorus, Magnesium, Albumin, and Calcium); Hepatic panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)

2.3.4.3 Hepatitis B surface antigen, Hepatitis surface antibody and Hepatitis B core antibody, HCV antibody, HIV 1/2 antibody

2.3.4.4 PT/PTT/ thrombin time, fibrinogen, d-dimer

2.3.5 Pregnancy Testing

2.3.5.1 Urine or serum β-hCG (female subjects only). Females must follow pregnancy testing requirements as outlined in the REVLIMID REMS®™ program.

2.3.6 Radiographic studies

2.3.6.1 CT of neck, chest, abdomen and pelvis. May be performed in conjunction with staging ^{18}FDG-PET scan

2.3.6.2 MRI (Brain)

2.3.7 Confirmation of Diagnosis

Lymphoma diagnostic confirmation at the NCI Laboratory of Pathology will occur in all cases. Confirmation of KS, KSHV-MCD will occur only if indicated.

2.3.7.1 Where pathological tissue for confirmation of the diagnosis has not already been obtained, biopsy of involved tissue will be performed. Pleural fluid will be tested for cytopathology, molecular pathology and flow cytometry.

2.3.7.2 Where diagnostic biopsies are obtained, the specimens will be handled as follows:

- Place sufficient tissue for diagnostic purposes directly into formalin and submit to Laboratory of Pathology for histopathology and immunohistochemistry including KSHV-LANA, KSHV-vIL-6 and EBV.
- Additional research tests may be performed on the screening tissue biopsy *only* where the participant has enrolled on study 01-C-0038 (Collection of Blood, Bone Marrow, Tumor, or Tissue Samples from Patients with HIV Infection, KSHV Infection, Viral-related Pre-Malignant Lesions, and/or Cancer)

2.3.8 Other Procedures

2.3.8.1 12 lead EKG

2.3.8.2 Echocardiogram

2.4 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.5 TREATMENT ASSIGNMENT PROCEDURES

Cohort

Number	Name	Description
1	Cohort 1	Participants with PEL and KSHV-associated large cell lymphoma

Arm

Number	Name	Description
1	Arm 1	Lenalidomide, Rituximab, Prednisone, Etoposide, Doxorubicin, Vincristine, and Cyclophosphamide

Arm Assignment

Participants in cohort 1 will be assigned to arm 1.

2.6 BASELINE EVALUATION

Screening clinical laboratory and radiographic studies may be used for baseline evaluation provided they were obtained within 3 weeks prior to study therapy, *except* baseline CBC and acute care, mineral and hepatic panel are required within 1 day prior to first dose of therapy. For females of child-bearing potential, a pregnancy test is required 10-14 days prior to first dose of lenalidomide. Pregnancy test must also be repeated within 1 day before the first dose to continue to meet eligibility criteria for the subject to commence therapy. Baseline clinical and research blood tests will generally be performed within 1 day before the first dose of therapy, except for labs required for planning management of co-morbidities*, which may be performed up to 3 weeks prior to initiation of therapy, or research studies with specific scheduling requirements, which may be performed within 7 days before the first dose of therapy†. Pregnancy test repeated within 1 day before the first dose must continue to meet eligibility criteria (Section **2.1.1.6**) for the subject to commence therapy. See Section **3.4**.

2.6.1 Clinical Examination

- Complete history and physical examination; recording of performance status
- Ophthalmology Exam in participants with vision symptoms and/or CD4 <100

2.6.2 Clinical Laboratory Data

- CBC with differential and reticulocyte count.
- Acute care panel (Sodium, Potassium, Chloride, CO2, Creatinine, Glucose, and Urea Nitrogen)
- Mineral panel (Phosphorus, Magnesium, Albumin, and Calcium)
- Hepatic panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
- C-reactive protein
- Creatine kinase, uric acid, LDH
- Urinalysis
- Fe, ferritin, transferrin, haptoglobin
- Folate, 25-OH Vitamin D
- Lipids, Thyroid stimulating hormone (TSH); free T4
- Quantitative Ig, IgE, serum kappa and lambda free light chains
- Fecal occult blood testing
- Lymphocyte phenotype TBNK (requires simultaneous CBC and automated differential)
- * HIV and HBV viral load if seropositive at screening, HCV RNA PCR in all participants
- EBV, CMV viral load
- KSHV viral load (blood)
- * RPR
- Pregnancy testing: Urine or serum β-hCG (female subjects only)
- 24 hour urine collection to evaluate renal function

2.6.3 Staging and Research Tests

- Bone marrow aspirate and biopsy (optional)
- Cerebrospinal fluid (Cell count, cytopathology, KSHV, EBV, flow cytometry and Ig PCR)
- Effusion (KSHV, EBV, Ig PCR, flow cytometry, cytopathology, microbiology: for collaborating labs –See Section **5.1.3**)
- Bronchoalveolar lavage (if bronchoscopy indicated – See Section **5.1.3**)
- † Flow cytometry (blood)
- † Molecular pathology (blood and effusion)
- KSHV and EBV viral load (blood)
- KSHV viral load (saliva)
- Research blood to AML

2.6.4 Imaging

- CT Scan (neck, chest, abdomen, pelvis)
- Chest X-ray (PA/lateral and decubitus) in participants with pleural effusions
- ^{18}FDG PET
- Pulmonary function tests
- Bronchoscopy in participants with CXR abnormalities or by investigator discretion

- Endoscopy for participants with GUAIAC positive stool or by investigator discretion
- KS photography and measurement (See Section **6.4.4.1.1**)

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a Phase I/II single center study of DA-EPOCH-R^2 in participants with KSHV-positive aggressive B cell lymphomas.

The phase I portion of the study will evaluate safety and tolerability of lenalidomide 25 mg administered by mouth daily on days 1-10 of a 21 day cycle in combination with DA-EPOCH-R administered days 1-5 of a 21 day cycle (days 6-10 during cycle 1 only to allow for a 5 day lenalidomide run-in), in participants with PEL to determine safety of the combination and recommended phase II lenalidomide dose. If this dose is not tolerable, additional dose levels of lenalidomide 20 mg orally daily for 10 days and lenalidomide 15 mg oral daily for 10 days will be explored using a traditional 3+3 design.

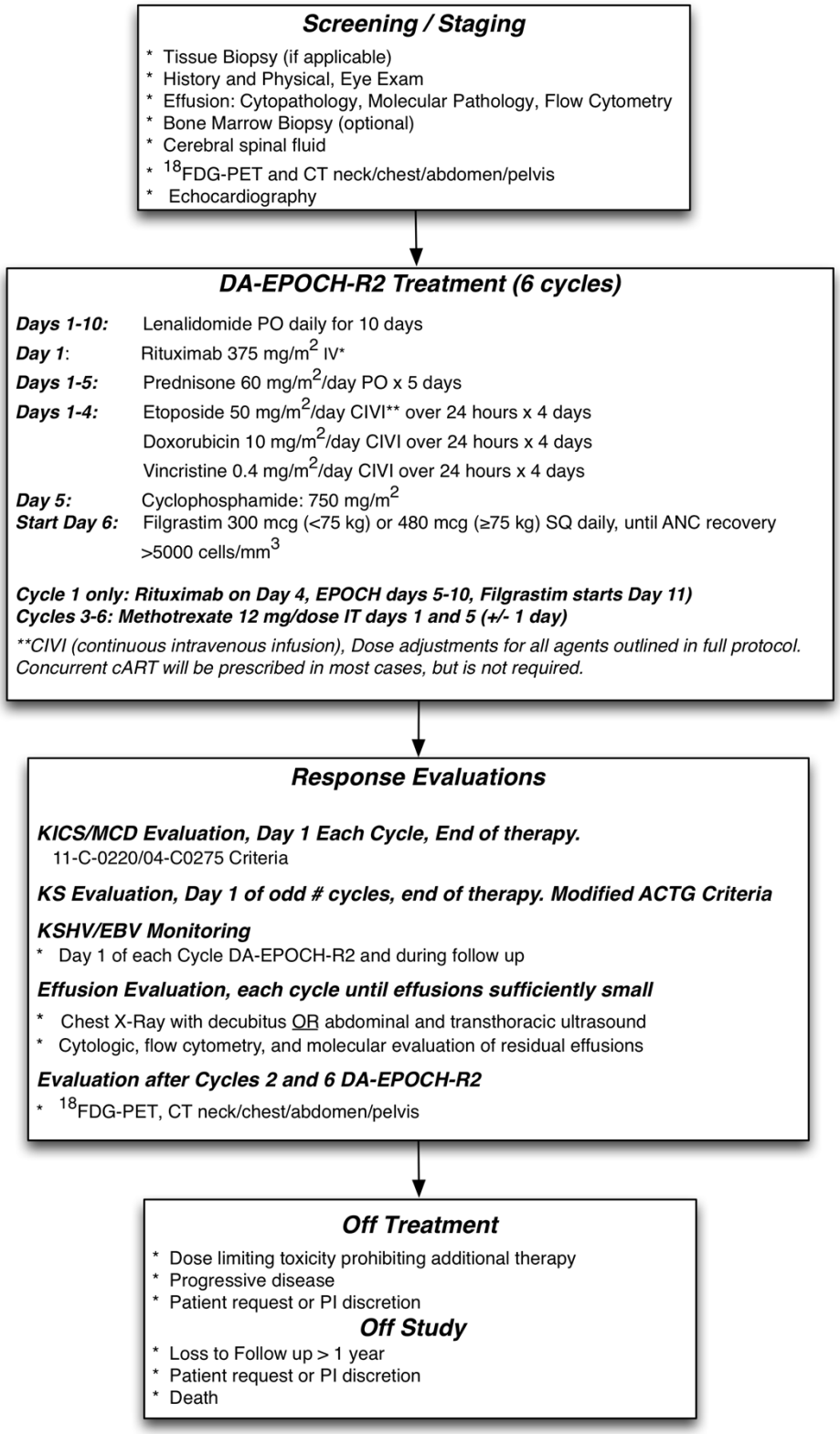
This will follow a 3+3 design.

If ≤1 of three have a DLT, expand to cohort to 6 participants. If ≥2 of 6 have a DLT, go to next lower dose level of lenalidomide.

The study will proceed to Phase II at the highest dose level in which ≤1 of 6 participants have a DLT during Phase I.

The phase II portion of the study will use the recommended phase II dose of lenalidomide as determined in the phase I portion of the study in combination with DA-EPOCH-R in order to determine overall survival in treatment naïve PEL. Participants may be co-enrolled in other NIH Natural History (i.e., 04-C-0275 or 11-C-0220) or Tissue Procurement (i.e., 01-C-0038) protocols.

3.1.1 Study Schema



3.1.2 Treatment Plan Outline

The treatment plan during phase I and phase II will include lenalidomide days 1-10 in combination with EPOCH-R days 1-5 during cycles 2-6:

During cycle one only, there will be a 5 day lenalidomide run-in prior to initiation of EPOCH-R to evaluate the effects of lenalidomide on tumor, immunologic and viral factors. Lenalidomide will be given days 1-10 but EPOCH will be given on days 6-10 for the first cycle. Also during cycle one only rituximab therapy will be given on day 4 to evaluate the effects of lenalidomide with rituximab on tumor, immunologic and viral factors. This will allow for evaluation of effect of lenalidomide monotherapy alone and then in combination with rituximab in regulating the inflammatory milieu, as well as potential cytotoxic effect as single agent lenalidomide in participants with PEL. During cycle 1, participants with urgent requirement for chemotherapy, as determined by baseline ECOG performance status (PS) and organ function (below) will have a shorter run-in of lenalidomide. Treatment during the 5 day run-in will be based on the following ***guidelines***. All participants may proceed directly to EPOCH-R² without a run-in period at PI discretion. Schedule changes and dose delays up to 7 days that are due to unavoidable scheduling conflicts (i.e., Federal Holidays, inclement weather, U.S. Government shutdown, etc.) will not be reported as protocol deviations.

- Participants with **ECOG PS 0-3** with no limiting organ function attributed to lymphoma, KSHV-MCD or KICS. Organ dysfunction will be defined as any of the following: ANC<1000/mm³, platelets <75,000/mm³, creatinine clearance < 60 mL/min, bilirubin (total) > 1.5 times the upper limit of normal, AST and/or ALT > 3 times the upper limit of normal (See Sections **2.1.2.6** - **2.1.2.7** exclusion criteria cut-offs) at baseline will have **5 days of lenalidomide plus rituximab therapy** as follows:
    - Lenalidomide days 1 to 5
    - Evaluation (clinical response and correlatives) of lenalidomide monotherapy will be done prior to administration of rituximab
    - Rituximab therapy on day 4.
    - Evaluation (response and correlatives) of rituximab therapy after lenalidomide will be done on day 6 prior to the start of EPOCH-R² therapy.
    - Lenalidomide will continue in combination with EPOCH on day 6-10.
- Participants with ECOG PS 4 and/or any performance status, but organ function compromised by lymphoma to the extent outlined above (Also, see Sections **2.1.2.5** - **2.1.2.7**) EPOCH will begin on day 3 **of the first cycle** as follows:
    - Lenalidomide days 1-10
    - Rituximab monotherapy will be administered on day 2
    - Evaluation (response and correlatives) of rituximab therapy will be done on day 3, prior to the start of EPOCH-R² therapy.
- Response/effect of lenalidomide monotherapy and rituximab will be evaluated with respect to effect on clinical parameters using established criteria for KS, KSHV-MCD or KICS, effect on KSHV viral load, vIL6 and inflammatory cytokines in blood and effusions, and effect on effusion cell counts and chemistries.
- EPOCH-R², 6 cycles, each cycle lasts 21 days.
- During cycle 1, EPOCH will be administered on days 6-10 after the lenalidomide run-in.

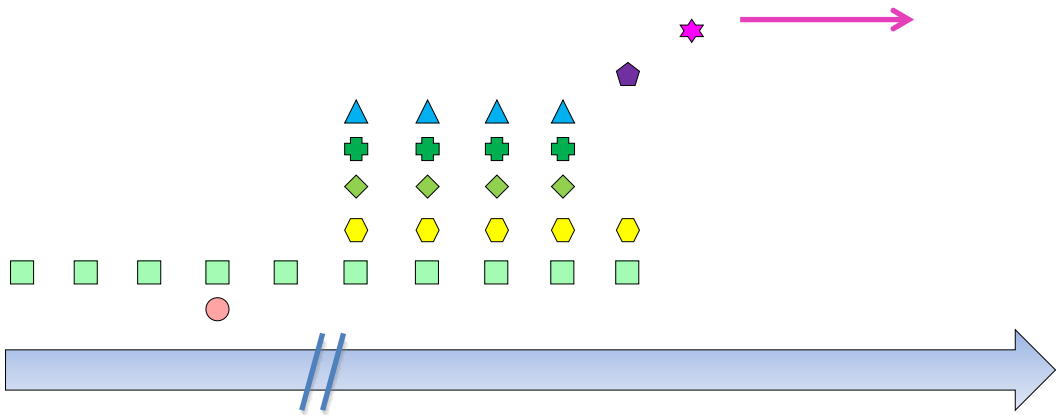
- During cycle 1, rituximab will be administered on day 4 prior to the start of EPOCH.
- During cycles 2 to 6, rituximab will be administered on day 1 of each cycle. Rituximab administration will be completed before continuous infusion of etoposide + doxorubicin + vincristine commences.
- During each cycle, etoposide + doxorubicin + vincristine administration will be completed before cyclophosphamide is administered on day 5 (Day 10 on Cycle 1).
- Participants that are shown to have leptomeningeal disease at screening will be treated according to the guidelines in Section **3.2.2.2**. For all other participants, CNS prophylaxis with intrathecal methotrexate will be given according to guidelines in Section **3.2.2.3**.
- Evaluations for DA-EPOCH-R^2 will be studied with respect to effect on KSHV viral load, inflammatory cytokines in blood and effusions (effusions when present and accessible), effect on effusion cell counts and chemistries (if indicated), and effect on clinical parameters using established criteria for KS, KSHV-MCD or KICS.
  Lymphoma response after 6 cycles will be based on a modification of the International Working Group Response Criteria for Malignant Lymphoma[83] that takes into account evaluation of effusions in KSHV-associated diseases.

Figure 8

A: Cycle 1 Lenalidomide Monotherapy Run-in plus Dose Adjusted EPOCH-R² (Initial dose levels shown)

Lenalidomide PO daily at assigned dose level days 1 to 10
Rituximab 375 mg/m^2 IV on day 4
Prednisone 60 mg/m^2/day PO days 6 to 10
Etoposide 50 mg/m^2/day CIVI** days 6 to 9
Doxorubicin 10 mg/m^2/day CIVI** days 6 to 9
Vincristine 0.4 mg/m^2/day CIVI** days 6 to 9
Cyclophosphamide 750 mg/m^2 day 10
Filgrastim 300- 480 mcg SQ daily day 11 until ANC recovery ≥5000 cells/mm^3 after neutrophil nadir

** CIVI (continuous intravenous infusion)

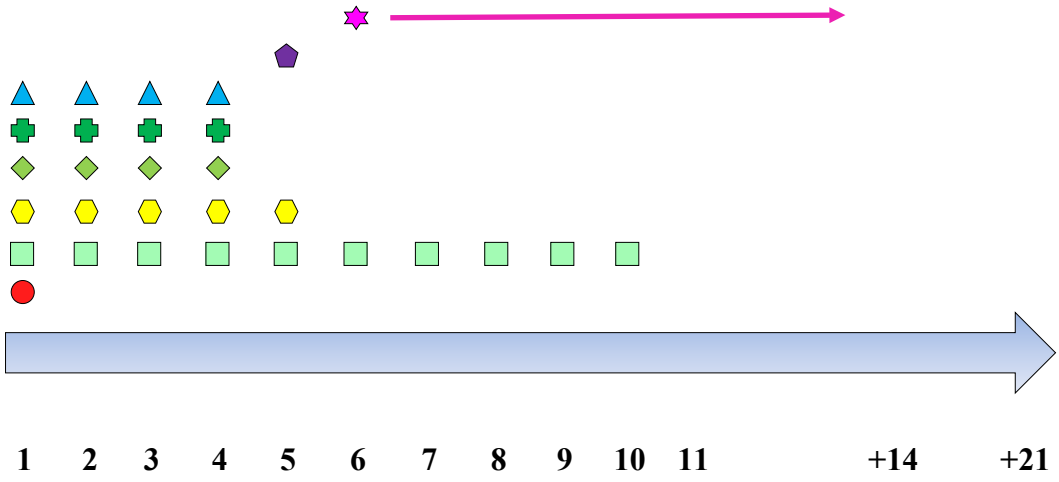


Day 1 2 3 4 5 6 7 8 9 10 11 +14 +21

B: Cycles 2-6 Lenalidomide plus Dose Adjusted EPOCH-R² (Initial dose levels shown)

- Lenalidomide PO daily at assigned dose level days 1 to 10
- Rituximab 375 mg/m^2 IV on day 1
- Prednisone 60 mg/m^2/day PO days 1 to 5
- Etoposide 50 mg/m^2/day CIVI** days 1 to 4
- Doxorubicin 10 mg/m^2/day CIVI** days 1 to 4
- Vincristine 0.4 mg/m^2/day CIVI** days 1 to 4
- Cyclophosphamide 750 mg/m^2 day 5
- Filgrastim 300- 480 mcg SQ daily day 6 until ANC recovery ≥5000 cells/mm^3 after neutrophil nadir

** CIVI (continuous intravenous infusion)



3.1.3 Dose Limiting Toxicity

Following is the definition of dose-limiting toxicity (DLT). It should be noted that neither preexisting manifestations of HIV infection, nor of therapy for HIV infection, nor of KS (including bleeding from KS), nor of MCD will be considered dose-limiting. DLTs will be assessed over the first 6 weeks (2 cycles) of DA-EPOCH-R² administration.

If a participant meets any of the criteria defined below, they will be considered as having dose-limiting toxicity.

- Any Grade 4 adverse event at least possibly attributable to lenalidomide AND not probably or definitely attributable to HIV or a KSHV-associated malignancy resulting in a dose delay in DA-EPOCH-R² > 2 weeks due to failure to resolve to grade 3 or lower. This will NOT include lymphopenia or CD4 lymphopenia
- Any Grade 3 cardiac toxicity that is at least possibly attributable to lenalidomide AND not attributable to HIV or a KSHV-associated malignancy resulting in a dose delay in DA-EPOCH-R² > 2 weeks due to failure to resolve to grade 2 or lower OR failure to become asymptomatic at rest or with mild activity. Excludes:
    - Grade 3 changes in ejection fraction that are asymptomatic (American Heart Association Class I)
    - Grade 3 hypertension, in which antihypertensive agents are being adjusted
- Any Grade 3 central nervous system adverse event at least possibly attributable to lenalidomide AND not attributable to HIV or a KSHV-associated malignancy resulting in a dose delay in DA-EPOCH-R² > 2 weeks due to failure to resolve to grade 2 or lower
- Grade 3 hepatic failure (asterixis, encephalopathy) at least possibly attributable to lenalidomide AND not attributable to HIV or a KSHV-associated malignancy resulting in a dose delay in DA-EPOCH-R² > 2 weeks due to failure to resolve to grade 2 or lower
- Grade 3 acute kidney injury or proteinuria at least possibly attributable to lenalidomide AND not attributable to HIV or a KSHV-associated malignancy resulting in a dose delay in DA-EPOCH-R² > 2 weeks due to failure to resolve to grade 2 or lower
- Grade 3 bronchopulmonary hemorrhage, dyspnea, or pulmonary edema, at least possibly attributable to lenalidomide AND not attributable to HIV or a KSHV-associated malignancy resulting in a dose delay in DA-EPOCH-R² > 2 weeks due to failure to resolve to grade 1 or lower
- Any drug-related death

3.1.4 Dose De-escalation

Dose de-escalation during phase I portion will proceed in cohorts of 3–6 participants. The MTD is the dose level at which no more than 1 of up to 6 participants experiences DLT during the *first two cycles* of DA-EPOCH-R² treatment. If applicable, the MTD will be a dose below that at which at least 2 (of ≤6) participants have DLT as a result of the drug. If a participant did not experience DLT and did not finish treatment, he or she will not be evaluable for toxicity and will be replaced in the dose level. **Table 1** shows the dose de-escalation schedule for lenalidomide during the phase I portion of the study. Participants will receive the same dose during the lenalidomide run-in and cycles 1-6.

Table 1: Phase I lenalidomide dose de-escalation

Dose Level	Lenalidomide mg/day
1	25
-1	20
-2	15

Dose escalation will follow the rules outlined in **Table 2** below.

Table 2: Phase I dose de-escalation rules

Number of Participants with DLT at a Given Dose Level	De-escalation Decision Rule
0 out of 3	Enter up to 3 participants at the same dose level
1 out of 3	Enter up to 3 more participants at this dose level, of these 3 additional subjects: • If 0 of these 3 additional participants (a total of 1 out of 6) experience DLT, continue the current dose level. • If 1 or more of the additional 3 (≥ 2 out of 6 total) of this group suffer DLT, then dose de-escalation to next level down.
≥ 2	Dose de-escalation to next dose level down. If this occurs at dose level -2 (15 mg), safety data will be evaluated, and either the study will be discontinued, or additional dose levels will be defined.
≤1 of 6 at highest dose level	This is the MTD and will be the dose further evaluated in the phase II portion of the trial

3.2 DRUG ADMINISTRATION

3.2.1 Lenalidomide

Participants will receive lenalidomide 25 mg daily for 10 consecutive days. Starting in Phase II, this dose may be adjusted for renal dysfunction per **Table 4**. Please see **Appendix A-Performance Status Criteria** and Section **3.1.2**. for the ECOG performance status based lengths for lenalidomide run-in during cycle 1.

3.2.1.1 Lenalidomide

3.2.1.1.1 Lenalidomide (REVLIMID®) will be provided to research subjects for the duration of their participation in this trial at no charge to them or their insurance providers. Lenalidomide will be provided in accordance with the Celgene Corporation's

REVLIMID REMS®™ program. Per the standard REVLIMID REMS®™ program requirements, all physicians who prescribe lenalidomide for research subjects enrolled into this trial, and all research subjects enrolled into this trial, must be registered in and must comply with all requirements of the REVLIMID REMS®™ program. Further information about the Revlimid REMS®™ program is available at www.celgeneriskmanagement.com.

3.2.1.1.2 Drug will be ordered by Faxing an Order form with Authorization number to Biologics, Inc. Clinical Research Services, FAX 919-256-0794, Phone 800-693-4906. For the initial order and at time of IRB renewal, documentation of IRB Approval will also be faxed. Lenalidomide will then be shipped on a per participant basis from Biologics, Inc., to the CCR Pharmacy: c/o Hope Decederfelt, 10 Center Drive Room 1C230, Bethesda, MD, 20892.

3.2.1.1.3 Only enough lenalidomide for one cycle of therapy will be supplied to the participant each cycle. This is in accordance with the REVLIMID REMS®™ program.

3.2.1.1.4 Females must follow pregnancy testing requirements as outlined in the REVLIMID REMS®™ program.

3.2.1.1.5 Lenalidomide will be self-administered on an outparticipant basis at the assigned dose once daily. Subjects will be instructed to take lenalidomide at approximately the same time every morning. Lenalidomide capsules should be swallowed whole, and should not be broken, chewed or opened.

3.2.1.1.6 Lenalidomide should be taken without food, at least 2 hours before or 2 hours after a meal.

3.2.1.1.7 If a dose of lenalidomide is missed, it should be taken as soon as possible on the same day. If it is missed for the entire day, it should not be made up, rather it should be taken at the next scheduled time point. Participants who take more than the prescribed dose of lenalidomide should be instructed to seek emergency medical care if needed and contact study staff immediately.

3.2.1.1.8 Subjects will keep a daily diary recording lenalidomide administration including the time of administration, and any clinical toxicity or other observations made during the cycle (**Appendix E: Participant Drug Administration Diary**). This will be used as only an aide memoire for subjects. The clinical research team maintains the primary source record of events including toxicities.

3.2.1.1.9 Females of childbearing potential should not handle or administer lenalidomide unless they are wearing gloves.

3.2.1.1.10 Thromboprophylaxis with aspirin 81 mg, enoxaparin 0.5 mg/kg daily (up to 40 mg), or an acceptable alternative agent will continue throughout therapy (days 1-28 of each cycle), as described in Section **4.3.4**. Hold thromboprophylaxis for a platelet count less than 50,000/mm^3.

3.2.1.1.11 Dose delays for non-medical purposes will be avoided whenever possible. Treatments and corresponding evaluations may be rescheduled to the closest day possible (within 7 days) without constituting a protocol deviation (e.g., for Federal holidays or unforeseen circumstances such as travel difficulties, snow closures and the like).

3.2.1.1.12 Accurate records will be kept of all study drug administration (including dispensing and dosing) will be made in the source documents.

3.2.2 Cycles 1 through 6

3.2.2.1 DA-EPOCH-R²

Plan for 6 cycles of modified dose-adjusted EPOCH-R². Each cycle is 21 days in duration.
All participants initiate therapy at Dose Level 1 of modified DA-EPOCH-R² shown below unless evidence of hepatic dysfunction as defined in Section **3.3.3.1**, in which case vincristine dosage will be adjusted. For cycle 1, rituximab will not be administered on day 1. Participants will receive rituximab on day 4. Subsequent treatment doses of DA-EPOCH-R² are determined by hematological toxicity experienced during the previous cycle according to the dose-adjustment paradigm in Section **3.3**. Dose adjustment is based on measurements of twice weekly CBC only (e.g., Monday and Thursday, or Tuesday and Friday), even if additional CBCs are obtained. For drug administration and preparation instructions see Section **14**.

Premedicate 30-60 minutes (±10 minutes) before rituximab infusion with

- 650 mg acetaminophen PO unless participant unable to tolerate PO intake
- 50 mg diphenhydramine PO or IV
- 8 mg dexamethasone IV(optional, consider Cycle 1 only)
- **Note:** These medications may also be administered at least 30 minutes post rituximab infusion.

Table 3 Administration of Drugs in EPOCH-R²

Drug	Initial Dose	Route	Treatment days
CYCLE 1			
Rituximab	375 mg/m²	IV	Day 4
Etoposide	50 mg/m²/day	CIVI over 24 hours	6, 7, 8, 9 (96 hours)
Vincristine	0.4 mg/m²/day	CIVI over 24 hours	6, 7, 8, 9 (96 hours)
Doxorubicin	10 mg/m²/day	CIVI over 24 hours	6, 7, 8, 9 (96 hours)
Cyclophosphamide	750 mg/m²	IV over 30 minutes	Day 10
Lenalidomide	* mg	PO	Days 1 to 10
Prednisone	60 mg/m²/day**	PO	Days 6 to 10
Filgrastim	300 mcg/day (<75 kg) 480 mcg/day (≥75 kg)	SC	Days 11 to ANC recovery to ≥ 5000/mm³ after neutrophil nadir
CYCLES 2-6			
Rituximab	375 mg/m²	IV	Day 1
Etoposide	Per Dose Adjustment***	CIVI over 24 hours	1, 2, 3, 4 (96 hours)
Vincristine	Per Dose Adjustment***	CIVI over 24 hours	1, 2, 3, 4 (96 hours)
Doxorubicin	Per Dose Adjustment***	CIVI over 24 hours	1, 2, 3, 4 (96 hours)
Cyclophosphamide	Per Dose Adjustment***	IV over 30 minutes	Day 5
Lenalidomide	* mg	PO	Days 1 to 10
Prednisone	60 mg/m²/day**	PO	Days 1 to 5
Filgrastim	300 mcg/day (<75 kg) 480 mcg/day (≥75 kg)	SC	Days 6 to ANC recovery to ≥ 5000/mm³ after neutrophil nadir

1. Begin the infusional agents after rituximab infusion is completed on cycles 2-6.
2. Infusional agents should be administered through a central venous access device.
3. Begin cyclophosphamide after infusions are completed and administer over 30 minutes.
4. A total of 5 doses of Prednisone 60 mg/m2 per day will be administered on 5 consecutive days.
5. CIVI (continuous intravenous infusion)
6. * During cycle 1, rituximab will be administered on day 4 during lenalidomide run-in
7. ** refers to Phase I dose level or recommended phase II dose of lenalidomide
8. *** Prednisone dose will be rounded to the nearest 20 mg based on body surface area (see Section **14.8**)
9. See **Table 5. Drug doses per dose level in modified DA-EPOCH-R2**

3.2.2.2 Treatment for CNS involvement

Participants with leptomeningeal lymphoma or if the CSF is positive for malignant cells by cytology or flow cytometry will receive monotherapy with intrathecal methotrexate 12 mg or cytarabine 50 mg (if intolerant to methotrexate). Triple intrathecal therapy (methotrexate 5 mg, cytarabine 50 mg, hydrocortisone 50 mg) as clinically indicated may be administered at PI discretion. Administer leucovorin 25 mg PO (or IV) 24 hours after each dose of methotrexate. Due to unforeseeable events, the above therapy may be modified. All decisions should be discussed with the PI. Delivery should be isovolumetric (mL CSF out=mL drug in) with participants preferably in lateral decubitus position during LP. Participants should remain in prone or Trendelenburg position for 30 minutes post LP to facilitate drug circulation throughout the CNS.

CNS Treatment Schedule:

- Induction: Twice weekly for 2 weeks beyond negative cytology and/or flow cytometry, for a minimum of 4 weeks (obtain CSF for cytology, protein, glucose, and cell count weekly).
- Consolidation: Weekly for 6 weeks (obtain CSF for cytology, protein, glucose, and cell count weekly). Before moving on to maintenance therapy, collect CSF for cytology, flow cytometry and molecular pathology to confirm continued absence of CNS PEL.
- Maintenance: Monthly for 6 months (obtain CSF for cytology, flow cytometry molecular pathology, protein, glucose, cell count).
- Alternative chemotherapy regimens or radiotherapy should be considered for resistant leptomeningeal disease.

3.2.2.3 CNS Prophylaxis

- All participants who do not have leptomeningeal involvement at baseline will receive CNS prophylaxis during cycles 3 through 6.
- Administer methotrexate 12 mg/dose IT on days 1 and 5 every 21 days for a total of 8 doses.
- Administer leucovorin 25 mg PO (or IV) 24 hours after each methotrexate dose.
- If signs or symptoms of chemical arachnoiditis occur, administer 10 mg of hydrocortisone intrathecally with the methotrexate.
- Cytarabine 50 mg/dose IT on days 1 and 5 every 21 days for the remainder of a total of 8 cumulative doses may be used in place of methotrexate after discussion with the principal investigator if methotrexate is contraindicated. The days of IT therapy may be adjusted to accommodate participant schedule, travel or federal holidays.

3.2.3 CCR Self-Administered Study Drugs Policy

All oral self-administered investigational agents will be properly accounted for, handled, and disposed in accordance with existing federal regulations and principles of Good Clinical Practice.

All oral study drugs will be recorded in the participant diaries found in **Appendix E: Participant Drug Administration Diary**. This will be used as a memory aide for subjects. A clinical research team maintains the primary source record.

3.3 DOSE MODIFICATION

All treatment modifications should be discussed with the senior clinical investigators on the study. Before each cycle, participants will be evaluated for possible toxicities that may have occurred after the previous dose(s). Toxicities will be assessed according to the NCI Common Terminology Criteria for Adverse Events version 5 (CTCAE v5.0) (http://ctep.cancer.gov/reporting/ctc.html). Each adverse event (AE) attributed to lenalidomide will be carefully recorded, so that the dose modifications can be made accordingly. If multiple toxicities are noted, the dose adjustments and/or delays will be made according to guidelines that address the most severe toxicity. Dose Modifications are listed in Section **3.3.1**.

3.3.1 Lenalidomide Dose Modification

Intrapatient dose modifications based on changes in creatinine clearance will be allowed.

Table 4: Lenalidomide dose modifications or interruption during subsequent cycles.

Toxicity	Action	Resumption of therapy
Neutropenia Grade 4 neutropenia (ANC < 500/mm^3)	No dose modifications. May co-administer filgrastim 300 mcg (<75 kg) or 480 mcg (≥75 kg) up to 24 hours before EPOCH if ANC < 1000 cells/uL on Day1	NA
Thrombocytopenia Platelets <75,000/mm^3 on Day 1	If attributed to lymphoma or KSHV, no change. If attributed to therapy, see EPOCH adjustment below.	NA
Creatinine Clearance 30 to 60 mL/min	10 mg once daily	NA
Creatinine Clearance <30 mL/min (not requiring dialysis)	15 mg every other day	NA
Creatinine Clearance <30 mL/min (requiring dialysis)	5 mg once daily. On dialysis days, administer the dose following dialysis. For continuous dialysis, administer 5 mg once daily.	NA

3.3.2 DA-EPOCH-R² Dose Modification

3.3.2.1 Dose modifications during DA-EPOCH-R² will be based on predefined dose levels in **Table 5**. These dose levels are largely based on hematologic toxicities observed during the previous cycle, as defined below Table 5. Special considerations/additional modifications for neuropathies, ileus, renal dysfunction, or other non-hematologic toxicities are noted below.

Table 5. Drug doses per dose level in modified DA-EPOCH-R²

Drug	Dose Level							
	-2	**-1**	**1**	**2**	**3**	**4**	**5**	**6**
Rituximab (mg/m^2)	375	375	375	375	375	375	375	375
Prednisone (mg/m^2/day)	60	60	60	60	60	60	60	60
Vincristine (mg/m^2/day)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Doxorubicin (mg/m^2/day)	10	10	10	12	14.4	17.3	20.7	24.8
Etoposide (mg/m^2/day)	50	50	50	60	72	86.4	103.7	124.4
Cyclophosphamide (mg/m^2)	480	600	750	750	750	750	750	750
Lenalidomide mg/day	No intrapatient modifications based on hematologic toxicities							

3.3.3 Additional DA-EPOCH-R^2 dose modifications

- If there is concern for the onset or worsening of KS (either visceral or cutaneous), rituximab may be held after cycle 1 per PI discretion. For those with concurrent KS and KSHV-MCD or KICS, the symptoms/laboratory abnormalities attributed to these conditions need to have resolved per PI discretion prior to omission of rituximab for subsequent studies.
- Participants with CD4 <100 cells/mm^3, albumin <2.5 g/dL, and/or ECOG ≥3 may be initiated at dose level -1 at PI discretion
- Lenalidomide will be administered days 1-10 and will not be dose-adjusted for hematologic toxicities
- Filgrastim 300 mcg/day (<75kg) or 480 mcg/day (≥75kg) SC will be administered on day 6 or Day 11 in Cycle 1 (~24 hours after completion of cyclophosphamide) and will be given once daily by subcutaneous injection until ANC has recovered to ≥ 5000/mm3 after neutrophil nadir or up to 24 hours before starting subsequent cycle.
- Vincristine dose will be reduced by 25%- 50% for ileus, grade 3 constipation, or hepatic toxicity, and by 25-100% (depending on grade) for other grade 2 or greater neurotoxicity
- Intrapatient dose EPOCH dose-adjustments are made based on hematotoxicity from previous cycle based on the following criteria:
    - nadir ANC ≥500/mm^3 on all measurements: increase 1 dose level
    - nadir ANC <500/mm^3 for <7 days: same dose level
    - nadir ANC <500/mm^3 for ≥7 days: decrease one dose level

OR

- platelet count <25,000/mm³ attributable to chemotherapy: decrease one dose level

- For EPOCH dosing starting on Day 1, if ANC ≥1000/mm³ and platelets ≥75,000/mm³ begin treatment
- If ANC< 1000/mm³ or platelets < 75,000/mm³** on day 1, delay up to 1 week. G-CSF may be started for ANC < 1000/mm³ and stopped 24 hours before treatment. If counts are still low after 1week delay, decrease 1 dose level below last cycle.

Important: Measurement of ANC nadir is based on twice weekly CBC only (3 days apart). Only use twice weekly CBC for dose-adjustment, even if additional CBC's are obtained. Twice weekly CBCs may be discontinued after ANC has recovered to ≥5000/mm³ and filgrastim has been discontinued unless clinically indicated.

****Note**: This does not apply to participants who have low platelets at baseline due to lymphoma, immune-mediated mechanism caused by lymphoma, HIV, KSHV or KICS. In those cases, no delay or dose reduction is required. The dose adjustments for these participants will be based solely on the ANC nadir and the PI or designee's discretion.

3.3.3.1 Modifications for non-hematologic toxicity DA-EPOCH-R²

Sensory neuropathy

Grade	Vincristine dose	Lenalidomide
2	0.4 mg/ m²/day	Continue
3	0.2 mg/ m²/day	Continue
4	0	Continue

Motor neuropathy

Grade	Vincristine dose (%)	Lenalidomide
1	0.4 mg/ m²/day	Continue
2	0.3 mg/ m²/day	Continue
3	0.1 mg/ m²/day	Continue
4	0	Continue

Hepatic dysfunction

Bilirubin (mg/dL)	Vincristine dose
>1.5 to <3	0.3 mg/ m²/day
≥3	0.2 mg/ m²/day

Vincristine dose may be re-escalated as hyperbilirubinemia improves.

Ileus

Constipation commonly occurs in participants receiving vincristine so participants should receive stool softeners as indicated (see Section **4.3.3**). Occasionally, symptomatic ileus may occur and this should be treated with a vincristine dose reduction. Because the severity of ileus is dose related, it is usually unnecessary to stop the vincristine altogether. Furthermore, because the therapy administered in this study is potentially curative, every effort should be made to not unnecessarily reduce vincristine doses. The guidelines for the management of symptomatic ileus (see Section **4.3.2**) on a previous cycle should be followed.

Renal dysfunction secondary to lymphoma

Etoposide dosage should be reduced 25% on cycle one for creatinine clearance < 50 mL/min. Etoposide should be returned to full dosage (or escalated if indicated) after creatinine clearance >50 mL/min is achieved. No other dose modifications for abnormal renal indices will be made for enrolled participants.

Other non-hematologic toxicity (excluding asymptomatic laboratory abnormalities)

Symptom grade	Management
≤ grade 2	Treat symptomatically
≥ grade 3	Hold next dose until ≤grade 2 and, if further therapy is medically indicated.reduce DA-EPOCH-R² by 1 dose level. If further toxicity at the reduced dose level, reduce DA-EPOCH-R² by another dose level After 2 dose reductionsparticipant will be removed from study

3.3.3.2 Dose Modification for Obese Participants

All dosing is based on the participant's BSA as calculated from actual weight. There is no clearly documented adverse impact of treatment of obese participants when dosing is performed according to actual body weight. Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation.

3.3.3.3 Rituximab Infusional related Adverse Events

Pretreatment for rituximab with diphenhydramine and acetaminophen using standard medical practice will be used in all participants. Pretreatment with 8mg dexamethasone, given intravenously or orally, is optional. Side effects of rituximab may be infusion rate related and may be reduced by slower administration or premedication. Thus, dose reductions of rituximab will not be made. Rituximab will be discontinued for the duration of the cycles in participants with grade 4 allergic reactions. At the discretion of the PI, rituximab may be administered on the following cycles using slower infusion rates.

3.4 STUDY CALENDAR

- Screening studies must be performed within 3 weeks or less prior to starting treatment. Positive serologies and pathology specimens performed earlier than 3 weeks prior to enrollment are acceptable for screening. See Section **2.3** for additional information.
- Baseline staging studies are generally performed within 7 days of starting therapy, except ophthalmology exam (if performed) which should be completed in the last 4 weeks prior to starting therapy. Baseline labs should be performed within one day of starting therapy and must be performed as outlined in Section **2.6**.
- All study assessments beyond day 1 cycle 1 may be performed within ± 7 days of what is indicated in order to accommodate weekends, holidays or other scheduling difficulties.
- Cycle 1 day 1 assessments may be performed up to 1 day prior to initiating study therapy and do not need to be repeated if performed within this timeframe at screening or baseline.
- Follow up every month for 3 months then every 3 months for the rest of the first year, then every 6 months for 4 years. First safety follow-up visit should be performed within 30 days (± 7 days) of cycle 6 day 21. At these visits, information will be collected about

the status of their PEL and any new primary malignancies. If participants are unable or refuse to come in for follow-up visits, an attempt will be made to collect as much information as possible about their status by communication with the participant and/or their local physician. Failure to make such visits will not be considered a protocol deviation.

3.4.1 Study Calendar Cycle 1

			DA-EPOCH-R[2] (*Cycle 1 only*)				
Procedure	***Screening***	***Baseline***	**Day 1**	**Day 4**	**Day 6[1]**	**Day 10**	**Days 11-21**
History and PE, ROS	X	X			X		
Ophthalmology Exam[16]		X					
Vital signs	X	X	X	X	X	X	
Performance Status	X	X					
NIH Advanced Directives Form[15]		X					
Labs							
CBC/diff Acute Care Panel/ Mineral Panel, Hepatic Panel	X	X		X	X	X	X[2]
Reticulocytes, creatine kinase, uric acid, lactate dehydrogenase, CRP		X			X		
Fe and transferrin, ferritin, haptoglobin		X					
Folate, 25-OH Vit D, RPR		X					
Stool, fecal occult blood		X					
Urine or serum pregnancy test	X	X					
UA		X					
24-hour urine study to correlate with PK		X					
PT/PTTT, thrombin time, fibrinogen, D-dimer	X						
Lipids, TSH, Free T4		X					
Quant Ig, IgE, free light chains		X			X		
Serology[4] HBV/HCV/HIV	X						
PCR[4] HBV[5]/HCV, HIV[5]; CMV/EBV		X					
KSHV Viral load (Whitby) (Also see Correlative Studies Calendar)		X			X		
Lymphocytes TBNK (requires CBC w diff)		X			X		
Pathology							
Lymphoma (Surgical or cytopathology) dx confirmation	X						
KS, KSHV-MCD dx confirmation if indicated	X						
Bone Marrow Biopsy (optional)		X					
CSF Cell count, cytopathology, KSHV, EBV, flow cytometry and molecular pathology		X					
Radiological Assessments							
CT, N/C/A/P	X						
[18]FDG-PET		X					
MRI Brain	X						
CXR: PA/lat/decub[8]		X			X		
Ultrasound[8]					X		
Other Specific Assessments							
EKG, ECHO	X						
PFTs		X					
Bronchoscopy/Endoscopy[9]		X					
Photography		X					
KS Measurements		X					
Adverse Events		X			X		X
Concomitant Medications[13]	X	X					

3.4.2 Study Calendar Cycles 2-6 and Follow up

	DA-EPOCH-R² (*Cycles 2-6)*			**Off protocol therapy**	
Procedure	**Day 1[1]**	**Day 5**	**Days 6-21**	**Follow-up through year 5**	**Long term survival**
History and PE, ROS	X			X	
Vital signs	X	X		X	
Performance Status	X			X	
Labs					
CBC/diff Acute Care Panel/ Mineral Panel, Hepatic Panel	X	X	X[2]	X	
Reticulocytes, creatine kinase, uric acid, lactate dehydrogenase, CRP, ferritin	X			X	
Fe and transferrin, ferritin, haptoglobin	X			X	
Folate, 25-OH Vit D, RPR				X	
Stool, fecal occult blood	X[3]			X[3]	
Urine or serum pregnancy test	X				
UA	X			X	
24-hour urine study	X (C6)				
PT/PTT, thrombin time (TT), fibrinogen, D-dimer	X			X	
Lipids, TSH, Free T4	X			X	
Quant Ig, IgE, free light chains	X			X	
PCR[4] HBV[5]/HCV, HIV[5]; CMV/EBV[5]	X			X	
KSHV Viral load (Whitby), also see Correlative Studies Calendar	X				
Lymphocytes TBNK (requires simultaneous CBC w diff)	X			X	
Pathology					
CSF	X[6]				
Radiological Assessments					
CT, N/C/A/P	X[7]			X[7]	
[18]FDG-PET	X[7]			X[7]	
CXR: PA/lat/decub[8]	X[8]				
Ultrasound[8]	X[8]				
Other Specific Assessments					
EKG, ECHO					
PFTs				X[9]	
Bronchoscopy/Endoscopy[9]				X[9]	
Photography	X[10]			X[10]	
KS Measurements[11]	X			X	
Response Evaluation[11]	X			X[11]	
Adverse Events	X			X[12]	
Concomitant Medications[13]	X			X	
Annual phone call[14]					X

1. Day 1 or the first day of EPOCH-R² (+/- 3 days).
2. Twice a week CBC with Diff until ANC recovery to ≥ 5000/mm³ after the neutrophil nadir.
3. Repeat if abnormal at baseline until negative.

4. Viral studies include: HBV serology (HBsAg, anti-HBs, anti-HBc), HCV serology (anti-HCV); HCV RNA quantitative viral load, EBV/CMV PCR viral load, -HIV 1 antibody**. Follow up viral load only for those viruses detected at screening, or HIV viral load in all participants to be performed at baseline and day 1 cycle 1, then every 3 cycles.**
5. HIV viral load (HIV-RNA quantification) only in participants with positive serology (Anti-HIV 1 antibody). HBV PCR if indicated due to positive serology.
6. Intrathecal methotrexate is being administered days 1 and 5 of cycles 3 through 6. Cell counts, chemistries, and cytopathology are obtained at time of IT methotrexate administration on day 1 of cycles 3 through 6 will be sent for studies outlined in Section **5.1.3**.
7. Cycle 3, Day 1 (+/- 7 days); end of DA-EPOCH-R^2 therapy, and at 3 months follow up, or as clinically needed to evaluate and document suspected progressive disease anytime during any cycle of therapy or follow up. ^{18}FDG PET is performed prior to Cycle 3 and at the end of therapy. In addition, new abnormal findings may be investigated by ^{18}FDG-PET and/or pathologic evaluation if clinically indicated.
8. Chest X-ray, abdominal and/or transthoracic ultrasound as indicated in participants with pleural or peritoneal effusions at specified time points until resolution. In participants with disease measurable/assessable by CT but NOT chest X-ray, abdominal or thoracic ultrasound, who have received at least 14 days of lenalidomide.
9. For participants with lung abnormalities on CT Chest (Bronchoscopy and PFTs) OR stool fecal occult blood test (guaiac) positive, to be performed at the end of therapy or if considered clinically indicated by PI.
10. Every odd number cycle until resolution of cutaneous KS during therapy, and at off study visit.
11. Response evaluation:
    - Clinical KSHV-MCD or KICS evaluation, Day 1 each cycle and during follow up visits.
    - KS Measurements, Day 1 of odd numbered cycles and days that photography is performed; during follow up visits
    - Lymphoma response, Cycle 3 day 1 (+/- 5 days), , 28- day safety visit after completion of cycle 6 (± 7 days), every 3 months in follow-up for the first year post-treatment, or as clinically needed to evaluate and document suspected progressive disease anytime during any cycle of therapy or follow up.
    - Please see Section **6.4** for Response criteria for further information on individual assessments.
12. AEs to be monitored for one month after the completion of the last cycle of therapy or until return to baseline or stabilization of event.
13. Supportive medications including aspirin, G-CSF, concomitant antimicrobials (including antiretroviral therapy and prophylactic agents), blood products including albumin, and steroids only
14. Participants who remain on study beyond 5 years may be contacted up to once per year by telephone or clinic visit for survival.
15. As indicated in Section **12.3**, all subjects will be offered the opportunity to complete an NIH advanced directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.
16. Ophthalmology exam (if performed) should be completed in the last 4 weeks prior to starting therapy.

3.5 COST AND COMPENSATION

3.5.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs.

3.5.2 Compensation

Participants will not be compensated on this study.

3.5.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to documenting removal from study, effort must be made to have all subjects complete a safety visit approximately 28 days following the last dose of study therapy.

3.6.1 Criteria for removal from protocol therapy

- Completion of protocol therapy and 28-day post end of treatement study visit (standard of care cART therapy not included in definition of protocol therapy)
- Progressive disease (does not include progressive leptomeningeal disease). Participants may remain on study for follow-up if they are receiving subsequent secondary systemic lymphoma-directed therapy.
- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity as defined in Sections **3.1.3** & **3.3**
- Investigator discretion
- Second malignancy, other than KSHV-associated malignancies or resectable non-melanomatous skin cancer
- Positive pregnancy test

3.6.2 Off-Study Criteria

- Lost to follow-up for greater than one year
- Participant requests to be withdrawn from study
- Death
- Investigator Discretion

3.6.3 Lost to follow-up

A participant will be considered lost to follow-up if he or she fails to return for 5 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit for up to 6 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 CONCURRENT MEDICATIONS/TREATMENTS

4.1.1 Antiretroviral therapy

- Generally, cART will be prescribed for HIV infected subjects.
- Participants requiring modification of cART regimen due to contraindicated agents (i.e. cobicistat or ritonavir) or HIV resistance will generally have their cART regimen modified during the screening and baseline assessment period.
- Integrase inhibitor based regimens are preferred for co-administration with EPOCH
- Referring physicians may manage this component of care while liaising with study physician investigators, at the discretion of the Principal Investigator or Lead Associate Investigator.
- Recommended combination therapy will be based on Department of Health and Human Services Guidelines for treatment of HIV infection: http://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv-guidelines/0
  However, the need for concurrent chemotherapy administration may require deviation from these guidelines in some cases. Certain antiretroviral agents with CYP3A4 inhibition are contraindicated.

4.1.2 Hepatitis B antigenemia

- For participants with serologic evidence of chronic persistent/active HBV infection, consideration will be given toward treating with lamivudine 150 mg PO daily, or other cART medications that also have demonstrated anti-HBV activity
- Treatment with DA-EPOCH-R^2 will be modified or discontinued in any participant who develops active HBV infection or hepatitis as directed by the PI to balance the risks of hepatitis reactivation with death from suboptimal treatment of lymphoma.

4.2 CONTRAINDICATED THERAPIES

- HIV protease inhibitors (including ritonavir) and cobicistat containing once a day pills (i.e. Stribild® [elvitegravir + cobicistat + emtricitabine + tenofovir disoproxil fumarate]) are contraindicated with EPOCH.
- Agents that interact with CYP450 Enzymes

- Strong inhibitors of CYP3A subfamily enzymes (e.g. ketoconazole) could increase exposure and should be avoided while subjects are receiving EPOCH
- Strong inducers of CYP3A (e.g. rifampin) could decrease exposure and should be avoided while subjects are receiving EPOCH
- Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated list such as http://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/druginteractionslabeling/ucm093664.htm. You may also consult **Appendix F: SELECT Strong inducers and inhibitors of CYP450 3a4**.

4.3 SUPPORTIVE CARE

4.3.1 General Principles of Supportive Care

Medications may be administered as clinically indicated, or at the discretion of the Principal Investigator, with the exception of other specific therapies for PEL. Additional supportive measures, specific to this participant population, will be allowed.

1) Management and serial evaluation of pleural, peritoneal, and pericardial effusions that, if not specifically addressed, generally lead to progressive decline in participant performance status and failure of cytotoxic therapy.
2) Use of filgrastim in participants who develop neutropenia on lenalidomide alone.
3) Participants with concurrent KS or recurrent KSHV-MCD may receive additional therapy directed at KS or MCD during post-treatment clinical follow-up if clinically indicated.

4.3.2 Management of Symptomatic Ileus

- Clinical ileus of < 8 days duration with abdominal pain requiring opioid analgesics and/or persistent nausea/vomiting for >2 days: Reduce vincristine dose by 25% (0.3 mg/ m^2/day)
- Clinical ileus of 8-12 days duration with abdominal pain requiring opioid analgesics and/or persistent nausea/vomiting for > 2 days: Reduce vincristine dose by 50% (0.2 mg/ m^2/day)
- Clinical ileus of > 12 days duration with abdominal pain requiring opioid analgesics and/or persistent nausea/vomiting for > 2 days: Hold vincristine on next cycle. May restart at a 50% reduction (0.2 mg/ m^2/day) on subsequent cycle.

4.3.3 Recommended Bowel Regimen

(G*oal of at least one soft bowel movement every 24 hours while on study*)

- Adults: docusate/sennosides 50mg/8.6 mg tablet or capsule. Take two tablets or capsules once a day, on Days 1-5 of Cycles 2-6 of DA-EPOCH-R^2; Days 6-10 of Cycle 1. Additional use beyond Day 5 (or Day 10 of Cycle 1) may be indicated. If needed can double the frequency to two tablets or capsules every 12 hours. If needed add oral lactulose 15-30 mL/dose prn q 6 hourly.

4.3.4 Thromboprophylaxis

- Subjects will receive thromboprophylaxis for the duration of lenalidomide therapy (including 'rest' days), ceasing 28 days following the last cycle of lenalidomide.
- Recommended thromboprophylaxis is aspirin 81mg PO once daily.

- In subjects intolerant of the above, an appropriate alternate regimen may be used. Alternatives include but are not limited to enoxaparin 0.5mg/kg to a maximum of 40mg SC once daily Enoxaparin dose may be rounded to accommodate available commercial prefilled syringes. Enoxaparin may also be considered in subjects with additional thrombotic risk factors, such as immobility or significant KS-associated edema. Participants receiving therapeutic anticoagulation for another indication (for example atrial fibrillation), whether with warfarin, enoxaparin, or another agent, may continue that agent in lieu of additional or alternative thromboprophylaxis
- Neither aspirin nor low molecular weight heparin will be administered when platelet counts are <50,000 cells/mm^3.

4.3.5 Pneumocystis jirovecii (PCP) prophylaxis

- All participants will receive *Pneumocystis jirovecii* prophylaxis during EPOCH-R^2 treatment regardless of CD4 count.
- Participants will continue to receive Pneumocystis jirovecii prophylaxis after completion of EPOCH-R2 at least until their CD4 count is > 200 cells/mm3, but may continue for 6 months after completing EPOCH-R^2 in those with higher CD4 counts at the discretion of the investigator.
- Recommended therapy is trimethoprim 160 mg + sulfamethoxazole 800 mg (Bactrim® DS) per day PO on three days weekly.
- In participants intolerant of the above, an appropriate alternate regimen may be used. Alternatives include but are not limited to dapsone 50–100 mg PO daily or 100 mg/dose PO twice weekly; atovaquone 1500 mg PO daily; aerosolized pentamidine monthly.

4.3.6 HSV I/II and VZV prophylaxis

- Valacyclovir 500 mg twice daily during therapy and 6-months to 1 year after completing EPOCH-R^2.

4.3.7 Fungal infections

- Oral Candidiasis if asymptomatic, recommend clotrimazole troches. If symptomatic, recommend oral fluconazole 200 mg daily. Fluconazole interacts with many drugs, and can alter EPOCH pharmacokinetics and therefore must be held on at least days 6 – 10 of the cycle during EPOCH administration.
- Esophageal Candidiasis oral fluconazole 200 mg daily. Fluconazole interacts with many drugs, and can alter EPOCH pharmacokinetics and therefore must be held on at least days days 6 – 10 of the cycle during EPOCH administration.
- Other Fungal Infections require consultation with Infectious Diseases.

4.3.8 Mycobacterium avium complex (MAC) prophylaxis

- Consider for participants whose CD4 cells are below 50-75/mm^3, and for those with historic CD4 nadir ≤75/mm^3 if HIV viral load is detectable.
- Recommend azithromycin 1200 mg once weekly, but other agents are acceptable

4.3.9 Febrile Neutropenia

Subjects who develop febrile neutropenia will be hospitalized and treated with appropriate broad-spectrum intravenous antibiotics. See Section **3.3** for treatment modifications and guidelines for filgrastim use pertaining to neutropenia.

4.3.10 Anemia

If a subject develops symptomatic anemia, or if the hemoglobin falls below 8.0 g/dL transfusion may be considered. Appropriate evaluation for etiology of the anemia, including but not limited to lenalidomide, HIV and its therapy, should be initiated.

4.3.11 Thrombocytopenia

Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should be given for a platelet count below 10,000/mm^3. For subjects with risk factors for bleeding, including fever, platelet transfusion may be considered for platelet counts below 20,000/mm^3. If invasive procedures are planned or the participant develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count > 50,000/mm^3.

4.3.12 Opportunistic Infections

Subjects who develop opportunistic infections, including but not limited to *Pneumocystis jirovecii* pneumonia, mycobacterial diseases, cytomegalovirus (CMV), and fungal infections will be treated using standard regimens. All opportunistic infections will be discussed with the Principal Investigator. Consultation with the Infectious Disease Service is recommended for subjects diagnosed with mycobacterium tuberculosis or fungal infections other than oral candidiasis.

4.3.13 Nutritional Assessment and Psychological Support

- KS and lymphoma may compromise nutritional status. Careful attention will be paid to nutritional status, and consultation with nutrition healthcare workers to optimize caloric intake will be undertaken as necessary.
- The chronic, incurable and potentially life-threatening nature of this disease and the stigmatizing nature of visible lesions is a profound psychological stressor. All such subjects on the study will be informed of and encouraged to see a NIH Social Worker for evaluation and support.

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 BIOSPECIMEN COLLECTION

Note: Throughout this section, tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.1.1 Lenalidomide Pharmacokinetics

Blood samples for the determination of lenalidomide plasma concentration will be obtained from each participant on cycle 1, day 1 (lenalidomide alone), cycle 1, day 7 (lenalidomide+EPOCH) and cycle 6, day 1 (lenalidomide + EPOCH). This may be adjusted per PI discretion or that of the PI's clinical designee if participant is receiving accelerated treatment. Samples will be collected at 1 hour (± 15 min), 4 hours (± 15 min) and 24 hours (± 1 hour) post lenalidomide.

Lenalidomide measurements in cerebral spinal fluid (CSF), the collection resulting from an already-scheduled spinal tap Cycle 3 Day 1, and in pleural fluids resulting from effusions will be performed if feasible with timing of sample in relation to lenalidomide dosing noted. These samples will generally be peak levels performed 1-2 hours after a dose of lenalidomide (Timing will be based on feasibility of specimen collection in relation to the procedure required to obtain the specimen). A blood specimen from the corresponding time point will be drawn for comparison.

All bioanalytical measurements will be conducted on an uHPLC with tandem mass spectrometric detection using an assay developed and validated by the Clinical Pharmacology Program (CPP).

Because lenalidomide clearance has been previously shown to be largely dependent on a participant's renal function[84], a 24 hr urine collection for creatinine clearance will be performed both at baseline and on cycle 6. The plasma pharmacokinetic measurements will primarily be used to compare single-agent lenalidomide PK to PK when lenalidomide is administered with the EPOCH regimen. Changes in renal function after 6 cycles of therapy will also be analyzed for correlations with lenalidomide PK.

If significant toxicity occurs in a participant, a single blood sample (purple top tube) can be drawn to assess that participant's pharmacogenomics using the DMET Plus (Affymatrix) genotyping platform that tests for 1,936 genetic variations in 225 drug disposition genes, including 47 CYP (phase I metabolism) genes, 13 non-CYP (phase I metabolism) genes, 78 phase II metabolizing genes (including UGTs), 63 transporters, 4 genes involved in facilitation of drug transporters, 9 genes involved in global regulation of drug metabolizing/transporting proteins, 4 drug binding proteins, and 4 drug targets. An additional blood sample (green top tube) can be drawn in order to measure each of the EPOCH drugs and lenalidomide to identify which has elevated plasma concentrations that may be causing the toxicity.

5.1.2 Tenofovir Pharmacokinetics

The available drug-drug interaction data with antiretrovirals and chemotherapy is limited. The current era of antiretroviral treatment (ART) provides more simplified and effective antiretroviral regimens.[34] Tenofovir disoproxil fumurate (TDF) and tenofovir alafenamide (TAF) are both oral pro-drugs and comprise first line ARV regimens for the initial treatment of HIV. TDF and TAF are converted to tenofovir (TFV) in plasma and subsequently phosphorylated to its main intracellular metabolite of interest, tenofovir-diphosphate (TFV-dp).[35, 36] Although both TDF and TAF are converted *in vivo* to parent TFV in plasma, the total plasma exposure to TFV is significantly higher following TDF administration. Higher and prolonged exposure to plasma TFV is reported to result in its uptake into various off target sites such as kidney and bone. In fact, renal impairment including Fanconi syndrome and proximal renal tubulopathy as well as perturbations in bone metabolism have been described.[18, 40] TFV is eliminated unchanged in the urine by a combination of glomerular filtration and proximal tubular secretion.[35] Twenty to thirty percent of TFV is described to be actively transported into renal proximal tubule cells by organic anion transporter, OAT1/3, in the basolateral membrane. TFV is then secreted to the tubular lumen by the apical membrane transporters multi-drug resistance proteins MRP-4 and MRP-2.

In regards to treatment agents in the current study- vincristine and cyclophosphamide (as part of EPOCH) are reported to interact with OAT1/3 and MRP2/4 transporters[37] and theoretically may cause excessive entry or reduced outflow of TFV from the proximal tubule cells, resulting in intracellular accumulation (TFV-dp) and potentially increased renal toxicity. The additional agents contained within EPOCH including etoposide, prednisone, doxorubicin, and rituximab are

not reported to stimulate OAT1/3 or MRP2/4. The only available drug-drug interaction data on TFV and the study treatment agents described herein this protocol, comes from a Phase II trial evaluating the safety and efficacy of lenalidomide in HIV infected participants with progressive KS. TDF 300mg + lenalidomide 25mg daily Days 1-21 for 24 weeks revealed no significant changes in plasma TFV C_{24} concentrations.[38] Given the lack of pharmacokinetic data between this chemotherapy regimen and TDF/TAF based antiretroviral therapy, the objective of this exploratory study will be to evaluate the effects of lenalidomide combined with modified DA-EPOCH and rituximab on the steady state pharmacokinetics of plasma tenofovir (TFV) and intracellular tenofovir di-phosphate (TFV-dp).

Blood samples for determination of lenalidomide maximum plasma concentrations (C_{max}) is an already planned investigation during Cycle 1 day 1 (lenalidomide alone), cycle 1 day 7 (lenalidomide + EPOCH), and cycle 6 day 1 (lenalidomide + EPOCH) at 1 hour post-dose lenalidomide. Tenofovir (TFV) maximum plasma concentrations (C_{max}) will also be determined at the same aforementioned time points from plasma aliquots without the need for additional blood draws.

The full pharmacokinetic profile of TFV, namely area under the dosing curve (AUC), has not been described when given concomitantly with lenanalidomide + EPOCH and hence will require additional blood draws. Blood samples for determination of tenofovir (TFV) plasma concentrations will be obtained from each participant at the following days and time points:

a. Baseline visit (pre-study treatment): trough concentration before tenofovir disoproxil fumurate/alafenamide (TDF/TAF) based ART administration. Participants will be instructed to take their antiretrovirals after trough concentrations are drawn.
b. Cycle 1 day 1 and 2 (lenalidomide alone) at the following time points: 1 hour (± 15 min), 4 hours (± 15 min) and 24 hours (± 1 hour) post lenalidomide dose. The timing of the PK draw must be documented on the participant sample.
c. Cycle 1 day 7 and 8 (lenalidomide + EPOCH) at the following time points: 1 hour (± 15 min), 4 hours (± 15 min) and 24 hours (± 1 hour) post lenalidomide dose
d. Cycle 6 day 1and 2 (lenalidomide + EPOCH) at the following time points: 1 hour (± 15 min), 4 hours (± 15 min) and 24 hours (± 1 hour) post lenalidomide dose

Blood samples for PBMC collection and determination of tenofovir-diphosphate (TFV-dp) concentrations will be obtained from each participant at the following days and time points:

a. Baseline visit (pre-study treatment): trough concentration before tenofovir disoproxil fumurate/alafenamide (TDF/TAF) based ART administration. Participants will be instructed to take their antiretrovirals after trough concentrations are drawn.
b. Cycle 1 day 1/2 (lenalidomide alone) at 1 hour (± 15 min), 4 hours (± 15 min) and 24 hours (± 1 hour) post lenalidomide dose
c. Cycle 1 day 7/8 (lenalidomide + EPOCH) at 1 hour (± 15 min), 4 hours (± 15 min) and 24 hours (± 1 hour) post lenalidomide dose
d. Cycle 6 day 1/2 (lenalidomide + EPOCH) at 1 hour (± 15 min), 4 hours (± 15 min) and 24 hours (± 1 hour) post lenalidomide dose

5.1.2.1 Sample Collection

- For lenalidomide and tenofovir plasma PK, blood will be collected in 6 mL sodium heparin tubes (green top) to which 0.1% hydrochloric acid has been added. For intracellular

tenofovir-diphosphate PK, PBMCs will be processed from a 10 ml blood collection in K_2 EDTA (purple top) tubes. See Table in Section **5.1.4.**

- 1 mL CSF will be collected without additive, and placed on wet ice for transport to the Figg laboratory.
- Optional 10 mL blood for pharmacogenetic studies will be collected in 10 mL EDTA (lavender top)

5.1.2.2 Sample Processing

- Research nurses will page the Blood Processing Core (BPC) within the CPP (Page 102-11964) immediately prior to blood draw to request a chilled 6 mL green-top sodium heparin tube (BD, Franklin Lakes, NJ).
- The date and exact time of each blood draw should be recorded on the sample tube and the PK sheet.
- Please e-mail NCIBloodcore@mail.nih.govat least 24 hours before transporting samples (the Friday before is preferred).
- For sample pickup, page 102-11964.
- For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).
- For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.
- After drawing, blood samples must be thoroughly mixed for drug stability and placed immediately on wet ice for transport to the Figg laboratory.
- Samples will then be centrifuged within 30 minutes of collection, for 10 minutes at 2000g (4°C), and plasma supernatants will be transferred for storage as indicated in Section **5.2.1**.
- Samples will be barcoded as described in Section **5.2.1.**

5.1.2.3 Blood Processing Core (BPC) Storage

- All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.
- Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to participants without Labmatrix access. The data recorded for each sample includes the participant ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer locations. Participant demographics associated with the clinical center participant number are provided in the system. For each sample, there are notes associated with the processing method (e.g. delay in sample processing, storage conditions on the ward, etc.).
- Barcoded samples are stored in bar-coded boxes in locked freezers at either -20° C or -80° C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per IRB approved protocol) and that any unused samples must be returned to the BPC.

- Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.
  If, at any time, a participant withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the participant, if so requested). The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**.
- Sample bar-codes are linked to participant demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to participant information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.1.3 Correlative Studies

Specimens will be collected for several planned correlative studies. Many samples will be processed and stored to be run in batches. When blood volume is limiting, these will be considered secondary to tests needed to make clinical decisions. Also, they may be delayed or omitted if collecting the specimens may pose a danger to the participant, or if they cannot be done or processed (because of Federal Holidays, inclement weather, US Government shutdown, etc.). Planned assays, by lab, include:

1. Whitby Lab: KSHV viral load, EBV viral load, flow cytometry, KSHV gene expression, and cell signaling assays, KSHV T-cell response
2. **AIDS Monitoring laboratory: Multiplex cytokines/chemokines and growth factor assays, flow cytometry
3. **Yarchoan lab: viral IL6 assay
4. ** Maldarelli lab: HIV single copy assays, HIV genetic diversity (See Section **5.1.4**)
5. Laboratory of Pathology, CCR Flow Cytometry Unit (Stetler-Stevenson lab) flow cytometry and Molecular Diagnostics (Raffeld Lab), Immunoglobulin heavy and light chain PCR

** Blood Biospecimens for storage (See Section **5.1.3.1.1**) will be collected and stored at AML for processing in batches (See Section **5.1.4** for collection schedule):

5.1.3.1 Sample collection for correlative studies

5.1.3.1.1 Blood

- Whitby lab for KSHV and EBV viral load and correlative studies
  - One 8.5 mL ACD tube (BD Vacutainer® Ref 364606 yellow top)
- Stetler-Stevenson/Flow Cytometry
  - 20 mL in Na Heparin (2 x 10 mL Green Top Tube) delivered stat to Flow Cytometry Lab (B1-B58)
- Raffeld/Molecular Pathology
  - 4 mL in Citrate tube (Light blue)
- Biospecimens for flow cytometry and storage at AML will be collected in the following tubes:

- Two 6 mL EDTA tubes (BD Vacutainer® Ref 367863 lavender top) for flow cytometry
- Two 8.5 mL ACD tubes (BD Vacutainer® Ref 364606 yellow top)
- Two 10 mL serum tubes (BD Vacutainer® Ref 367820 red top tubes)
- Two 10 mL sodium heparin (BD Vacutainer® Ref 367878 green top tube) for viable cells for batched flow cytometry (Stetler-Stevenson)
- 3 to 4 10 mL EDTA (BD Vacutainer® Ref 366643 lavender top tube) for measurement of single copy HIV VL

5.1.3.1.2 Saliva

Collected in 50 mL polypropylene conical tube (Becton-Dickinson labware) with 2 mL scope mouth wash (swish and spit), to Whitby Lab

5.1.3.1.3 Effusions (If applicable and feasible)

- Laboratory Medicine: Cell count (EDTA); protein, albumin, amylase, triglycerides, LDH (Red top)
- Stetler-Stevenson/Flow Cytometry
  - 20 mL in polypropylene conical tube (Becton-Dickinson labware) delivered stat to Flow Cytometry Lab (B1-B58)
- Raffeld/Molecular Pathology (Tina Pham, Room 2N-116A)
  - 14 mL in Sterile Tube (Corning Orange Top)
- Cytopathology (Room 2A-21)
  - 14 mL in Sterile Tube (Corning Orange Top)
- Whitby Laboratory
  - 50 mL polypropylene conical tube (Beckton-Dickenson labware) up to 2 L in a evacuated container (Baxter) on wet ice.
- Tosato Laboratory
  - 60 mL syringe with 2 mL preservation free heparin on wet ice delivered stat for development of cell cultures
- AML
  - 60 mL syringe with 2 mL preservation free heparin
- Ziegelbauer Laboratory
  - 10 – 20 mL on polypropylene conical tube on wet ice delivered stat

5.1.3.1.4 Cerebrospinal Fluid

- Laboratory Medicine: Cell count, protein, glucose (1-2 mL)
- Raffeld/Molecular Pathology (1-2 mL)
- Cytopathology (Room 2A-21) (2-4 mL)
- Whitby Laboratory (1 mL)
- AML (1mL)
- Laboratory of Pathology, CCR Flow Cytometry Unit (Stetler-Stevenson)

5.1.3.1.5 Bone Marrow Aspirates

- 2-5 mL in EDTA to Whitby lab

5.1.3.1.6 Bronchoalveolar Lavage

- Laboratory Medicine: Microbiology, Culture, PCR for: CMV, EBV, respiratory virus, pneumocystis jiroveci, legionella, chlamydia (10-20 mL)

- Raffeld/Molecular Pathology (5 mL)
- Cytopathology (Room 2A-21) (10 mL)
- Whitby Laboratory (5 mL)
- AML (5 mL)

5.1.4 Pharmacokinetics and Correlative Studies Calendar

Please also refer to Sections **5.1.1** and **5.1.2** for sample collection instruction. The individual sections listed in the calendar contain descriptions of the tests to be performed as well as laboratory locations for delivery. If limited effusion samples, distribution will be prioritized in order listed. Modification in volumes is possible after discussion with accepting laboratories.

Cycle 1					
Specimens (# of tubes)					
Days	BL/1	2	6	7	8
Pharmacokinetics – Please e-mail NCIBloodcore@mail.nih.govat least 24 hours before transporting samples (the Friday before is preferred). For sample pickup, page technician at 102-11964 immediately prior to blood and effusion collection. See Section **5.1.2.2** for additional information.					
Blood					
❿ Tenofovir plasma and intracellular levels before antiretroviral therapy (ART) dosing (pre-treatment at baseline) o Collect in one- 6 ml Figg lab supplied Na+ heparin tubes; immediately place on wet ice o Collect in one- 10ml Figg lab supplied K_2EDTA tubes for PBMC processing and collection	X (baseline) TFV and TFV-dp levels				
❿ Lenalidomide and tenofovir C_{max} o Draw 1 hour post dose o Collect in one-6 mL Figg lab supplied Na+ heparin tubes; immediately place on wet ice	X (D1)			X	
❿ Additional tenofovir plasma levels o Two sampling times: 4 hours and 24 hours post-dose o Collect in one- 6 mL Figg lab supplied Na+ heparin tubes at each time point; immediately place on wet ice	X (D1)	X		X	X
❿ Intracellular tenofovir-dp levels o Three sampling times: 1 hour, 4 hours, and 24 hours post dose o Collect in one- 10ml Figg lab supplied K_2EDTA tubes at each time point for PBMC processing and collection; immediately place on ice	X (D1)	X		X	X
Effusions – drawn based on feasibility ❿ 6 mL Figg lab supplied Na+ heparin tubes; immediately placed on wet ice	X				

Cycle 1					
Specimens (# of tubes)					
Days	BL/1	2	6	7	8
Blood					
Raffeld/ Molecular Path (See **5.1.3.1.1**) 4 mL in light blue citrate (1)	X		X		
Whitby lab T-cell studies (See **5.1.3.1.1**) ❿ 8.5 mL yellow top ACD (4)	X				
AML (See **5.1.3.1.1**)	X		X		
Storage 8.5 mL ACD (2);10 mL red top Serum (2)	X		X		
10 mL green top heparin (2) delivered stat to store viable PBMC for flow flow cytometry in Stetler-Stevenson/Flow (See **5.1.3.1.1**)	X				
Flow Cytometry: 6 mL EDTA (2)	X				
HIV Studies: 10 mL EDTA (7)	X	X	X		
Saliva					
Whitby lab (See **5.1.3.1.2**) ❿ In 50 mL polypropylene conical tube with 2 mL Scope mouthwash	X				
Effusions (See **5.1.3.1.3**)					
Laboratory Medicine ❿ 10 mL red top ❿ 6 mL in EDTA tube	X		X (optional)		
Cytopathology lab ❿ 14 mL in sterile tube (Corning orange top)	X		X		
Stetler-Stevenson/Flow) ❿ 20 mL in polypropylene conical tube delivered stat	X		X		
Whitby lab ❿ 50 mL polypropylene conical tube ❿ Up to 2 L in evacuated container on wet ice	X		X		
Tosato Lab • 60 mL syringe with 2 mL preservation free heparin on wet ice delivered stat	X				
AML 60 mL syringe with 2 mL preservation free heparin	X		X		
Ziegelbauer lab • 10 – 20 mL on polypropylene conical tube on wet ice delivered stat	X				
Yarchoan Lab (See **5.1.3.1.3**)	X				
BAL (See **5.1.3.1.6**)					
Lab medicine (10 – 20 mL)	X				
Raffeld/ Mol. pathology lab (5 mL)	X				
Cytopathology (10 mL)	X				
Whitby lab (5 mL)	X				
AML (5) mL	X				
Bone Marrow Aspirate					
Whitby lab (See **5.1.3.1.5**) (2 – 5 mL in EDTA)	X				

Cycle 1					
Specimens (# of tubes)					
Days	BL/1	2	6	7	8
CSF (See **5.1.3.1.4**)					
Lab medicine (1 – 2 mL)	X				
Raffeld/ Molecular Path (1 mL)	X				
Cytopathology (2- 4 mL)	X				
Whitby lab (1 mL)	X				
AML (1mL)	X				
Stetler-Stevenson/Flow (1-2 mL)	X				

BL: Baseline or Day 1; BAL, bronchoalveolar lavage; CSF, cerebral spinal fluid; C1, Cycle 1 only; C3, Cycle 3 only

Cycles 2-6		
Specimens (# of tubes)		
	Day 1(unless noted)	End of therapy
Pharmacokinetics – Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred). For sample pickup, page technician at 102-11964 immediately prior to blood and effusion collection. See Section **5.1.1** for additional information.		
Blood ❿ Lenalidomide and tenofovir C_{max} ○ Draw 1 hour post dose ○ Collect in one-6 mL Figg lab supplied Na+ heparin tubes; immediately place on wet ice ❿ Additional tenofovir plasma levels ○ Two sampling times: 4 hours and 24 hours post-dose ○ Collect in one- 6 mL Figg lab supplied Na+ heparin tubes at each time point; immediately place on wet ice ❿ Intracellular tenofovir-dp levels ○ Three sampling times: 1 hour, 4 hours, and 24 hours post dose ○ Collect in one- 10ml Figg lab supplied K_2EDTA tubes at each time point for PBMC processing and collection; immediately place on wet ice	X (C6, D1 and D2) X (C6, D1 and D2) X (C6, D1 and D2)	
CSF – drawn based on feasibility or if flow positive at baseline ❿ 1 mL without additive and placed on wet ice for transport to Figg Laboratory ❿ 1-2 mL placed on ice to Stetler-Stevenson/Flow Lab	X (C3, D1) C2-C6 D1 if positive at baseline)	
Effusions – drawn based on feasibility ❿ 6 mL Figg lab supplied sodium heparin tubes; immediately placed on wet ice for transport to Figg Lab	X (if thoracentesis is performed)	

Cycles 2-6		
Specimens (# of tubes)		
	Day 1(unless noted)	End of therapy
Blood [Pharmacogenetics] ➓ 10 mL EDTA lavender top tube	X (C2 or 3 if treatment-related AE based upon PI discretion)	
Blood (See **5.1.3.1.1**)		
Whitby lab ➓ 8.5 mL yellow top ACD (1)	X	X
Raffeld/ Molecular Path ➓ 4 mL in light blue citrate (1)		X
AML • 8.5 mL ACD, 2 unless noted as 1 tube (1) • 10 mL red top Serum (2) • 6 mL EDTA (2) for Flow Cytometry (Cycle 2, 5, end of therapy, one year) • 10 mL EDTA (1)	X	X
Saliva (See **5.1.3.1.2**)		
Whitby lab ➓ In 50 mL polypropylene conical tube with 2 mL Scope mouthwash	X	
Effusions (See **5.1.3.1.3**)		
Laboratory Medicine ➓ 10 mL red top ➓ 6 mL in EDTA tube	X	
Cytopathology lab ➓ 14 mL in sterile tube (Corning orange top)	X	
Stetler-Stevenson/Flow ➓ 20 mL in polypropylene conical tube delivered stat	X	
Whitby lab ➓ 50 mL polypropylene conical tube ➓ Up to 2 L in evacuated container on wet ice	X	
Tosato Lab • 10 – 40 mL in polypropylene conical tube on wet ice delivered stat	X	
AML 60 mL syringe with 2 mL preservation free heparin	X	
Ziegelbauer lab ➓ 10 – 20 mL on polypropylene conical tube on wet ice delivered stat	X	
Yarchoan Lab (See **5.1.3.1.3**)	X	
BAL (See **5.1.3.1.6**)		
Lab medicine ➓ 10 – 20 mL		
Raffeld/ Mol. pathology lab ➓ 5 mL		

Cycles 2-6		
Specimens (# of tubes)		
	Day 1(unless noted)	End of therapy
Cytopathology ❿ 10 mL		
Whitby lab ❿ 5 mL		
AML ❿ 5 mL		
Bone Marrow Aspirate (See **5.1.3.1.5**)		
Whitby lab ❿ 2 – 5 mL in EDTA		
CSF (See **5.1.3.1.4**)		
Lab medicine ❿ 1 – 2 mL	X (C3,6) (if CSF + for PEL at baseline collect C2-C6 D1)	
Raffeld/ Molecular Path ❿ 1-2 mL	X (C3,6) (if CSF + for PEL at baseline collect C2-C6 D1)	
Cytopathology ❿ 2- 4 mL	X (C3,6) (if CSF + for PEL at baseline collect C2-C6 D1)	
Whitby lab ❿ 1 mL	X (C3,6) (if CSF + for PEL at baseline collect C2-C6 D1)	
AML ❿ 1mL	X (C3,6)	
Flow cytometry: 1-2 mL placed on ice to Stetler-Stevenson/Flow Lab	X (C3,6) (if CSF + for PEL at baseline collect C2-C6 D1)	

BL: Baseline or Day 1; BAL, bronchoalveolar lavage; CSF, cerebral spinal fluid; C1, Cycle 1 only; C3, cycle 3 only

5.1.5 Description of Studies

5.1.5.1 Evaluation of the effect of lenalidomide alone and in combination with DA-EPOCH-R on HIV latency reversal and HIV reservoirs

Deliver samples:	AIDS Monitoring Laboratory, Leidos Biomedical Inc.

Plasma HIV RNA (Single copy assay) and cell associated HIV Studies (only in participants with HIV infection).

Send to Leidos-AML to be processed using a protocol specific SOP for separation of plasma and PBMCs, and frozen at ≤ -80°C (plasma) or cryopreserved (viable PBMCs). These samples will be transferred to the Maldarelli laboratory when ready for batched testing. Samples will be run only in participants whose HIV VL by conventional techniques is below the limit of detection (<50 copies mL).

5.1.5.1.1 Single Copy plasma HIV RNA (SCA)

Sample collection: 30-40 mL blood will be collected in 10 mL EDTA tubes and transported to AML. Samples will be centrifuged twice using an SOP developed for collection of HIV for single copy assays. Plasma and PBMCs will be aliqouted and stored at -80 degrees Celsius or colder (plasma) or Liquid Nitrogen (PBMCs). Single copy HIV RNA (SCA) will be evaluated batched samples from plasma stored from these specimens using the following assay:

In participants with HIV well controlled on cART, the plasma HIV viral load, as measured by commercial RNA PCR assays, is generally suppressed to <20 copies/mL, although "blips" up to 400 copies/mL are sometimes noted, and not necessarily associated with virologic failure. Plasma measurements of HIV RNA by PCR are thought to largely represent reactivated virus from HIV infected reservoirs in this setting [85], and persistence of HIV-1 viremia can still be detected and quantified using a "single copy" quantitative real time PCR assay targeting the HIV-gag RNA that is sensitive down to 0.3 copies/mL, using methods previously described [86].

If residual plasma HIV viral load in participants on cART represents a combination of spontaneous and induced latency reversal, measurements over time would be expected to correlate with the size of the HIV reservoir. "Steady-state" HIV plasma HIV RNA viral load will be used as a correlate of the total HIV reservoir, and should a participant obtain a persistently undetectable plasma HIV by the SCA, additional evaluation of the HIV reservoirs through cell based assays (beyond those outlined in Section **5.1**) or evaluation of tissues may be warranted.

It is unknown whether lenalidomide may lead to reversal of HIV latency, which would be expected to lead to changes in CD4+ T-cell transcription leading to cell activation, increased expression of HIV RNA, with the effect of leading to release of HIV RNA into plasma despite optimal cART.

The SCA will be employed to:

- Evaluate the kinetics of HIV latency reversal Cycle 1 Days 1 and 2, looking at baseline, 2-hours (TMax 3 hours), 24-hours.
- Additionally we will evaluate on Cycle 1, Day 6 prior to administration of DA-EPOCH; before dosing on cycle 2 day 1; at end of therapy and at an off –study evaluation. Compare the steady state HIV plasma RNA in participants on cART at baseline, after 1 cycle, after 6 cycles, and one month off therapy.

5.1.5.1.2 PBMC associated HIV RNA and HIV DNA

Sample collection: 30-40 mL blood will be collected in 10 mL EDTA tubes and transported to AML. Samples will be centrifuged twice using an SOP developed for collection of HIV for single copy assays. Plasma and PBMCs will be aliqouted and stored at -80 degrees Celsius or colder (plasma) or Liquid Nitrogen (PBMCs). PBMC associated HIV RNA and HIV DNA will be evaluated on batched samples from PBMCs stored from these specimens with the following objectives.

In addition to measuring plasma HIV RNA, measures of basal and induced HIV transcription will be evaluated using an assay quantifying cell-associated unspliced HIV using a semi-nested real-time quantitative PCR for HIV-gag US RNA in sorted CD4+ T-cells. Total cell associated DNA will also be measured. As with the SCA assay, the cell based HIV assays will be employed to:

- Evaluate the kinetics of HIV latency reversal during cycle 1, looking at baseline, 2-hours, and

24-hours.

- Compare the cell associated unspliced HIV RNA in participants on cART at baseline to that prior to cycle 2 and then at time of tumor progression and/or off therapy.

Cell-associated total HIV DNA will also be quantified in the same samples using real time PCR.

5.1.5.1.3 HIV molecular evolution

HIV molecular evolution will be evaluated on batched samples from plasma stored from the specimens collected as outlined in Section **5.1.3**.

Inferences about clonality of residual HIV reservoirs can be evaluated, in part, by exploring changes in the phylogenetics of plasma HIV using molecular phylogenetic approaches. Evaluation of clonality of HIV sequences may be particularly useful when evaluating changes in total HIV vial load by single copy assay, and may provide further support that changes in plasma HIV RNA correlate with changes in clonal "wild-type" HIV infected cells. The effects of lenalidomide combined with DA-EPOCH-R on HIV-1 molecular evolution in participants on cART will be evaluated by evaluating single genome sequences of HIV at baseline, cycle 2, and off therapy. Intra-patient HIV populations will be evaluated overtime using phylogenetic analyses, as previously described [87]. Additionally, HIV DNA sequencing will be evaluated in select participants.

5.1.5.2 Evaluation of the effect of the regimen on lymphocyte and monocyte cell populations and phenotypes by flow cytometry

(AIDS Monitoring Laboratory)

Specimen collection: 10 mL blood will be collected in EDTA for flow cytometry. Samples will be processed within 24 hours.

We will evaluate the effect of lenalidomide combined with DA-EPOCH-R on lymphocyte and monocyte numbers and phenotypes by flow cytometry. Assays will focus on T cell polyclonal stimulation, activation and exhaustion (Panel: CD3, CD4, CD8, CD27, CD38, CD45Ro, PD-1, HLA-DR, CD57) as well as monocyte activation (CD14, TF-PE, CCR2, CD16, CX3CR1 APC, CCR5 APC, CD3, CD2, CD19, CD20, CD56, HLADR) and PDL1 (CD14, CD15, and CD274).

Baseline (Day 1, Cycle 1) specimens will be compared to specimens Cycle 2, Cycle 5, end of therapy visit and 1 year.

Flow cytometry will be performed in the Laboratory of Randy Stevens, AML, under the supervision of Joe Adelsberger and Jeannette Higgins.

5.1.5.3 Whitby Laboratory

Send samples to:	Laboratory of Denise Whitby, Ph.D. •Leidos Biomedical, Inc. • 50 Boyles St• Building 535, Room 428A •Frederick, MD, 301-846-1714

KSHV Viral Load (blood, effusions and saliva)

DNA will be extracted from peripheral blood mononuclear cells (PBMC) using the Roche Magnapure 96 with quality and concentration was assessed by optical density using Nanodrop1000 (Thermo Scientific, Wilmington, DE). DNA concentration was adjusted to 250 ng per 10 µL for two quantitative real-time PCR assays developed using TaqMan® (Applied Biosystems, Foster City, CA). KSHV-viral load will be measured using previously described RT-PCR methods. Quantitative testing for KSHV will be performed in triplicate using a primer and probe set that amplifies a 176-base pair fragment of the K6 region. The primers are K6-10F 5′CGCCTAATAGCTGCTGCTACGG-3′ (nucleotides 27309–27330) and K6-10R 5′-TGCATCAGCTGCCTAACCCAG-3′ (nucleotides 27159–27330). The probe sequence was p-K6-10 5′-**R**-CACCCACCGCCCGTCCAAATTC-**Q**-3′ (nucleotides 27277–27298, GenBank accession number U75698) [88]. Negative control wells will be run in triplicate on each assay plate. The number of cellular equivalents, roughly estimating peripheral blood mononuclear cell concentration, will be determined using a quantitative assay for human endogenous retrovirus 3 (ERV-3)[89]. KSHV viral load will be reported as viral DNA copies per million PBMCs[90]. PCR will be performed using the GS Junior (Roche).

EBV viral load (blood only)

On prospective samples, EBV viral load will be run in parallel with KSHV viral load, using the same DNA extraction, quality control and PCR methodology. Sample collection and processing will be the same as for KSHV viral load. EBV viral load will be measured using RT-PCR using primers for the pol gene[91] and corrected for ERV-3[91].

5.1.5.3.1 KSHV-specific B-cell responses will be evaluated using a bead based multiplex serologic assay developed in the laboratory of Dr. Whitby in collaboration with HAMB. The breadth and intensity of anti-KSHV responses at baseline will be compared those from samples collected obtained after 6 cycles of therapy or at an off visit study, whichever comes first.

5.1.5.3.2 KSHV-Specific T-cell Responses will be measured using an ELISPOT assay that is currently being developed in the laboratory of Denise Whitby in collaboration with HAMB. The current ELISPOT evaluates T-cell responses to an array of overlapping peptides from potentially immunogenic KSHV-encoded proteins. Samples from baseline will be compared to those obtained after 6 cycles of therapy or at an off visit study, whichever comes first.

Effusion Studies

Effusion studies will be performed every cycle if residual effusions are present and only if samples are clinically feasible to obtain.

- Viable subpopulations of cells will be isolated by flow cytometric cell sorting from pleural effusion samples for following subsets:
    - T cells
    - B cells – including assessing CD138, PEL cells from other B cell subpopulations
    - Monocytes
    - Various subpopulations associated with the above and others

- Viral load quantitation of isolated subpopulation of cells
    - Assess viral load
    - Assess viral gene expression differences in isolated subpopulations

5.1.5.4 AIDS Monitoring Laboratory

Send samples to:	AIDS Monitoring Laboratory, Leidos Biomedical Inc.

Cytokines, Chemokines and Growth Factor Assays.

To be run on serum, plasma, CSF or effusion samples at AML using a custom 15-plex array (V-Plex Plus, Mesoscale Discovery, Gaithersburg, MD) that includes IFNg, IL1b, IL6, IL8, IL10, IL12p70, TNFa, VEGFA, IP-10, MCP1, IL12/IL23p40, IL17a, Eotaxin3, MIP-1a, IL7. Additional assays may be performed.

5.1.5.5 NCI Flow Cytometry, peripheral blood and pleural fluid

Deliver samples stat to:	Flow Cytometry Unit NCI, Building 10, Room 3S240

Effusion specimens will be processed within 12 h of collection and stained with a panel of antibodies[92]. Specimens will be acquired using an 8-color multiparametric approach on a 3-laser FACS Canto II (BD Biosciences, San Jose, CA) with DiVa 6.1.1 software and analyzed by FCS Express 3 software (DeNovo Software, Los Angeles, CA). Markers will include: surface and intracellular kappa and lambda light chains, CD3, CD4, CD5,CD8, CD13, CD14, CD16, CD19, CD30, CD34, CD38, CD45, CD56, CD57, CD138, and if possible, surface immune markers (e.g. MHC-1, ICAM-1, B7-2). The studies done will in part depend on the number of cells available for study. Blood specimens will stored viably at AML for batched analysis that includes an anti-LANA antibody in the panel.

5.1.5.6 Molecular Pathology (Raffeld)

Deliver samples to:	Tina Pham, Building 10, Room 3S-249

Immunoglobulin heavy and light chain PCR

DNA will be extracted from blood, bronchoalveolar lavage, and effusions and studied for the presence of clonal B-cell populations using multiplexed consensus PCR targeting the immunoglobulin heavy (IgH) and light (IgL) chains. For the IgH locus, a pair of consensus primers (FRIII-IGH/JH and FRII IGH/JH) will be employed[93] Additional reactions will be performed for the IGk locus using a Biomed II primer set.[94] Products will be analyzed on an ABI 3130 XL Genetic Analyzer, and analyzed using Genemapper software.

5.1.5.7 Laboratory Medicine

	Orders for the samples will be entered through CRIS and processed by standard DLM procedures

Effusion fluid will be tested for cell count, protein, albumin, amylase, triglycerides and LDH.

Cerebrospinal fluid will be tested for cell count, protein and glucose levels

Microbiology will perform the following clinical evaluations on BAL:culture; PCR for: CMV, EBV, respiratory virus, pneumocystis jiroveci, legionella, chlamydia

5.1.5.8 Cytopathology

	Orders for samples will be entered through CRIS and processed by standard LP procedures

Cerebrospinal fluid, bronchoalveolar lavage and pleural effusion samples will undergo cytopathology testing in the NIH Laboratory of Pathology. Cell pellets will be stored when feasible for indicated immunohistochemical stains.

5.1.5.9 Ziegelbauer Laboratory

Deliver samples stat to:	Laboratory of Joseph M. Ziegelbauer, Building 10, Room 5A-21

KSHV-encoded microRNA

Valuation of predicted KSHV microRNA target genes in KSHV associated malignancies and control tissues. Predictions of KSHV microRNA target genes are based on mRNA expression profiling in previous cell culture experiments. One of the principal techniques will involve extracting soluble proteins from participant samples and probing them with antibodies in western blotting assays in order to aid in ongoing microRNA target validations. Other techniques to look at gene expression will also be used. Also, analyses of gene expression in collected tissues such as peripheral blood mononuclear cells may be made, and KSHV gene sequences may be studied.

5.1.5.10 Tosato Laboratory

Deliver samples:	Laboratory of Giovanna Tosato, Building 37, Room 4124

Pleural effusions will be collected for development of mesothelial and primary effusion cell cultures. Co-culture experiments will be performed to explore the role of microenvironment signaling in the survival and proliferation of PEL.

5.1.5.11 Yarchoan Laboratory

Deliver samples:	AIDS Monitoring Laboratory, Leidos Biomedical Inc. for storage
Grouped samples will be delivered to:	Laboratory of Robert Yarchoan, MD Building 10, Room 5A25, Bethesda, MD 240-760-6075

Viral-IL6.

Performed in the laboratory of Dr. Robert Yarchoan, using a sandwich ELISA as previously described by our group[13].

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through the Clinical Trial Data Management system. Should a CRIS screen not be available,the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

It is understood that per the NCI policy regarding the Requirements for the Research Use of Stored Human Specimens and Data, prospective NIH IRB approval and continuing IRB oversight must be obtained for research involving identified or coded samples or data where investigators can

identify the source. This policy applies to research protocols where the remaining research activities are limited to data analysis and to the subsequent research use of specimens or data previously collected under a now terminated protocol. The following guidelines describe how these principles apply to this specific protocol.

5.2.1 Clinical Pharmacology Program

- Plasma supernatants of centrifuged samples will be transferred to individually labeled tubes, barcoded, anonymized, and stored at -80 °C until analysis. Participant data will be entered into a secure and encrypted LabSamples database maintained by the Clinical Pharmacology Program, Office of the Clinical Director.

5.2.2 AIDS Monitoring Laboratory, Leidos Biomedical Inc.

- Many samples on this study will be processed and stored in the AIDS Monitoring Laboratory (AML) run by Leidos Biomedical Inc. in the NCI-Frederick facility located with Fort Detrick. The samples are stored under code, and the information linking these unique codes to the subjects is kept on the AML database. The laboratory informatics system conforms to NIH Information Technology Security Requirements and NIH Protection of Human Research Subjects Guidelines. All laboratory staff is trained to adhere to NIH Information Technology Security Requirements and NIH Protection of Human Research Subjects Guidelines. Computers used to access inventory systems require username and password for login. The laboratory database is housed in a secure, protected environment and backups are performed routinely. Access to specimen information, clinical data, and stored specimens is limited to approved laboratory staff and the investigator in charge of the study (or individuals authorized by the investigator).

➢ Specimen Withdrawal for Research Purposes

- The protocol team will inform the AML staff when tests are to be run with the specimens, and the samples used for testing will be tracked by the AML. This information will in turn be shared with the protocol team. The research nurse on the study will be in charge of tracking this information for the protocol team.

5.2.3 Specimens Sent to the Whitby Laboratory

- Some of the specimens are sent to the laboratory of Dr. Denise Whitby, also in Leidos Biomedical, Inc. in the NCI-Frederick facility located with Fort Detrick. The Whitby lab maintains CLIA certification for measurement of KSHV viral load. This is a locked laboratory, and a log is kept of the specimens and when they are utilized. The samples sent are coded by the protocol research team. Specimens collected for KSHV viral load have participant identifiers as required for CLIA certified tests. Additional research specimens are identified by unique identifiers. Biospecimens are logged in by Dr. Whitby's laboratory and are run in batch when enough specimens are collected. Records are kept when the specimens are used for analysis.

  Denise Whitby PhD
  Leidos Biomedical, Inc.
  50 Boyles St, Building 535, Room 428A

Frederick, MD
301-846-1714

5.2.4 Specimens Sent to the Yarchoan Laboratory

- A limited number of samples are sent to Dr. Yarchoan's laboratory. This is a locked laboratory, and a log is kept of the specimens and when they are utilized.

Robert Yarchoan, MD
Building 10, Room 5A25
Bethesda, MD
240-760-6075

5.2.5 Specimens Sent to the Tosato Laboratory

- A limited number of samples are sent to Dr. Tosato's laboratory. This is a locked laboratory, and a log is kept of the specimens and when they are utilized. The samples sent are coded by the protocol research team and have no participant identifiers. They are logged in by Dr. Tosato's laboratory and are run in batch when enough specimens are collected. Records are kept when the specimens are used for analysis.

Giovanna Tosato, MD
Building 37, Room 4124
Convent Drive
Bethesda, MD
240-760-6144

5.2.6 Specimens Sent to the Ziegelbauer Laboratory

- A limited number of samples are sent to Dr. Ziegelbauer's laboratory. This is a locked laboratory, and a log is kept of the specimens and when they are utilized. The samples sent are coded by the protocol research team and have no participant identifiers. They are logged in by Dr. Ziegelbauer's laboratory and are run in batch when enough specimens are collected. Records are kept when the specimens are used for analysis.

Joseph Ziegelbauer, PhD
Building 10, Room 5A21
10 Center Drive
Bethesda, MD
240-858-3267

5.2.7 Specimens Sent to the Maldarelli Laboratory

- A limited number of samples are sent to Dr. Maldarelli's laboratory. This is a locked laboratory, and a log is kept of the specimens and when they are utilized. The samples sent are coded by the protocol research team and have no participant identifiers. They are logged in by Dr. Maldarelli's laboratory and are run in batch when enough specimens are collected. Records are kept when the specimens are used for analysis.

Frank Maldarelli, MD
Building 10, 5A06
10 Center Dr

Bethesda, MD
240-760-6082

5.2.8 Clinical Center Processing and Storage of Clinical Specimens

Many routine samples and a sample of the biopsy specimens are sent to the Laboratory of Pathology (CCR), Department of Laboratory Medicine, and Department of Transfusion Medicine at the NIH Clinical Center. These samples will be handled according to the procedures of these departments. Results for clinical testing are generally available via the CRIS electronic medical record.

Co-enrollment on 01-C-0038

If subjects have co-enrolled on study 01-C-0038 (Collection of Blood, Bone Marrow, Tumor, or Tissue Samples from Subjects with HIV Infection, KSHV Infection, Viral-related Pre-Malignant Lesions, and/or Cancer), then the samples may also be tested under the specifications of that study. Similarly, if subjects have co-enrolled on other studies approved by the NIH IRB that call for maintaining and testing the samples, then they may be transferred to those studies.

Handling of Specimens at Study Termination

At the termination of the protocol, if participants have been co-enrolled on study 01-C-0038 (Collection of Blood, Bone Marrow, Tumor, or Tissue Samples from Patients with HIV Infection, KSHV Infection, Viral-related Pre-Malignant Lesions, and/or Cancer), then excess specimens and accompanying data will be coded and linked, and stored under 01-C-0038. Also, if subjects have co-enrolled on other studies approved by the NIH IRB that call for maintaining the samples, then they will be maintained on those protocols. Otherwise, the unused samples will be destroyed.

Loss or Destruction of Samples

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**. Any new use of the samples, specimens, or data will require prospective IRB approval or Office of Human Subject Research determination of IRB exemption.

6 DATA COLLECTION AND EVALUATION

6.1 SUMMARY

Members of the HIV/AIDS Malignancy Branch clinical research team will collect data on study subjects according to the Study Calendar (Section **3.4** and Section **5.1.3**). Complete records will be maintained on each participant including supplementary information obtained from outside laboratories, radiology reports, or physician's records. These will serve as the primary source material that forms the basis for the research record. The primary source documentation will assure the following:

- The participant satisfied each eligibility criterion.
- Signed informed consent was obtained prior to registration and treatment.
- Treatment was given according to protocol or any protocol deviations documented and justified.
- Toxicity and response were assessed according to protocol.
- Drug accountability records were kept on each participant.

Clinical data will be coded for database entry. Data will be stored in the CCR clinical trials database. Dr. Robert Yarchoan, the Principal Investigator, will be responsible for the protocol.

6.2 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through 28 days after the end of treatment. Beyond 28 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the participant's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

6.2.1 Specific data elements that will be collected and recorded in the database:

1. All data elements of the required for disease measurement
2. All transfusions of blood products, including albumin
3. All infections, and associated antibiotics employed
4. All hospitalizations, regardless of reason and the reason for hospitalization and the duration of hospitalization
5. As participants will potentially remain on protocol until the time they die, the name(s) of subsequent regimens and date(s) received if participant has progressive disease.
6. All Unexpected Grade 2 adverse events
7. Only the following **expected** Grade 2 adverse events:
    a. Neuropathy-motor
    b. Neuropathy-sensory
    c. Constipation
    d. Ileus

e. Allergic Reaction
f. Hemorrhagic Cystitis
g. Febrile Neutropenia
h. Hypothyroidism
i. Rash
j. Edema

8. All other adverse events grade 3 or higher
9. **Special Note**: Record only the highest grade of each adverse event **(and the dates corresponding to the highest grade)** during each cycle of treatment.

6.2.2 Specific data elements that will not be collected or recorded in the database:

- Grade 1 adverse events
- Grade 2 expected adverse events, except as listed above
- Any adverse events that occur during subsequent regimens administered for progressive disease.
- Concomitant medications
- Results of physical exams
- Vital Signs

6.2.3 Specific Adverse Event Reporting Exceptions

6.2.3.1 Events that Do Not Meet the Definition of an SAE

Elective hospitalizations to administer, or to simplify trial treatment or trial procedures (e.g., an overnight stay to facilitate chemotherapy and or correlative studies) are not considered SAEs. However, all events leading to unplanned hospitalizations or unplanned prolongation of an elective hospitalization (e.g., undesirable effects of any administered treatment) must be documented and reported as SAEs.

6.2.3.2 Events not to be Considered as AEs/SAEs

Medical conditions present at the initial trial visit that do not worsen in severity or frequency during the trial are defined as baseline medical conditions, and are not to be considered AEs.

6.2.3.3 AE/SAEs Observed in Association with Disease Progression

Progression of the disease/disorder being studied, assessed by measurement of lesions on radiographs or other methods as well as associated clinical signs or symptoms (including laboratory abnormalities) should not be reported as an (S)AE, unless 1) the subject's general condition is more severe than expected for the participant's condition and/or 2) unless the outcome is fatal within the adverse event reporting period and/or 3) the condition is at least possibly attributed to therapy.

6.3 DATA SHARING PLANS

What data will be shared?

I will share human data generated in this research for future research as follows:

X Coded, linked data in an NIH-funded or approved public repository (Clinicaltrials.gov).

X Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

X Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

X An NIH-funded or approved public repository. Insert name or names: clinicaltrials.gov.

X BTRIS (automatic for activities in the Clinical Center)

X Approved outside collaborators under appropriate individual agreements.

X Publication and/or public presentations.

When will the data be shared?

X At the time of publication or shortly thereafter.

6.4 RESPONSE CRITERIA

In this study, in addition to lymphoma response criteria, 3 additional possible response criteria are being evaluated. The response evaluation schedule and criteria are as follows:

6.4.1 NCI Criteria for Primary Effusion Lymphoma

Subjects will be evaluated for response on day 1 of cycle 3 (±7 days), and at the end of DA-EPOCH-R^2 therapy, and at 3 months follow up. Lymphoma will be based on a modification of the International Working Group Response Criteria for Malignant Lymphoma[83] that utilizes^{18}FDG positron emission tomography /computerized tomography, but also takes into account evaluation of effusions in KSHV-associated diseases as described below. Pathologic documentation of lymphoma will require cytopathologic/histopathologic evidence of disease. Flow cytometry for KSHV-associated lymphomas have not been validated, and therefore flow cytometry will not be used alone in assessment of response, but along with KSHV viral load and Ig PCR, will be considered an adjuvant tool for assessing disease status. Novel flow cytometry methods will be explored, and the protocol may be amended to include flow cytometry in future response criteria.

6.4.1.1 Disease Parameters

Measurable sites of disease are defined as lymph nodes, lymph node masses, or extranodal discrete masses that are sites of lymphoma. Each measurable site of disease must be greater than 1.5 cm in the long axis regardless of short axis measurement or greater than 1.0 cm in the short axis regardless of long axis measurement, and clearly measurable in 2 perpendicular dimensions. Measurement must be determined by imaging evaluation. All other sites of disease are considered assessable, but not measurable.

Up to 6 measurable sites of disease, clearly measurable in 2 perpendicular dimensions, will be followed for each subject. Measurable sites of disease should be chosen such that they are representative of the subject's disease (this includes splenic and extranodal disease). If there are lymph nodes or lymph node masses in the mediastinum or retroperitoneum larger than 1.5 cm in 2 perpendicular dimensions, at least 1 lymph node mass from each region should always be included. In addition, selection of measurable lesions should be from as disparate regions of the body as possible.

All other sites of disease will be considered assessable. Assessable disease includes objective evidence of disease that is identified by radiological imaging, physical examination, or other procedures as necessary, but is not measurable as defined above. Importantly, this will include *effusions*. Other examples of assessable disease include bone lesions; mucosal lesions in the gastrointestinal tract; *pleural, peritoneal, or bowel wall thickening*; disease limited to bone marrow; and groups of lymph nodes that are not measurable but are thought to represent lymphoma. In addition, if more than 6 sites of disease are measurable, then these other sites of measurable disease may be included as assessable disease. Special considerations for evaluation of effusions include:

- Volumetric measurements of drainage, if applicable
- Pleural effusions should be evaluated by decubitus X-Ray as well as other appropriate imaging. If fluid is not free-flowing, it will be noted to be loculated
- **Radiographic evidence of effusions is inadequate evidence of lymphoma**. Effusions must be evaluated cytopathologically. Additional evaluations including effusion chemistries, cell counts, flow cytometry, and KSHV viral load, and Ig PCR may be employed.

6.4.1.2 Complete Response

For CR determination, all the following criteria must be met:

1. Complete disappearance of all detectable evidence of disease and disease-related symptoms, if present before therapy.
2. All lymph nodes and nodal masses must have regressed on CT to normal size (≤1.5 cm in the greatest transverse diameter [GTD] for nodes >1.5 cm before therapy, regardless of the short axis). Previously involved nodes that were between 1.1 cm and 1.5 cm in the long axis and more than 1.1 cm in the short axis before treatment must have decreased to ≤1.0 cm in the short axis after treatment. All splenic and hepatic nodules and other extranodal disease must have disappeared.
3. PET scan must be negative (for the combined CT+PET assessment of CR). A post-treatment residual mass of any size is permitted as long as it is PET-negative.
4. The spleen and/or liver, if enlarged before therapy on the basis of physical examination or CT scan, should not be palpable on physical examination and should be considered normal size by imaging studies.
5. Bone marrow biopsies are not required for staging. If a bone marrow biopsy is done for clinical purposes, and if the bone marrow was involved with lymphoma before treatment, the infiltrate must have cleared on repeated bone marrow biopsy. The biopsy sample on which this determination is made must be adequate (with a goal of >20 mm unilateral core). If a sample is intermediate by morphology, it should be negative by IHC (if bone marrow was involved before therapy and a radiological CR was achieved, but with no bone marrow assessment after treatment, the response should be classified as a PR.).
6. If the CSF was involved at baseline, repeat CSF must show no cytopathology evidence of disease
7. No new sites of disease are detected during assessment.

6.4.1.3 Complete Response, unconfirmed (CRu)

(Modification specific to abnormalities noted in KSHV-associated lymphomas and other KSHV-associated malignancies)

1. Participants who otherwise meet criteria for CR, but who have residual effusions *with no cytopathologic evidence of disease* will be classified as CRu. Such participants will be reclassified as CR if effusions subsequently resolve; or if they persist, but have no cytopathologic evidence of lymphoma with 12 months follow up off therapy.
2. Participants who otherwise meet criteria for CR, but who have residual splenic abnormalities on CT or residual bone lucencies, but no corresponding FDG-PET abnormalities will be classified as CRu. Such participants will be reclassified as CR if splenic abnormalities resolve; or if they persist, but have no pathologic evidence of lymphoma with 12 months follow-up off therapy.

6.4.1.4 Partial Response

For PR determination, all the following criteria must be met:

1. A ≥50% decrease in the sum of the product of the diameters (SPD) of up to 6 of the largest dominant nodes or nodal masses.
2. No increase should be observed in the size of other nodes, liver, or spleen, meeting the criteria for PD.
3. Splenic and hepatic nodules must regress by ≥50% in the SPD or, for single nodules, in the GTD.
4. With the exception of splenic and hepatic nodules, other organs should not have any measurable disease.
5. Bone marrow assessment is not required for PR determination.
6. No new sites of disease should be observed.
7. At least 1 PET-positive site of disease (required for the CT+PET assessment of PR) unless effusions are the evaluable site of disease.
8. For participants with effusions, at least 50% decrease in KSHV copy numbers per 10^6 mononuclear cells

6.4.1.5 Stable disease

Stable disease is defined as:

1. A subject is considered to have stable disease when he or she fails to attain the criteria needed for a CR or PR, but does not fulfill those for PD.
2. The PET should be positive at, at least 1 previously involved site of disease, with no new areas of lymphoma involvement on the post-treatment CT or PET (for the combined CT+PET assessment of stable disease) OR in participants with effusions, PET may be negative, but there is persistent cytopathologic evidence of KSHV-associated lymphoma. Not meeting criteria for partial response based on KSHV-viral load.

6.4.1.6 Progressive Disease or Relapsed Disease

Progressive disease or relapsed disease (after CR) is defined as:

Lymph nodes should be considered abnormal if the long axis is ≥1.6 cm, regardless of the short axis length. If a lymph node has a long axis from 1.1 cm to 1.5 cm, it should be considered

abnormal only if its short axis is >1.0 cm. Lymph nodes ≤1.0 cm x ≤1.0 cm will not be considered abnormal for the assessment of PD/relapsed disease.

1. Appearance of any new nodal lesion ≥1.6 cm in GTD or ≥1.1 cm in short axis during or after the end of therapy, even if other lesions are decreasing in size.
2. Appearance of any new unequivocal extra-nodal lesion measuring >1.0 cm in GTD, not thought to be benign by the reviewer, even if other lesions are decreasing in size.
3. At least a 50% increase from the nadir in the SPD of any previously involved nodes, or in a single involved node, or in the size of other lesions (e.g., splenic or hepatic nodules). To be considered PD, a lymph node with a diameter of the short axis of <1.0 cm must increase by ≥50% and to a size of 1.5 x 1.5 cm or more than 1.5 cm in the long axis.
4. At least a 50% increase from the nadir in the longest diameter of any single previously identified node more than 1 cm in its short axis.

For the combined CT+PET assessment of PD, lesions should be PET-positive or the lesion was PET-positive before therapy unless the lesion was too small to be detected with current PET systems (smaller or equal to 1.5 cm in the long axis by CT). Any previously involved FDG-positive site that became negative and subsequently became positive will be considered PD. Increased FDG uptake in a previously unaffected site should only be considered PD after confirmation with other modalities.

Pathology confirmation of KSHV-associated lymphoma is required when there is an appearance on CT of a new lesion ≥1.5 cm in its long axis and is PET-negative.

For effusions, cytopathology confirmation of lymphoma is required. For disease to be considered progressive in participants with persistent effusions, they must meet all the following criteria:

- 25% increase in KSHV copy numbers per 10^6 mononuclear cells
- Cytopathologic evidence of progressive disease (at least 25% increase in estimate of percent of total
- Worsening clinical status by KICS or KSHV-MCD criteria (See **6.4.2** and **6.4.3**)
- Persist one cycle

New effusions with pathologic evidence of lymphoma that persist at least 1 cycle

6.4.2 KSHV-MCD

Subjects with KSHV-MCD will be evaluated on day 1 (±5 days) of each cycle and during long term follow up using KSHV-MCD Clinical Benefit Response Criteria that has been developed as a tool for assessment of participants receiving therapy for KSHV-MCD, and does not depend on radiographic findings. As such, it allows for same-day assessment of clinical benefit utilizing common symptoms and clinical laboratories. To evaluate clinical benefit in participants with KSHVMCD receiving therapy, clinical and laboratory responses are assessed in aggregate, and compared to baseline. These criteria allow for assessment of participants who may be deriving meaningful clinical benefit from therapy, but have not necessarily achieved an overall complete response by the original NCI KSHV-MCD response criteria.

Evaluation of KSHV-MCD is complicated by the heterogeneous and non-specific nature of many symptoms and signs of KSHV-MCD and the common intercurrence of other pathologies in this group. For this reason, assessment of responses other than complete response is made on the basis

of eight *indicator abnormalities* (four symptom groups and four laboratory parameters) that are most closely associated with disease activity:

Indicator abnormalities assessed in responses	
Symptoms	**Laboratory abnormalities**
Fatigue (includes lethargy)	Thrombocytopenia
Gastrointestinal (includes nausea and anorexia, altered bowel habit and abdominal discomfort)	Anemia
Respiratory (includes airway hyperreactivity, dyspnea and cough)	Hypoalbuminemia

For the purposes of response assessment, clinical symptoms attributed to MCD will be assigned an NCI-CTCAE grade equivalent, with response assessment based on changes in grade severity or symptom resolution.

Increases in hemoglobin in participants who have received a transfusion do not count towards PR or CR for 3 weeks, and increases in albumin or platelet count in participants who have received a transfusion do not count towards PR or CR for 7 days.

6.4.2.1 KSHV-MCD Clinical Benefit Response Criteria

Complete Response (CR)

Full resolution of all clinical symptoms and laboratory abnormalities (whether or not these are indicator abnormalities) probably or definitely attributable to MCD, lasting at least 3 weeks.

Partial Response (PR)

PR is assessed based on the eight indicator abnormalities above. At least 50% of the abnormalities probably or definitely attributed to KSHV-MCD must improve by the minimum amounts specified below to attain PR.

Only abnormalities present in a specific participant at baseline may count toward the achievement of a PR (e.g. if six of indicator abnormalities are present at baseline, at least three must meet the specified criteria to be considered a PR).

Improvement in symptoms require at least 1 CTCAE grade equivalent improvement. For symptom groups (e.g. gastrointestinal and respiratory), where multiple symptoms within the group are present at least half of those attributable to KSHV-MCD must improve by at least 1 CTCAE grade equivalent to consider the group as a whole to be improved.

Improvement for each laboratory parameters requires either normalization of the lab value or else the following:

- C-reactive protein reduction to ≤50% of baseline
- Hemoglobin increment 2g/dL not explained by transfusion
- Platelet increment ≥50,000/mm^3 not explained by transfusion
- Albumin increment ≥1g/dL not explained by transfusion

To be considered a partial response, there may be no new indicator abnormalities probably or definitely attributed to KSHV-MCD; no indicator symptom may worsen by ≥1 CTCAE grade

equivalent; and no indictor laboratory abnormality may worsen by the amount given in the criteria for progressive disease.

Stable disease (SD)

No change in signs and symptoms of KSHV-MCD that meet criteria for any of CR, PR or PD.

Progressive disease (PD)

PD is assessed based on the eight indicator abnormalities above. At least two indicator abnormalities must deteriorate by the minimum amounts specified below to constitute PD. The development of new indicator abnormalities not present in a specific participant at baseline is incorporated in the assessment of PD

Deterioration in signs and symptoms require at least 1 CTCAE grade equivalent increase in severity. For symptom groups (e.g. gastrointestinal and respiratory), where multiple symptoms within the group are present at least half of those attributable to KSHV-MCD must increase in severity by at least 1 CTCAE grade equivalent to consider the group as a whole to have deteriorated.

Deterioration for each laboratory parameter requires an abnormal laboratory value meeting the following criteria:

- C-reactive protein increase by ≥50% of baseline (or the upper limit of normal, whichever is greater)
- Hemoglobin decrement 2g/dL not otherwise explained
- Platelet decrement ≥25,000/mm^3 not otherwise explained
- Albumin decrement ≥0.5g/dL

6.4.3 Evaluation of KSHV-associated Inflammatory Cytokine Syndrome (KICS)

Subjects meeting criteria for KICS will be evaluated on day 1 (±5 days) of each cycle and during long term follow up using NCI KICS Clinical Benefit Response criteria

The evaluation of the response of KICS to an agent or regimen is difficult to grade by means of commonly used oncologic definitions. The following clinical benefit response criteria were therefore devised, based on similar HAMB criteria for clinical benefit response assessment in KSHV-MCD, to allow streamlined same-day assessment of clinical benefit for participant receiving therapy for KICS utilizing common symptoms and laboratory tests.

Clinical and laboratory responses are assessed in aggregate, and compared to baseline. This allows for assessment of participants who may be deriving meaningful clinical benefit from therapy, but have not necessarily achieved a complete response. We anticipate that the results obtained will shed light on the benefits attained with therapy and also provide guidance to refine response criteria in subsequent trials in this disease.

Evaluation of KICS is complicated by the heterogeneous and non-specific nature of many of its symptoms and signs and the common intercurrence of other pathologies in this group. For this reason, assessment of responses other than complete response is made on the basis of eight *indicator abnormalities* (four symptom groups and four laboratory parameters) that are currently considered to be most closely associated with disease activity, and which form the basis for initiating protocol therapy:

6.4.3.1 Indicator Abnormalities Assessed in KICS Responses

Symptoms Laboratory Abnormalities

- Fever (temperature ≥38°C; includes chills and rigors)
- Elevated C-reactive protein
- Fatigue (includes lethargy)
- Thrombocytopenia
- Gastrointestinal (includes nausea and anorexia)
- Anemia
- Respiratory (includes airway hyperreactivity and cough)
- Hypoalbuminemia

6.4.3.1.1 Clinical benefit response will be evaluated at the beginning of each cycle and each follow-up visit. The baseline for response criteria evaluations will be based on the Day 1 Cycle 1 evaluation for clinical and laboratory parameters for any given therapy. Any new abnormalities that are probably or definitely attributed to KICS and that occur between baseline and the end of the final cycle of a given therapy must also resolve in order to be considered a complete response. Only those abnormalities attributed as probably or definitely related to the disease will be included in the response assessment.

6.4.3.1.2 For the purposes of response assessment, clinical symptoms attributed to KICS will be assigned an NCI-CTCAE grade equivalent, with response assessment based on changes in grade severity or symptom resolution.

6.4.3.1.3 Increases in hemoglobin in participants who have received a transfusion do not count towards PR or CR for 3 weeks, and increases in albumin or platelet count in participants who have received a transfusion do not count towards PR or CR for 7 days.

6.4.3.1.4 Best clinical and laboratory response and end-of therapy clinical and laboratory response will be described for each subject.

6.4.3.1.5 The frequency of associated radiographic abnormalities (lymphadenopathy, splenomegaly, hepatomegaly or effusions) and the clinical importance, if any, of resolution of these abnormalities in treating KICS remains to be defined. For this reason, while participants with radiographic abnormalities will undergo response assessment at each CT scan as described below, radiographic responses (best and end-of-therapy) will be reported for each applicable subject independent of clinical and laboratory response.

6.4.3.1.6 Other biological markers (clinical and research KSHV viral load, cytokines including vIL-6 and hIL-6, immunoglobulins and serum free light chains) will be assessed for research purposes but will not constitute a component of response assessment.

6.4.3.2 Clinical Benefit Response Definitions

Complete Response (CR)

Full resolution of all clinical symptoms and laboratory abnormalities (whether or not these are indicator abnormalities) probably or definitely attributable to KICS, lasting at least 3 weeks.

Partial Response (PR)

assessed based on the eight indicator abnormalities above. At least 50% of the abnormalities probably or definitely attributed to KICS must improve by the minimum amounts specified below to attain PR. Only abnormalities present in a specific participant at baseline may count toward the

achievement of a PR (e.g. if six indicator abnormalities are present at baseline, at least three must meet the specified criteria to be considered a PR).

Improvement in symptoms require at least 1 CTCAE grade equivalent improvement. For symptom groups (e.g. gastrointestinal and respiratory), where multiple symptoms within the group are present at least half of those attributable to KICS must improve by at least 1 CTCAE grade equivalent to consider the group as a whole to be improved.

Improvement in for each laboratory parameters requires either normalization of the lab value or else the following:

- C-reactive protein reduction to ≤50% of baseline
- Hemoglobin increment 2g/dL not explained by transfusion
- Platelet increment ≥50,000/mm^3 not explained by transfusion
- Albumin increment ≥1g/dL not explained by transfusion

To be considered a partial response, there may be no new indicator abnormalities probably or definitely attributed to KICS; no indicator symptom may worsen by ≥1 CTCAE grade equivalent; and no indictor laboratory abnormality may worsen by the amount given in the criteria for progressive disease.

Stable disease (SD)

No change in signs and symptoms of KICS that meet criteria for any of CR, PR or PD.

Progressive disease (PD)

PD is assessed based on the eight indicator abnormalities above. At least two indicator abnormalities must deteriorate by the minimum amounts specified below to constitute PD. The development of new indicator abnormalities not present in a specific participant at baseline is incorporated in the assessment of PD

Deterioration in signs and symptoms require at least 1 CTCAE grade equivalent increase in severity. For symptom groups (e.g. gastrointestinal and respiratory), where multiple symptoms within the group are present at least half of those attributable to KICS must increase in severity by at least 1 CTCAE grade equivalent to consider the group as a whole to have deteriorated.

Deterioration for each laboratory parameter requires an abnormal laboratory value meeting the following criteria:

- C-reactive protein increase by ≥50% of baseline.
- Hemoglobin decrement 2g/dL not otherwise explained (for example, by intercurrent bleeding).
- Platelet decrement ≥25,000/mm^3 not otherwise explained.
- Albumin decrement ≥0.5g/dL.

6.4.3.3 Radiographic Response Definitions

The lymphoma response criteria outlined in Section **6.4.1** will be utilized in this study.

6.4.4 Kaposi's Sarcoma

Subjects will be assessed according to the response schedule established in the Study Calendar (Section **3.4**). Kaposi's sarcoma will be evaluated using a modified version (consistent with other

HAM Branch studies) of the AIDS Clinical Trial Group Oncology Committee staging and response definitions for Kaposi's sarcoma[95]. It should be noted that there is some observer variability in the evaluation of the number, size, nodularity, and color of lesions, and this must be taken into account when measurements are interpreted.

- For evaluation of less than complete responses in subjects with more than 50 lesions at entry, only the previously selected 1 - 3 representative areas that contain at least 20 lesions will be considered. However, complete responses still require the absence of any detectable disease over the entire body (i.e. not confined to the representative areas).

6.4.4.1 Methods of Evaluation for Measurable Disease

6.4.4.1.1 KS Tumor Photography

Scheduled as per study calendar (Section **3.4**)

Whole body photographs will be obtained upon entry into the study. At this time, 5 lesions (hereafter called marker lesions), representative of the participant's disease and, if possible, located on separate areas of the body will be selected. These marker lesions should be lesions that have never been treated with local therapies such as radiation therapy or intralesional injections. An attempt will be made to distribute the "marker" lesions between the representative areas (described below in Section **6.4.4.1.3.1**) and the rest of the body. Detailed photographs of these lesions will be obtained with a metric rule beside them.

6.4.4.1.2 Documentation of Marker Lesions

Scheduled as per study calendar (Section **3.4**)

The size, color and nodularity of the marker lesions will be recorded. Documentation will depend on the number of lesions.

6.4.4.1.3 Documentation of Extent of Disease

Scheduled as per study calendar (Section **3.4**)

6.4.4.1.3.1 *Subjects with 50 or more KS lesions:* for subjects with 50 or more lesions at entry, between 1 and 3 representative areas will be selected at baseline and these will be used for each subsequent evaluation. Representative areas are sections of the body (e.g. the back, a leg, an arm, etc.), which contain at least 20 KS lesions. The total number of lesions in these representative areas will be counted and a record made of whether they are flat or raised. If, in the course of treatment, a single lesion breaks up into 2 or more smaller lesions whose area does not extend beyond the boundary of the initial lesion, these lesions will still be counted as single lesions for the purpose of assessing total numbers in defining a response to therapy.

6.4.4.1.3.2 *Subjects with fewer than 50 KS lesions:* for subjects with less than 50 lesions at entry, the total number of lesions will be counted and a record made of whether they are flat or raised.

6.4.4.1.4 Additional studies for visceral KS involvement: additional studies, including but not limited to, gastrointestinal endoscopy and bronchoscopy will be performed at baseline where clinically indicated, based on clinical evaluation of the participant. Abnormal studies will be repeated at the end of EPOCH-R².

6.4.4.2 KS Response Criteria

6.4.4.2.1 Complete Response

- The absence of any detectable residual disease, including tumor associated edema, persisting for at least 4 weeks.
- For subjects with pigmented macular skin lesions persisting after apparent complete response, a biopsy of at least one representative lesion is required to document the absence of malignant cells. If a lesion has not been biopsied, the participant may be classified as having a clinical CR.
- For subjects with visceral disease, the diagnostic radiologic or endoscopic study should be repeated if not medically contraindicated and found to be negative for evidence of disease. If such procedures are medically contraindicated but the participant has no clinical evidence of visceral disease, the participant may be classified as having a clinical CR.

Clinical Complete Response

- The absence of any detectable residual disease, including tumor associated edema, persisting for at least 4 weeks.
- For subjects with pigmented macular skin lesions persisting after apparent complete response, if a representative lesion has not been biopsied.

For subjects with visceral disease, the diagnostic radiologic or endoscopic study should be repeated if not medically contraindicated and found to be negative for evidence of disease. If such procedures are medically contraindicated but the participant has no clinical evidence of visceral disease, the participant may be classified as having a clinical CR.

6.4.4.2.2 Partial Response

- No progressive disease (see below and noting, that single lesions which split up into 2 or more smaller lesions during the course of treatment will still be counted as one); no new lesions occurring in previously uninvolved areas of the body; no new visceral sites of involvement or the appearance or worsening of tumor-associated edema or effusions and:
- A 50% or greater decrease in the number and/or size of previously existing lesions lasting for at least 4 weeks *or*
- Complete flattening of at least 50% of all previously raised lesions (i.e., 50% of all previously nodular or plaque-like lesions become macular) lasting for at least 4 weeks *or*
- A 50% decrease in radiologically measurable visceral lesions sustained without evidence of re-growth for at least 4 weeks *or*
- A 50% decrease in radiologically measurable visceral lesions sustained without evidence of re-growth for at least 4 weeks *or*
- Subjects who otherwise meet the criteria for a CR but still have residual tumor-associated edema or effusions will be classified as having a PR.

6.4.4.2.3 Progressive Disease

- For those criteria that involve measurement of lesions in the clinic, the designation of progression should be made, when feasible, only when the criteria below have been met in two measurements spaced at least 1 week apart. For the assignment of progressive disease

for the primary outcome analysis, progression will be defined in comparison to baseline measurements.

- An increase of 25% or more over baseline in the number of lesions and/or the size (sum of the products of the largest perpendicular diameters) of the marker lesions *or*
- A change in character from macular to plaque-like or nodular of at least 25% of the lesions *or*
- New visceral sites of involvement or progression of visceral disease *or*
- The development of new or increasing tumor-associated edema or effusion that lasts at least 1 week and interferes with the participant's normal activities.

  Stable Disease
- Any tumor measurement not meeting the criteria for Complete Response, Partial Response, or Progressive Disease.

6.4.5 Definitions

Evaluable for toxicity: All participants will be evaluable for toxicity from the time of their first treatment with lenalidomide or modified DA-EPOCH-R^2 (whichever occurs first).

Evaluable for objective response: Only those participants who have received at least two cycles of modified DA- EPOCH-R^2, and have had their lymphoma re-evaluated will be considered evaluable for response. (Note: Participants who exhibit objective disease progression prior to the end of cycle 2 will also be considered evaluable.)

6.4.6 Duration of Responses

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). This will be measured primarily for KSHV-lymphoma, but also for KS, KSHV-MCD, and KICS separately as indicated based on baseline assessment.

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease in subjects mot achieving a PR or better, measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements. If applicable, this can be used to describe KSHV-lymphoma, KS, KICS or KSHV-MCD criteria.

6.4.7 Overall Survival

Overall Survival (OS) is defined as the duration of time from start of treatment to time of progression of KSHV-lymphoma or death, whichever occurs first.

6.4.8 Progression-Free Survival

Progression-Free Survival (PFS) is defined as the duration of time from start of treatment to time of progression of KSHV-lymphoma or death, whichever occurs first.

6.4.9 Event-Free Survival

Event-Free Survival (EFS) is defined as the duration of time from start of treatment to time of progression of KSHV-lymphoma

6.5 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each participant while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when participants are being actively treated on the trial to discuss each participant. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior participants.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each participant to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR PROTOCOL SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see Section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32).

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to Section **6.2.3.3**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in Section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Reporting pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.4.1 Maternal exposure

If a participant becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy becomes known.

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (Section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies should be followed up and documented.

8.4.2 Paternal exposure

Male participants should refrain from fathering a child or donating sperm during the study and for 28 days after the last dose of Lenalidomide.

Pregnancy of the participant's partner is not considered to be an AE. The outcome of all pregnancies occurring from the date of the first dose until 28 days after the last dose should, if possible, be followed up and documented. Pregnant partners may be offered the opportunity to participate in an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy for safety reporting purposes.

8.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

All events listed below must be reported in the defined timelines to OSROSafety@mail.nih.gov.

CCR OSRO will send all reports to the manufacturer as described below.

8.5.1 Serious Adverse Events

Serious adverse events (SAE) are defined above. If any SAE is required to be reported to the sponsor according to Section **8** (i.e. within 28 days of receiving drug or assessed as being possibly related to drug administration), the sponsor should inform Celgene of the SAE within 1 business day of being aware of the event. This must be documented on a Celgene SAE form or FDA 3500A or MEDWATCH form. This form must be completed and supplied to Celgene within 24 hours/1 business day. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the investigational product(s), if available. Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up MEDWATCH. A final report to document resolution of the SAE is required.

The Celgene tracking number (RV-CL-OTHER-PI-007819) and the institutional protocol number should be included on SAE reports (or on the fax cover letter) sent to Celgene. A copy of the fax transmission confirmation of the SAE report to Celgene should be attached to the SAE and retained with the participant records.

All adverse experience reports must include the participant number, age, sex, weight, severity of reaction (e.g. mild, moderate, severe), relationship to drug (e.g. probably related, unknown relationship, definitely not related), date and time of administration of test medications and all concomitant medications, and medical treatment provided. The investigator is responsible for evaluating all adverse events to determine whether criteria for "serious" and as defined above are present. The investigator is responsible for additional reporting of certain adverse events to Celgene as described below.

In addition, if new primary malignancies that do not fall under the criteria for expedited reporting in Section **7.2.1** are reported to the sponsor, the sponsor should inform Celgene of the malignancy within 7 working days of receiving the report using the same forms.

8.5.2 IND Annual Reports

A copy of the FDA Annual Report should be provided to Celgene Corporation as a supporter of this study as follows.

Celgene Corporation
Attn: Medical Operations
86 Morris Avenue
Summit, NJ 07901
Tel: (908) 673-9000

8.5.3 Pregnancies

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on IP, or within 28 days of the subject's last dose of lenalidomide, are considered immediately reportable events. Lenalidomide is to be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to Celgene Drug Safety immediately by facsimile (See contact information below), or other appropriate method, using the Pregnancy Initial Report Form, or approved equivalent form.

The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the female subject until completion of the pregnancy, and must notify Celgene Drug Safety immediately about the outcome of the pregnancy (either normal or abnormal outcome) using the Pregnancy Follow-up Report Form, or approved equivalent form.

If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE to Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to the IP should also be reported to Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

Male Subjects

If a female partner of a male subject taking investigational product becomes pregnant, the male subject taking Lenalidomide should notify the Investigator immediately, and the pregnant female partner should be advised to call their healthcare provider immediately.

Celgene Drug Safety Contact Information:

Celgene Corporation
Global Drug Safety and Risk Management
86 Morris Avenue
Summit, NJ 07901
Fax: (908) 673-9115
E-mail: drugsafety@celgene.com

8.6 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and,
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

The primary objective of phase I is to determine the maximum tolerated dose and/or recommended phase II dose of lenalidomide in combination with DA-EPOCH-R. We expect dose level 1 will be tolerable, in which case there will be 6 participants treated in Phase I. However, should dose de-escalation be required, with three dose levels and 3-6 participants per dose level, it is possible that 6-18 participants may be required in phase I.

10.1 SAMPLE SIZE FOR PHASE II

The primary objective of phase II of this study is to determine in a pilot fashion if the proposed regimen of 6 cycles of lenalidomide in combination with, dose-adjusted etoposide, prednisone,

vincristine, cyclophosphamide, doxorubicin, and rituximab (DA- EPOCH-R^2) is able to be associated with a one-year overall survival in participants with PEL and no previous lymphoma therapy, which is consistent with a rate suggesting a potential improvement in this therapy compared to that experienced by other similar participants. Data from prior trials of participants with PEL treated with anthracycline-based chemotherapy indicate median survivals less than 6 months, which would translate to a 12-month survival of <25% assuming exponential failure curves.

This pilot study will use a standard approach for determining the sample size for a single arm survival curve based on comparison to a stated fixed null value, such as 1 year survival[96]. If 15 evaluable participants were enrolled over 36 months and there was an additional 12 months of follow-up after the last participant has enrolled, a one-tailed 0.10 alpha level test would have 80% power to determine if a single arm OS curve would demonstrate a result consistent with 45% or better OS at 12 months and ruling out 20% or worse OS at 12 months.

It is anticipated that 1-2 participants with PEL per year may enroll on this study and thus approximately 3-6 years will be required to enroll participants for the Phase I part of this study, and 9-12 years will be required in order to enroll 15 evaluable participants for the Phase II part of the study. In order to allow for a small number of inevaluable participants, the accrual ceiling for the Phase II PEL cohort will be set at 18.

Treatment naïve participants with PEL treated in phase I at the Phase II dose will be considered evaluable for OS in the phase II cohort, which may potentially decrease accrual time. The number of participants accrued during Phase II may therefore range from 9 to a ceiling of 18 (if no Phase I subjects are evaluable for the primary outcome, and maximal numbers of participants are accrued to the additional cohorts.

Overall, we expect this study to require 15-24 subjects (6+9 to 6+18). The phase II PEL requirement is 15 participants, but up to 18 are allowed to account for inevaluable participants. With up to 18 participants in phase I and 18 in phase II, the overall accrual ceiling will be set at 36 participants.

Given 15 participants with KSHV-associated lymphomas seen by the HAMB in the past 4 years even without a prospective treatment study, and no competing studies, we believe this is an achievable goal. With an open study and promotion, we hope for a more rapid accrual.

Early stopping rule: Although this is a limited size trial, we have included the following stopping rule in order to avoid over-accruing participants in the event that the combination is not effective. Based on the enrollment of 10 treatment naïve PEL participants, treated at the Phase II dose (including those that were treated in the phase I portion of the study), and potentially followed for 12 months from their date of enrollment, if fewer than 3 participants have completed DA-EPOCH-R^2 and survived to 12 months, no further participants will be enrolled. This stopping rule will apply as soon as this can be determined, and accrual does not need to be held due to inadequate follow up time of these first 8-10 participants. Depending on the accrual rate and the times at which deaths are noted, this may mean that accrual could end when as few as 8 participants have been enrolled (if none of the 8 survive to 12 months and accrual is slow) or as many as 12-13 have enrolled (if participants continue to enroll at a rate of 5 per year and whether an adequate number who have survived one year is not known until the 10th participant has been potentially followed for up to a year, and 2-3 more have entered). It is expected that a majority of the participants accrued will be diagnosed as PEL.

Additional endpoints: Response rates and PFS will also be estimated and reported using appropriate 90% and 95% confidence intervals as secondary endpoints.

A variety of correlative parameters will also be obtained and compared longitudinally to baseline values. These comparisons will be made using non-parametric tests and the results of these tests will be presented without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENTS

11.1 AGREEMENT TYPE

The study agent, Lenalidomide, is obtained under CRADA #3095 with Celgene.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

The protocol is designed for analysis of adult subjects with KSHV associated lymphomas, primary effusion lymphoma or large cell lymphoma arising in the setting of KSHV associated MCD. KSHV infection in the US is associated with certain ethnic and behavioral groups, including African immigrants and men who have sex with men (MSM)[97-100]; most of the women who have participated in previous HAMB studies of KSHV-associated diseases have been immigrants from Africa. KS and other KSHV-associated diseases are much more common in subjects with intercurrent immunodeficiencies, including HIV, and it is anticipated that the majority of study participants will be drawn from these groups. Including both HIV-infected and HIV-uninfected individuals may enable a greater understanding of the similarities and differences of lenalidomide safety, toxicity and response in these groups.

Strategies for recruitment will include announcements on www.clinicaltrials.gov, letters to referring physicians, targeting HIV providers and those who provide primary care to the African immigrant community, and AIDS treatment bulletins, and internet based recruitment strategies.

12.2 PARTICIPATION OF CHILDREN

Children are not included in this research protocol as the diseases under study are rare in children in the United States and there are currently no dosing or adverse event data available for the use of lenalidomide in combination with EPOCH-R in this population.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **12.5**), all subjects ≥ 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study.

Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for to assess ongoing capacity of the subjects and to identify an LAR, as needed.

12.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The investigational nature and objectives of this study, the procedures and treatments involved and their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained to the participant or the participant's surrogate, and a signed informed consent document will be obtained.

While the study is performed based on background data suggesting that DA-EPOCH-R^2 may be of benefit to participants with primary effusion lymphoma or large cell lymphoma arising in the setting of KSHV associated MCD, there is no assurance that this will be the case and it is quite possible that there may be no direct benefit to the participant volunteers on this study. The potential benefit is that the protocol therapy may result in control of lymphoma and resolution of symptoms in those participants to whom it is administered.

The potential risk for participants receiving treatment is that the protocol therapy may be toxic, ineffective, or both. In addition, the study requires multiple, sometimes invasive, evaluations. The time commitment and potential morbidity associated with tumor cytopathology as well as blood sampling is a potential risk and discomfort to study participants. Risks will be carefully monitored throughout the duration of the study.

12.5 RISKS/BENEFITS ASSESSMENT

12.5.1 Known Potential Risks

The risks to individual study subjects are reasonable in relation to the anticipated benefits. This protocol explores a combination therapy regimen with a strong preclinical rationale in treating KSHV associated lymphomas, which includes lenalidomide, a derivative of another agent (thalidomide) that was shown to have promising clinical activity in our prior study in KS as well as DA-EPOCH-R which has been extensively studied at the NCI. DA-EPOCH-R has an acceptable toxicity profile at similar or higher doses to that used here in studies in other solid tumors, and promising clinical activity in other solid tumors and hematologic malignancies. Potential therapeutic benefits include tumor regression, and additional therapeutic options for participants for difficult to manage KSHV related lymphoma. The risks include potential toxicities of lenalidomide alone or in combination with HIV and its therapies and additional studies associated with participation in a clinical study. The comparative risks and benefits are acceptable for an early phase clinical study, when compared to the alternatives for participants KSHV related lymphomas.

12.5.1.1 Study drugs

Potential adverse reactions attributable to the administration of the study drugs utilized in this trial are discussed in Section **14**.

12.5.1.2 Saliva collection

No physical risks are associated with saliva collection.

12.5.1.3 Blood draws

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting. Up to 138 mL of blood may be collected at any visit but no more than 550 ml in an 8 week period.

12.5.1.4 Urine collection

No physical risks are associated with urine collection.

12.5.1.5 Stool collection

No physical risks are associated with stool collection.

12.5.1.6 EKG

Side effects of EKG are skin irritation where EKG electrodes are placed.

12.5.1.7 Echocardiogram

Side effects of an echocardiogram are discomfort from the transducer being firmly placed against the chest.

12.5.1.8 Ophthalmology exam

Risks of complication may include rare reactions to eye drops that may cause dry mouth, flushing, dizziness, nausea, vomiting, or narrow-angle glaucoma.

12.5.1.9 Pulmonary function test (PFTs)

PFTs are usually safe for most people. Risks of complication include dizziness, asthma attack, or collapsed lung.

12.5.1.10 Endoscopy/bronchoscopy

Risks of endoscopy include infection, bleeding, or perforation of the duodenum, esophagus, or stomach.

Risks of bronchoscopy include bleeding, infection, bronchial perforation, bronchospasm, laryngospasm, pneumothorax.

12.5.1.11 Bronchoalveolar Lavage

There are very few side effects related to this procedure. In rare instances, participants have developed cough, temporary chills, fevers and muscle aches and a temporary decrease in lung function.

12.5.1.12 Effusion collection

Side effects of pleural effusion collection include pain, bleeding, bruising in the area where the needle or tube was inserted or lung collapse.

12.5.1.13 Bone marrow biopsy and aspiration

Bone marrow biopsy is minimally invasive and is typically a very safe procedure. Usually hipbone is numbed with anesthesia. Using a needle, the solid and liquid portion of bone marrow is taken out. This procedure causes some pain. Very rarely, infection or bleeding may occur at the needle site.

12.5.1.14 Lumbar puncture

Lumbar puncture is a relatively safe procedure. Side effects may include pain, bleeding, brainstem herniation, post-procedure headache.

12.5.1.15 Local anesthesia

Biopsy may be done under local anesthesia. Potential side effects of local anesthesia include drowsiness, headaches, blurred vision, twitching muscles or shivering, continuing numbness, weakness or pins and needles sensation.

12.5.1.16 Conscious sedation

Some procedures may be performed under sedation. The common side effects of conscious sedation include drowsiness, delayed reflexes, hypotension, headache, and nausea. These are generally mild and last no more than a few hours.

12.5.1.17 Imaging

In addition to the radiation risks discussed below, CT scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, headache, difficulty breathing, increased heartrate and swelling.

12.5.1.18 MR imaging

This research study involves MRIs. The main risk is an allergic reaction to an IV administered contrast agent. This risk is in the range of 1 to 1.5 %. There is also a risk of claustrophobia during MRI and feeling uncomfortable because of the loudness of the scanner. People with kidney disease are at risk for a serious reaction to gadolinium contrast called "nephrogenic systemic fibrosis (NSF)". This condition always involves the skin and can also involve the muscles, joints, and internal organs. NSF has resulted in a very small number of deaths. A blood test of participants' kidney function may be done within the month before an MRI scan with gadolinium contrast. Participants will not receive gadolinium for a research MRI scan if their kidney function is below the safe level.

12.5.1.19 Ultrasound

No physical risks are associated with ultrasound procedures.

12.5.1.20 Medical photography

No physical risks are associated with these procedures. This will not include photography of the face to protect identity. However, anonymity cannot be guaranteed as a specific body feature could be identified from the photograph.

12.5.1.21 Risks from radiation exposure

On this study, participants may receive up to 3 CT scans/year, 4 FDG PET/CT scans/year, and 3 chest x-ray/year. The total radiation dose for research purposes will be approximately 8.73 rem. The risk of getting cancer from the radiation exposure in this study is 0.9% and of getting a fatal cancer is 0.4%.

12.6 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with

the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825

12.6.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.6**.

12.6.2 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in Section **2.3.1** may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or

termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted, and data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the/each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 LENALIDOMIDE (IND # 131663)

14.1.1 Source

Lenalidomide is obtained under a CRADA with Celgene. Lenalidomide (Revlimid®) will be provided to research subjects for the duration of their participation in this trial at no charge to them or their insurance providers. Lenalidomide will be provided in accordance with the Celgene Corporation's Revlimid REMS®™ program. Per standard Revlimid REMS®™ program requirements, all physicians who prescribe lenalidomide for research subjects enrolled into this

trial, and all research subjects enrolled into this trial, must be registered in, and must comply with all requirements of the Revlimid REMS®™ program.

Drug will be shipped on a per participant basis by the contract pharmacy to the clinic site for IND studies. Only enough lenalidomide for one cycle of therapy will be supplied to the participant each cycle.

14.1.2 Toxicity

Lenalidomide was safe at doses of up to 50 mg in a phase I single dose study in normal, healthy male volunteers, and doses ranging from 5 - 50 mg have been evaluated in patients; DLTs seen in subjects receiving higher doses were predominantly hematopoietic (i.e., anemia, neutropenia, thrombocytopenia). Infection, rash and thrombo-embolic events have also been reported as the most common Grade 3-4 AEs. At least 3 cases of second primary malignancies have been observed in other ongoing studies.

14.1.3 Formulation and preparation

Lenalidomide will be supplied as 10-mg, 15-mg, and 25-mg capsules for oral administration.

14.1.4 Labeling

Lenalidomide supplies are dispensed in individual bottles of capsules. Each bottle will identify the contents as study medication. In addition, the label will bear Celgene's name, quantity contained and the standard caution statement as follows: "Caution: New drug - Limited by Federal law to investigational use." Lenalidomide should not be handled by females of child bearing potential unless they are wearing impermeable (latex, nitrile, polyisoprene or other "chemo") gloves.

The study drug label must be clearly visible. Additional labels must not cover the Celgene label.

14.1.5 Stability and Storage

At the study site, all investigational study drugs will be stored in a locked, safe area to prevent unauthorized access.

The study drug should be stored at room temperature away from direct sunlight and protected from excessive heat and cold.

14.1.6 Administration procedures

Lenalidomide capsules should be swallowed whole with water, and should not be broken, chewed or opened. Lenalidomide should be taken orally at about the same time each day, either with or without food.

If a dose of lenalidomide is missed, it should be taken as soon as possible on the same day. If the scheduled dose is more than 12 hours late, it should not be made up, rather it should be taken at the next scheduled time point.

Participants who take more than the prescribed dose of lenalidomide should be instructed to seek emergency medical care if needed and contact study staff immediately.

14.1.7 Special Handling Instructions

Females of childbearing potential should not handle or administer lenalidomide unless they are wearing impermeable (latex, nitrile, polyisoprene or other "chemo") gloves.

14.1.8 Disposal and Destruction

Celgene will instruct the Investigator on the return or destruction of unused study drug. If any study drug is lost or damaged, its disposition should be documented in the source documents. Study drug supplies will be retained at the clinical site pending instructions for disposition by Celgene.

14.1.9 Incompatibilities

Lenalidomide is not a substrate of hepatic metabolic enzymes *in vitro*. Lenalidomide is a weak substrate of P-gp. Lenalidomide is eliminated predominantly through renal excretion of the unchanged drug. It is not a substrate of human renal transporters such as MATE1, MRP2, OAT1, OAT3, ACT2, OCTN1 or OCTN2. Thus, it is unlikely that substrates or inhibitors of these transporters would effect human excretion of lenalidomide in humans.

Five clinical pharmacology studies have been conducted to assess drug interactions with lenalidomide. Co-administration of a single dose of digoxin with multiple doses of lenalidomide increased digoxin levels by 15% in healthy volunteers. Periodic monitoring of plasma digoxin levels is recommended during administration of lenalidomide.

Lenalidomide PK was not demonstrated to be altered during co-administration of warfarin, dexamethasone, or quinidine; like wise lenalidamide was not shown to alter the metabolism of the drugs.

14.2 RITUXIMAB

(For complete information, please see package insert)

Biosimilars are permitted in this protocol as they are highly similar to and have no clinically meaningful differences in terms of safety, purity, and potency (safety and effectiveness) from the filgrastim reference product. Rituximab and approved biosimilar products may be used interchangeably in this protocol between participants as needed based on product availability and NIH formulary status.

14.2.1 Supply

Rituximab (including biosimilars) is commercially available and will be supplied by the NIH CC Pharmacy. Rituximab (including biosimilars) is provided in 10 mL (100 mg) and 50 mL (500 mg) pharmaceutical grade glass vials at a concentration of 10 mg of protein per mL.

14.2.2 Storage

Rituximab for clinical use should be stored in a secure refrigerator at 2° to 8°C. Reconstitution and Dilution: Rituximab will be diluted in 0.9% Sodium Chloride or 5% Dextrose Injection to prepare a standard product with concentration equal to 2 mg/mL. Caution should be taken during the preparation of the drug, as shaking can cause aggregation and precipitation of the antibody. Rituximab solutions for infusion are stable at 2-8°C (36-46°F) for 24 hours and at room temperature for an additional 12 hours.

14.2.3 Administration

During cycle one only rituximab therapy will be given on day 4 to evaluate the effects of lenalidomide with rituximab on tumor, immunologic and viral factors Subsequent treatment doses of DA-EPOCH-R^2 are determined by hematological toxicity experienced during the previous cycle

according to the dose-adjustment paradigm in Section **3.3**. Acetaminophen 650mg PO and 50 mg diphenhydramine hydrochloride PO/IV will be administered within 60 minutes prior to starting each infusion of rituximab. Dexamethasone 8mg IV will be also administered as optional premedication within 60 minutes before infusion. A central intravenous line will be established. During the rituximab infusion, the participant's vital signs (blood pressure, pulse, respiration, temperature) should be monitored according to the standard of care. Available at the bedside prior to rituximab administration will be epinephrine 1:1000 (1mg/mL) for subcutaneous injection, diphenhydramine hydrochloride for intravenous injection, and resuscitation equipment for the emergency management of anaphylactic reactions.

First dose: The initial dose rate at the time of the first rituximab infusion should be 50mg/hour (25 mL/hr) for the first 30 minutes. If no toxicity is seen, the dose rate may be escalated gradually in 50 mg/hour (25 mL/h) increments at 30 minute intervals) to a maximum of 400 mg/hour (maximum rate = 200 mL/h).

Second and Subsequent Doses (select the appropriate administration timing):

90-minute Administration

If the first dose of rituximab was well tolerated, subsequent doses may be administered over 90 minutes with 20% of the total dose given in the first 30 minutes, and remaining 80% of the total dose administered over the subsequent 60 minutes; e.g.:

Two-Step Rate Escalation

Volume to administer (X mL)

1st portion (0 – 30 minutes)

2nd portion (30 – 90 minutes)

Special Note: The 90-minute infusion scheme is not recommended for participants with clinically significant cardiovascular disease or high circulating lymphocyte counts (≥5000/mcL).

Standard Administration for Second & Subsequent Infusions

Participants who tolerate initial treatment without experiencing infusion-related adverse effects but for whom the 90-minute infusion scheme during subsequent treatments is considered inappropriate, may receive subsequent rituximab doses at the Standard Rate for Subsequent Infusions, which is as follows:

Begin at an initial rate of 100 mg/hour (50 mL/h) for 30 minutes. If administration is well tolerated, the administration rate may be escalated gradually in 100-mg/hour (50-mL/h) at 30-minute intervals to a maximum rate of 400 mg/hour (maximum rate = 200 mL/h).

CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.

14.2.4 Safety Profile

No dose-limiting effects were observed in the Phase I/II studies. Reported adverse events including fever, chills, headache, nausea, vomiting, rhinitis, asthenia, and hypotension, occurred primarily during rituximab infusions and typically responded to an interruption of the infusion and resumption at a slower rate.

Fatal Infusion Reactions: Severe and fatal cardiopulmonary events, including angioedema, hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, and cardiogenic shock, have been reported. These severe reactions typically occurred during the first infusion with time to onset of 30-120 minutes.

Cardiac Events: Participants with preexisting cardiac conditions, including arrhythmia and angina, have had recurrences of these cardiac events during rituximab infusions.

Tumor Lysis Syndrome: Tumor lysis syndrome, some with fatal outcome, has been reported and is characterized in participants with a high number of circulating malignant cells ($\geq$25,000/mm^3) by rapid reduction in tumor volume, renal insufficiency, hyperkalemia, hypocalcemia, hyperuricemia, and hyperphosphatemia.

Renal Events: Rituximab has been associated with severe renal toxicity including acute renal failure requiring dialysis, and in some cases has led to death. Renal toxicity has occurred in participants with high numbers of circulating malignant cells ($\geq$25,000/mm^3) or high tumor burden who experience tumor lysis syndrome and in participants administered concomitant cisplatin.

Mucocutaneous Reactions: Severe bullous skin reactions, including fatal cases of toxic epidermal necrolysis and paraneoplastic pemphigus, have been reported in participants treated with rituximab. The onset of reaction has varied from 1 to 13 weeks following rituximab exposure.

Hematologic Events: In clinical trials, Grade 3 and 4 cytopenias were reported in 48% of participants treated with rituximab; these include: lymphopenia (40%), neutropenia (6%), leukopenia (4%), anemia (3%), and thrombocytopenia (2%). The median duration of lymphopenia was 14 days (range, 1 to 588 days) and of neutropenia was 13 days (range, 2 to 116 days). A single occurrence of transient aplastic anemia (pure red cell aplasia) and two occurrences of hemolytic anemia following Rituximab therapy were reported.

In addition, there have been a limited number of postmarketing reports of prolonged pancytopenia, marrow hypoplasia, and late onset neutropenia.

Infectious Events: Rituximab induced B-cell depletion in 70% to 80% of participants with NHL and was associated with decreased serum immunoglobulins in a minority of participants; the lymphopenia lasted a median of 14 days (range, 1-588 days). Infectious events occurred in 31% of participants: 19% of participants had bacterial infections, 10% had viral infections, 1% had fungal infections, and 6% were unknown infections. Serious infectious events (Grade 3 or 4), including sepsis, occurred in 2% of participants.

Hepatitis B Reactivation: Hepatitis B virus (HBV) reactivation with fulminant hepatitis, hepatic failure, and death has been reported in some participants with hematologic malignancies treated with rituximab. The majority of participants received rituximab in combination with chemotherapy. The median time to the diagnosis of hepatitis was approximately four months after the initiation of rituximab and approximately one month after the last dose.

Other Serious Viral Infections: The following additional serious viral infections, either new, reactivated or exacerbated, have been identified in clinical studies or postmarketing reports. The majority of participants received rituximab in combination with chemotherapy or as part of a hematopoietic stem cell transplant. These viral infections included JC virus (progressive multifocal leukoencephalopathy [PML]), cytomegalovirus, herpes simplex virus, parvovirus B19,

varicella zoster virus, West Nile virus, and hepatitis C. In some cases, the viral infections occurred up to one year following discontinuation of rituximab and have resulted in death.

Progressive multifocal leukoencephalopathy (PML)

PML is a rare disease caused by the reactivation of latent JC virus in the brain. Immunosuppression allows reactivation of the JC virus which causes demyelination and destruction of oligodendrocytes resulting in death or severe disability. Rare cases of PML, some resulting in death, have been reported in participants with hematologic malignancies who have received rituximab. The majority of these participants had received rituximab in combination with chemotherapy or as part of a hematopoietic stem cell transplant. Cases of PML resulting in death have also been reported following the use of rituximab for the treatment of autoimmune diseases. The reported cases had multiple risk factors for PML, including the underlying disease and long-term immunosuppressive therapy or chemotherapy. Most cases of PML were diagnosed within 12 months of their last infusion of rituximab.

Physicians should consider PML in any participant presenting with new onset neurologic manifestations. Consultation with a neurologist, brain MRI, and lumbar puncture should be considered as clinically indicated. In participants who develop PML, rituximab should be discontinued and reductions or discontinuation of any concomitant chemotherapy or immunosuppressive therapy should be considered.

Bowel Obstruction and Perforation: Abdominal pain, bowel obstruction and perforation, in some cases leading to death, were observed in participants receiving rituximab in combination with chemotherapy for DLBCL. In post-marketing reports, which include both participants with low-grade or follicular NHL and DLBCL, the mean time to onset of symptoms was 6 days (range 1−77) in participants with documented gastro-intestinal perforation. Complaints of abdominal pain, especially early in the course of treatment, should prompt a thorough diagnostic evaluation and appropriate treatment.

Immunogenicity: Participants may develop a human anti-chimeric antibody (HACA) response with rituximab treatment. The clinical significance of this is unclear.

Pregnancy: B-cell lymphocytopenia generally lasting less than 6 months can occur in infants exposed to rituximab in utero.

Immunization: Response rates may be reduced with non live vaccines.

Additional Safety Signals: The following serious adverse events have been reported to occur in participants following completion of rituximab infusions: arthritis, disorders of blood vessels (vasculitis, serum sickness and lupus-like syndrome), eye disorders (uveitis and optic neuritis), lung disorders including pleuritis and scarring of the lung (bronchiolitis obliterans), that may result in fatal outcomes, and fatal cardiac failure.

14.3 CYCLOPHOSPHAMIDE

(For complete information, please see package insert)

14.3.1 Supply

Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy from commercial sources.

14.3.2 Storage

All cyclophosphamide injectable products are stable at room temperature (not to exceed 30^{o}C). Reconstitute lyophilized cyclophosphamide for injection with appropriate amounts of 0.9% NaCl to produce a solution with concentration of 20 mg/mL. Discard solution after 24 hours at room temperature. Stable up to 6 days if refrigerated (2°-8^{o}C).

14.3.3 Administration

Cyclophosphamide will be further diluted in 100 mL of D5W or 0.9% NaCl and infused over 30 minutes. Participants will be instructed to drink an adequate amount of fluids and empty their bladders frequently during and after cyclophosphamide administration.

14.3.4 Toxicities

Myelosuppression, nausea and vomiting, hemorrhagic cystitis, and alopecia. Cystitis can be largely prevented by maintaining a good state of hydration and good urine flow during and after drug administration. Please refer to the package insert for a complete listing of all toxicities.

14.3.5 Hydration Guidelines

All participants should receive 0.9% Sodium Chloride Injection at the following volumes (based on cyclophosphamide dose levels) and rates with half given before and half given after the cyclophosphamide injection.

- Levels -2 to 6: 1 liter 0.9% NaCl @ 300-500 mL/hour, additional hydration may be given at the discretion of the Principal Investigator

14.4 DOXORUBICIN

(For complete information, please see package insert)

14.4.1 Supply

Doxorubicin HCl will be purchased by the NIH Clinical Center Pharmacy from commercial sources.

14.4.2 Toxicities

Myelosuppression, stomatitis, alopecia, nausea and vomiting, and acute and chronic cardiac toxicity, manifested as arrhythmias or a congestive cardiomyopathy, the latter uncommon at total cumulative doses less than 500 mg/m^2. The drug causes local necrosis if infiltrated into subcutaneous tissue. Please refer to the package insert for a complete listing of all toxicities.

14.5 VINCRISTINE

(For complete information, please see package insert)

14.5.1 Supply

Vincristine sulfate injection will be purchased by the NIH Clinical Center Pharmacy from commercial sources. Drug should be stored at 2°-8^{o}C and should be protected from light.

14.5.2 Toxicities

Peripheral neuropathy, autonomic neuropathy, and alopecia. Local necrosis if injected subcutaneously. Please refer to the package insert for a complete listing of all toxicities.

14.6 ETOPOSIDE

(For complete information, please see package insert)

14.6.1 Supply

Etoposide will be purchased by the NIH Clinical Center Pharmacy from commercial sources. Etoposide is commercially available as a concentrate for parenteral use in vials containing 100 mg, 500 mg, and 1 gram sterile, multidose vials. Each milliliter of solution contains 20 mg etoposide, 2 mg citric acid, 30 mg benzyl alcohol, 80 mg polysorbate 80, 650 mg of polyethylene glycol 300, and 30.5% alcohol.

14.6.2 Toxicities

Myelosuppression, nausea, vomiting, anaphylactic reactions, alopecia, and hypotension if infusion is too rapid. Please refer to the package insert for a complete listing of all toxicities.

14.7 ADMINISTRATION OF VINCRISTINE/DOXORUBICIN/ETOPOSIDE

Please also see **Appendix G: EPOCH Admixtures: Preparation and Administration NCI CC only.**

Stability studies conducted by the Pharmaceutical Development Section, Pharmacy Department, NIH Clinical Center, have demonstrated that admixtures of vincristine, doxorubicin, and etoposide in 0.9% Sodium Chloride Injection, USP at concentrations, respectively, of 1, 25, and 125 mcg/mL; 1.4, 35, and 175 mcg/mL; 2, 50, and 250 mcg/mL; and 2.8, 70, and 350 mcg/mL are stable for at least 36 hours at room temperature when protected from light. Also admixtures containing vincristine, doxorubicin and etoposide concentrations of 1.6, 40, and 200 mcg/mL are stable for at least 30 hours at 32°C. In this study, THE DAILY DOSE (i.e. a 24-hour supply) of vincristine, doxorubicin, and etoposide will be admixed together and delivered with a suitable infusion pump through a central venous access device. Supplies of the 3-drug admixture (etoposide + doxorubicin + vincristine) will be exchanged daily for each of the four consecutive days to complete a 96-hour infusion.

14.8 PREDNISONE

(For complete information, please see package insert)

14.8.1 Supply

Prednisone will be purchased by the NIH Clinical Center Pharmacy from commercial sources. Tablets should be stored in well-closed containers at temperatures between 15°-30°C.

14.8.2 Doses

Prednisone utilization may be simplified by using only 20- and 50-mg tablets to produce individual doses and by stratifying prednisone doses by a participant's body surface area (BSA) according to the chart below. These are recommendations and not requirements.

BSA (m_2) Each Dose	BSA (m_2) Each Dose
1.25 – 1.49	80 mg
1.5 – 1.83	100 mg

1.84 – 2.16	120 mg
2.17 – 2.41	140 mg
2.42 – 2.6	150 mg
2.61 – 2.69	160 mg
2.7 – 3	170 mg

14.8.3 Toxicities

Proximal muscle weakness, glucose intolerance, thinning of skin, redistribution of body fat, Cushingoid facies, immunosuppression, and propensity to gastrointestinal ulceration. Please refer to the package insert for a complete listing of all toxicities.

14.9 FILGRASTIM

(For complete information, please see package insert)

Biosimilars are permitted in this protocol as they are highly similar to and have no clinically meaningful differences in terms of safety, purity, and potency (safety and effectiveness) from the filgrastim reference product. Filgrastim and approved biosimilar products may be used interchangeably in this protocol between participants as needed based on product availability and NIH formulary status.

14.9.1 Supply

Filgrastim, including biosimilars, will be supplied by the NIH CC pharmacy.

14.9.2 Toxicities

Rare anaphylactic reactions with the first dose; bone pain at sites of active marrow with continued administration. Local reactions at injection sites. Constitutional symptoms; increased alkaline phosphatase, LDH, uric acid; worsening of pre-existing inflammatory conditions. Please refer to the package insert for a complete listing of all toxicities.

15 REFERENCES

1. Vitolo U, Chiappella A, Franceschetti S, Carella AM, Baldi I, Inghirami G, Spina M, Pavone V, Ladetto M, Liberati AM *et al*: **Lenalidomide plus R-CHOP21 in elderly patients with untreated diffuse large B-cell lymphoma: results of the REAL07 open-label, multicentre, phase 2 trial**. *Lancet Oncol* 2014, **15**(7):730-737.

2. Nowakowski GS, LaPlant B, Habermann TM, Rivera CE, Macon WR, Inwards DJ, Micallef IN, Johnston PB, Porrata LF, Ansell SM *et al*: **Lenalidomide can be safely combined with R-CHOP (R2CHOP) in the initial chemotherapy for aggressive B-cell lymphomas: phase I study**. *Leukemia* 2011, **25**(12):1877-1881.

3. Boulanger E, Duprez R, Delabesse E, Gabarre J, Macintyre E, Gessain A: **Mono/oligoclonal pattern of Kaposi Sarcoma-associated herpesvirus (KSHV/HHV-8) episomes in primary effusion lymphoma cells**. *Int J Cancer* 2005, **115**(4):511-518.

4. Boulanger E, Agbalika F, Maarek O, Daniel MT, Grollet L, Molina JM, Sigaux F, Oksenhendler E: **A clinical, molecular and cytogenetic study of 12 cases of human herpesvirus 8 associated primary effusion lymphoma in HIV-infected patients**. *Hematol J* 2001, **2**(3):172-179.

5. Luan SL, Boulanger E, Ye H, Chanudet E, Johnson N, Hamoudi RA, Bacon CM, Liu H, Huang Y, Said J *et al*: **Primary effusion lymphoma: genomic profiling revealed amplification of SELPLG and CORO1C encoding for proteins important for cell migration**. *J Pathol* 2010.

6. Wilson WH, Grossbard ML, Pittaluga S, Cole D, Pearson D, Drbohlav N, Steinberg SM, Little RF, Janik J, Gutierrez M *et al*: **Dose-adjusted EPOCH chemotherapy for untreated large B-cell lymphomas: a pharmacodynamic approach with high efficacy**. *Blood* 2002, **99**(8):2685-2693.

7. Little RF, Pittaluga S, Grant N, Steinberg SM, Kavlick MF, Mitsuya H, Franchini G, Gutierrez M, Raffeld M, Jaffe ES *et al*: **Highly effective treatment of acquired immunodeficiency syndrome-related lymphoma with dose-adjusted EPOCH: impact of antiretroviral therapy suspension and tumor biology**. *Blood* 2003, **101**(12):4653-4659.

8. Dunleavy K, Little RF, Pittaluga S, Grant N, Wayne AS, Carrasquillo JA, Steinberg SM, Yarchoan R, Jaffe ES, Wilson WH: **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**. *Blood* 2010.

9. Rappocciolo G, Hensler HR, Jais M, Reinhart TA, Pegu A, Jenkins FJ, Rinaldo CR: **Human herpesvirus 8 infects and replicates in primary cultures of activated B lymphocytes through DC-SIGN**. *J Virol* 2008, **82**(10):4793-4806.

10. Aoki Y, Tosato G, Fonville TW, Pittaluga S: **Serum viral interleukin-6 in AIDS-related multicentric Castleman disease**. *Blood* 2001, **97**(8):2526-2527.

11. Oksenhendler E, Carcelain G, Aoki Y, Boulanger E, Maillard A, Clauvel JP, Agbalika F: **High levels of human herpesvirus 8 viral load, human interleukin-6, interleukin-10, and C reactive protein correlate with exacerbation of multicentric castleman disease in HIV-infected patients**. *Blood* 2000, **96**(6):2069-2073.

12. Uldrick TS, Polizzotto MN, Aleman K, Wyvill KM, Marshall V, Whitby D, Wang V, Pittaluga S, O'Mahony D, Steinberg SM *et al*: **Rituximab plus liposomal doxorubicin in HIV-infected patients with KSHV-associated multicentric Castleman disease**. *Blood* 2014, **124**(24):3544-3552.

13. Uldrick TS, Wang V, O'Mahony D, Aleman K, Wyvill KM, Marshall V, Steinberg SM, Pittaluga S, Maric I, Whitby D *et al*: **An Interleukin-6-Related Systemic Inflammatory Syndrome in Patients Co-Infected with Kaposi Sarcoma-Associated Herpesvirus and HIV but without Multicentric Castleman Disease**. *Clin Infect Dis* 2010, **51**(3):350-358.

14. Boulanger E, Gerard L, Gabarre J, Molina J-M, Rapp C, Abino J-F, Cadranel J, Chevret S, Oksenhendler E: **Prognostic Factors and Outcome of Human Herpesvirus 8-Associated Primary Effusion Lymphoma in Patients With AIDS**. *J Clin Oncol* 2005, **23**(19):4372-4380.

15. Nowakowski GS, LaPlant B, Macon WR, Reeder CB, Foran JM, Nelson GD, Thompson CA, Rivera CE, Inwards DJ, Micallef IN *et al*: **Lenalidomide combined with R-CHOP overcomes negative prognostic impact of non-germinal center B-cell phenotype in newly diagnosed diffuse large B-Cell lymphoma: a phase II study**. *J Clin Oncol* 2015, **33**(3):251-257.

16. Jones KD, Aoki Y, Chang Y, Moore PS, Yarchoan R, Tosato G: **Involvement of interleukin-10 (IL-10) and viral IL-6 in the spontaneous growth of Kaposi's sarcoma herpesvirus-associated infected primary effusion lymphoma cells**. *Blood* 1999, **94**(8):2871-2879.

17. Aoki Y, Feldman GM, Tosato G: **Inhibition of STAT3 signaling induces apoptosis and decreases survivin expression in primary effusion lymphoma**. *Blood* 2003, **101**(4):1535-1542.

18. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM: **Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas**. *N Engl J Med* 1995, **332**(18):1186-1191.

19. Bower M, Fox P, Fife K, Gill J, Nelson M, Gazzard B: **Highly active anti-retroviral therapy (HAART) prolongs time to treatment failure in Kaposi's sarcoma**. *AIDS* 1999, **13**(15):2105-2111.

20. Portsmouth S, Stebbing J, Gill J, Mandalia S, Bower M, Nelson M, Gazzard B: **A comparison of regimens based on non-nucleoside reverse transcriptase inhibitors or protease inhibitors in preventing Kaposi's sarcoma**. *AIDS* 2003, **17**(11):F17-22.

21. Engels EA, Biggar RJ, Hall HI, Cross H, Crutchfield A, Finch JL, Grigg R, Hylton T, Pawlish KS, McNeel TS *et al*: **Cancer risk in people infected with human immunodeficiency virus in the United States**. *Int J Cancer* 2008, **123**(1):187-194.

22. Sullivan SG, Hirsch HH, Franceschi S, Steffen I, Amari EB, Mueller NJ, Magkouras I, Biggar RJ, Rickenbach M, Clifford GM: **Kaposi sarcoma herpes virus antibody response and viremia following highly active antiretroviral therapy in the Swiss HIV Cohort study**. *AIDS* 2010, **24**(14):2245-2252.

23. Olweny CL, Borok M, Gudza I, Clinch J, Cheang M, Kiire CF, Levy L, Otim-Oyet D, Nyamasve J, Schipper H: **Treatment of AIDS-associated Kaposi's sarcoma in Zimbabwe: results of a randomized quality of life focused clinical trial**. *Int J Cancer* 2005, **113**(4):632-639.

24. Biggar RJ: **Survival after cancer diagnosis in persons with AIDS**. *Journal of acquired immune deficiency syndromes* 2005, **39**(3):293.

25. Aoki Y, Tosato G: **Interactions between HIV-1 Tat and KSHV**. *Curr Top Microbiol Immunol* 2007, **312**:309-326.

26. Barillari G, Gendelman R, Gallo RC, Ensoli B: **The Tat protein of human immunodeficiency virus type 1, a growth factor for AIDS Kaposi sarcoma and cytokine-activated vascular cells, induces adhesion of the same cell types by using integrin receptors recognizing the RGD amino acid sequence**. *Proc Natl Acad Sci U S A* 1993, **90**(17):7941-7945.

27. Ensoli B, Barillari G, Salahuddin SZ, Gallo RC, Wong-Staal F: **Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients**. *Nature* 1990, **345**(6270):84-86.

28. Ensoli B, Gendelman R, Markham P, Fiorelli V, Colombini S, Raffeld M, Cafaro A, Chang HK, Brady JN, Gallo RC: **Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma**. *Nature* 1994, **371**(6499):674-680.

29. Albini A, Benelli R, Presta M, Rusnati M, Ziche M, Rubartelli A, Paglialunga G, Bussolino F, Noonan D: **HIV-tat protein is a heparin-binding angiogenic growth factor**. *Oncogene* 1996, **12**(2):289-297.

30. Albini A, Soldi R, Giunciuglio D, Giraudo E, Benelli R, Primo L, Noonan D, Salio M, Camussi G, Rockl W *et al*: **The angiogenesis induced by HIV-1 tat protein is mediated by the Flk-1/KDR receptor on vascular endothelial cells**. *Nat Med* 1996, **2**(12):1371-1375.

31. Mitola S, Soldi R, Zanon I, Barra L, Gutierrez MI, Berkhout B, Giacca M, Bussolino F: **Identification of specific molecular structures of human immunodeficiency virus type 1 Tat relevant for its biological effects on vascular endothelial cells**. *J Virol* 2000, **74**(1):344-353.

32. Chirivi RG, Taraboletti G, Bani MR, Barra L, Piccinini G, Giacca M, Bussolino F, Giavazzi R: **Human immunodeficiency virus-1 (HIV-1)-Tat protein promotes migration of acquired immunodeficiency syndrome-related lymphoma cells and enhances their adhesion to endothelial cells**. *Blood* 1999, **94**(5):1747-1754.

33. Aoki Y, Tosato G: **HIV-1 Tat enhances Kaposi sarcoma-associated herpesvirus (KSHV) infectivity**. *Blood* 2004, **104**(3):810-814.

34. IARC: **WHO Classification of Tumours of Haematopoetic and Lymphoid Tissues**, 4th edn. Lyon: IARC; 2008.

35. Knowles DM, Inghirami G, Ubriaco A, Dalla-Favera R: **Molecular genetic analysis of three AIDS-associated neoplasms of uncertain lineage demonstrates their B-cell derivation and the possible pathogenetic role of the Epstein-Barr virus**. *Blood* 1989, **73**(3):792-799.

36. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS: **Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma**. *Science* 1994, **266**(5192):1865-1869.

37. Nador RG, Cesarman E, Chadburn A, Dawson DB, Ansari MQ, Sald J, Knowles DM: **Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus**. *Blood* 1996, **88**(2):645-656.

38. Foster WR, Bischin A, Dorer R, Aboulafia DM: **Human Herpesvirus Type 8-associated Large B-cell Lymphoma: A Nonserous Extracavitary Variant of Primary Effusion Lymphoma in an HIV-infected Man: A Case Report and Review of the Literature**. *Clin Lymphoma Myeloma Leuk* 2016.

39. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD *et al*: **The 2016 revision of the World Health Organization classification of lymphoid neoplasms**. *Blood* 2016, **127**(20):2375-2390.

40. Cesarman E, Nador RG, Aozasa K, Delsol G, Said JW, Knowles DM: **Kaposi's sarcoma-associated herpesvirus in non-AIDS related lymphomas occurring in body cavities**. *Am J Pathol* 1996, **149**(1):53-57.

41. Strauchen JA, Hauser AD, Burstein D, Jimenez R, Moore PS, Chang Y: **Body cavity-based malignant lymphoma containing Kaposi sarcoma-associated herpesvirus in an HIV-negative man with previous Kaposi sarcoma**. *Ann Intern Med* 1996, **125**(10):822-825.

42. Ariad S, Benharroch D, Lupu L, Davidovici B, Dupin N, Boshoff C: **Early peripheral lymph node involvement of human herpesvirus 8-associated, body cavity-based lymphoma in a human immunodeficiency virus-negative patient**. *Arch Pathol Lab Med* 2000, **124**(5):753-755.

43. Teruya-Feldstein J, Zauber P, Setsuda JE, Berman EL, Sorbara L, Raffeld M, Tosato G, Jaffe ES: **Expression of human herpesvirus-8 oncogene and cytokine homologues in an HIV-seronegative patient with multicentric Castleman's disease and primary effusion lymphoma**. *Lab Invest* 1998, **78**(12):1637-1642.

44. Boulanger E, Daniel MT, Agbalika F, Oksenhendler E: **Combined chemotherapy including high-dose methotrexate in KSHV/HHV8-associated primary effusion lymphoma**. *Am J Hematol* 2003, **73**(3):143-148.

45. Guillet S, Gerard L, Meignin V, Agbalika F, Cuccini W, Denis B, Katlama C, Galicier L, Oksenhendler E: **Classic and extracavitary primary effusion lymphoma in 51 HIV-infected patients from a single institution**. *Am J Hematol* 2016, **91**(2):233-237.

46. Carbone A, Gloghini A, Cozzi MR, Capello D, Steffan A, Monini P, De Marco L, Gaidano G: **Expression of MUM1/IRF4 selectively clusters with primary effusion lymphoma among lymphomatous effusions: implications for disease histogenesis and pathogenesis**. *Br J Haematol* 2000, **111**(1):247-257.

47. Carbone A, Gloghini A, Larocca LM, Capello D, Pierconti F, Canzonieri V, Tirelli U, Dalla-Favera R, Gaidano G: **Expression profile of MUM1/IRF4, BCL-6, and CD138/syndecan-1 defines novel histogenetic subsets of human immunodeficiency virus-related lymphomas**. *Blood* 2001, **97**(3):744-751.

48. Dupin N, Diss TL, Kellam P, Tulliez M, Du MQ, Sicard D, Weiss RA, Isaacson PG, Boshoff C: **HHV-8 is associated with a plasmablastic variant of Castleman disease that is linked to HHV-8-positive plasmablastic lymphoma**. *Blood* 2000, **95**(4):1406-1412.

49. Oksenhendler E, Boulanger E, Galicier L, Du MQ, Dupin N, Diss TC, Hamoudi R, Daniel MT, Agbalika F, Boshoff C *et al*: **High incidence of Kaposi sarcoma-associated herpesvirus-related non-Hodgkin lymphoma in patients with HIV infection and multicentric Castleman disease**. *Blood* 2002, **99**(7):2331-2336.

50. Dargent JL, Lespagnard L, Sirtaine N, Cantinieaux B, Li R, Hermans P: **Plasmablastic microlymphoma occurring in human herpesvirus 8 (HHV-8)-positive multicentric Castleman's disease and featuring a follicular growth pattern**. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica* 2007, **115**(7):869-874.

51. Jenner RG, Maillard K, Cattini N, Weiss RA, Boshoff C, Wooster R, Kellam P: **Kaposi's sarcoma-associated herpesvirus-infected primary effusion lymphoma has a plasma cell gene expression profile**. *Proc Natl Acad Sci U S A* 2003, **100**(18):10399-10404.

52. Klein U, Gloghini A, Gaidano G, Chadburn A, Cesarman E, Dalla-Favera R, Carbone A: **Gene expression profile analysis of AIDS-related primary effusion lymphoma (PEL) suggests a plasmablastic derivation and identifies PEL-specific transcripts**. *Blood* 2003, **101**(10):4115-4121.

53. Dalton-Griffin L, Wilson SJ, Kellam P: **X-box binding protein 1 contributes to induction of the Kaposi's sarcoma-associated herpesvirus lytic cycle under hypoxic conditions**. *J Virol* 2009, **83**(14):7202-7209.

54. Wilson SJ, Tsao EH, Webb BL, Ye H, Dalton-Griffin L, Tsantoulas C, Gale CV, Du MQ, Whitehouse A, Kellam P: **X box binding protein XBP-1s transactivates the Kaposi's sarcoma-associated herpesvirus (KSHV) ORF50 promoter, linking plasma cell differentiation to KSHV reactivation from latency**. *J Virol* 2007, **81**(24):13578-13586.

55. Yu F, Feng J, Harada JN, Chanda SK, Kenney SC, Sun R: **B cell terminal differentiation factor XBP-1 induces reactivation of Kaposi's sarcoma-associated herpesvirus**. *FEBS letters* 2007, **581**(18):3485-3488.

56. Aoki Y, Tosato G: **Pathophysiological Role of Vascular Endothelial Growth Factory/Vascular Permeability Factor (VEGF/VPF) in Primary Effusion Lymphomas**. In: *3rd National AIDS Malignancy Meeting: May 26 - 27 1999; Bethesda*. National Cancer Institute: Abstract 89.

57. Aoki Y, Tosato G, Nambu Y, Iwamoto A, Yarchoan R: **Detection of vascular endothelial growth factor in AIDS-related primary effusion lymphomas**. *Blood* 2000, **95**(3):1109-1110.

58. Aoki Y, Yarchoan R, Wyvill K, Okamoto S, Little RF, Tosato G: **Detection of viral interleukin-6 in Kaposi sarcoma-associated herpesvirus-linked disorders**. *Blood* 2001, **97**(7):2173-2176.

59. Aoki Y, Tosato G: **Role of vascular endothelial growth factor/vascular permeability factor in the pathogenesis of Kaposi's sarcoma-associated herpesvirus-infected primary effusion lymphomas**. *Blood* 1999, **94**(12):4247-4254.

60. Nagy JA, Masse EM, Herzberg KT, Meyers MS, Yeo KT, Yeo TK, Sioussat TM, Dvorak HF: **Pathogenesis of ascites tumor growth: vascular permeability factor, vascular hyperpermeability, and ascites fluid accumulation**. *Cancer Res* 1995, **55**(2):360-368.

61. Nagy JA, Morgan ES, Herzberg KT, Manseau EJ, Dvorak AM, Dvorak HF: **Pathogenesis of ascites tumor growth: angiogenesis, vascular remodeling, and stroma formation in the peritoneal lining**. *Cancer Res* 1995, **55**(2):376-385.

62. Haddad L, El Hajj H, Abou-Merhi R, Kfoury Y, Mahieux R, El-Sabban M, Bazarbachi A: **KSHV-transformed primary effusion lymphoma cells induce a VEGF-dependent angiogenesis and establish functional gap junctions with endothelial cells**. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, UK* 2008, **22**(4):826-834.

63. Davis DA, Rinderknecht AS, Zoeteweij JP, Aoki Y, Read-Connole EL, Tosato G, Blauvelt A, Yarchoan R: **Hypoxia induces lytic replication of Kaposi sarcoma-associated herpesvirus**. *Blood* 2001, **97**(10):3244-3250.

64. Liu C, Okruzhnov Y, Li H, Nicholas J: **Human herpesvirus 8 (HHV-8)-encoded cytokines induce expression of and autocrine signaling by vascular endothelial growth factor (VEGF) in HHV-8-infected primary-effusion lymphoma cell lines and mediate VEGF-independent antiapoptotic effects**. *J Virol* 2001, **75**(22):10933-10940.

65. Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marme D: **Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1**. *Blood* 1996, **87**(8):3336-3343.

66. Ito T, Ando H, Suzuki T, Ogura T, Hotta K, Imamura Y, Yamaguchi Y, Handa H: **Identification of a primary target of thalidomide teratogenicity**. *Science* 2010, **327**(5971):1345-1350.

67. Zhu YX, Braggio E, Shi CX, Bruins LA, Schmidt JE, Van Wier S, Chang XB, Bjorklund CC, Fonseca R, Bergsagel PL *et al*: **Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide**. *Blood* 2011, **118**(18):4771-4779.

68. Lopez-Girona A, Mendy D, Ito T, Miller K, Gandhi AK, Kang J, Karasawa S, Carmel G, Jackson P, Abbasian M *et al*: **Cereblon is a direct protein target for immunomodulatory and antiproliferative activities of lenalidomide and pomalidomide**. *Leukemia* 2012, **26**(11):2326-2335.

69. Lu G, Middleton RE, Sun H, Naniong M, Ott CJ, Mitsiades CS, Wong KK, Bradner JE, Kaelin WG, Jr.: **The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins**. *Science* 2014, **343**(6168):305-309.

70. Kronke J, Udeshi ND, Narla A, Grauman P, Hurst SN, McConkey M, Svinkina T, Heckl D, Comer E, Li X *et al*: **Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells**. *Science* 2014, **343**(6168):301-305.

71. Shaffer AL, Emre NC, Lamy L, Ngo VN, Wright G, Xiao W, Powell J, Dave S, Yu X, Zhao H *et al*: **IRF4 addiction in multiple myeloma**. *Nature* 2008, **454**(7201):226-231.

72. Yang Y, Shaffer AL, 3rd, Emre NC, Ceribelli M, Zhang M, Wright G, Xiao W, Powell J, Platig J, Kohlhammer H *et al*: **Exploiting synthetic lethality for the therapy of ABC diffuse large B cell lymphoma**. *Cancer cell* 2012, **21**(6):723-737.

73. Li S, Pal R, Monaghan SA, Schafer P, Ouyang H, Mapara M, Galson DL, Lentzsch S: **IMiD immunomodulatory compounds block C/EBP{beta} translation through eIF4E down-regulation resulting in inhibition of MM**. *Blood* 2011, **117**(19):5157-5165.

74. Zhang LH, Kosek J, Wang M, Heise C, Schafer PH, Chopra R: **Lenalidomide efficacy in activated B-cell-like subtype diffuse large B-cell lymphoma is dependent upon IRF4 and cereblon expression**. *Br J Haematol* 2013, **160**(4):487-502.

75. Schafer PH, Gandhi AK, Loveland MA, Chen RS, Man HW, Schnetkamp PP, Wolbring G, Govinda S, Corral LG, Payvandi F *et al*: **Enhancement of cytokine production and AP-1 transcriptional activity in T cells by thalidomide-related immunomodulatory drugs**. *The Journal of pharmacology and experimental therapeutics* 2003, **305**(3):1222-1232.

76. Payvandi F, Wu L, Haley M, Schafer PH, Zhang LH, Chen RS, Muller GW, Stirling DI: **Immunomodulatory drugs inhibit expression of cyclooxygenase-2 from TNF-alpha, IL-1beta, and LPS-stimulated human PBMC in a partially IL-10-dependent manner**. *Cellular immunology* 2004, **230**(2):81-88.

77. Gorgun G, Calabrese E, Soydan E, Hideshima T, Perrone G, Bandi M, Cirstea D, Santo L, Hu Y, Tai YT *et al*: **Immunomodulatory effects of lenalidomide and pomalidomide on interaction of tumor and bone marrow accessory cells in multiple myeloma**. *Blood* 2010, **116**(17):3227-3237.

78. Dredge K, Marriott JB, Macdonald CD, Man HW, Chen R, Muller GW, Stirling D, Dalgleish AG: **Novel thalidomide analogues display anti-angiogenic activity independently of immunomodulatory effects**. *British journal of cancer* 2002, **87**(10):1166-1172.

79. Reddy N, Hernandez-Ilizaliturri FJ, Deeb G, Roth M, Vaughn M, Knight J, Wallace P, Czuczman MS: **Immunomodulatory drugs stimulate natural killer-cell function, alter cytokine production by dendritic cells, and inhibit angiogenesis enhancing the anti-tumour activity of rituximab in vivo**. *Br J Haematol* 2008, **140**(1):36-45.

80. Lentzsch S, LeBlanc R, Podar K, Davies F, Lin B, Hideshima T, Catley L, Stirling DI, Anderson KC: **Immunomodulatory analogs of thalidomide inhibit growth of Hs Sultan cells and angiogenesis in vivo**. *Leukemia* 2003, **17**(1):41-44.

81. Polizzotto MN, Uldrick TS, Hu D, Yarchoan R: **Clinical Manifestations of Kaposi Sarcoma Herpesvirus Lytic Activation: Multicentric Castleman Disease (KSHV-MCD) and the KSHV Inflammatory Cytokine Syndrome**. *Frontiers in microbiology* 2012, **3**:73.

82. Little RF, Wyvill KM, Pluda JM, Welles L, Marshall V, Figg WD, Newcomb FM, Tosato G, Feigal E, Steinberg SM *et al*: **Activity of Thalidomide in AIDS-Related Kaposi's Sarcoma**. *J Clin Oncol* 2000, **18**(13):2593-2602.

83. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ, Coiffier B, Fisher RI, Hagenbeek A, Zucca E *et al*: **Revised response criteria for malignant lymphoma**. *J Clin Oncol* 2007, **25**(5):579-586.

84. Chen N, Lau H, Kong L, Kumar G, Zeldis JB, Knight R, Laskin OL: **Pharmacokinetics of lenalidomide in subjects with various degrees of renal impairment and in subjects on hemodialysis**. *J Clin Pharmacol* 2007, **47**(12):1466-1475.

85. Cillo AR, Sobolewski MD, Bosch RJ, Fyne E, Piatak M, Jr., Coffin JM, Mellors JW: **Quantification of HIV-1 latency reversal in resting CD4+ T cells from patients on suppressive antiretroviral therapy**. *Proc Natl Acad Sci U S A* 2014, **111**(19):7078-7083.

86. Cillo AR, Vagratian D, Bedison MA, Anderson EM, Kearney MF, Fyne E, Koontz D, Coffin JM, Piatak M, Jr., Mellors JW: **Improved single-copy assays for quantification of persistent HIV-1 viremia in patients on suppressive antiretroviral therapy**. *Journal of clinical microbiology* 2014, **52**(11):3944-3951.

87. Kearney MF, Spindler J, Shao W, Yu S, Anderson EM, O'Shea A, Rehm C, Poethke C, Kovacs N, Mellors JW *et al*: **Lack of detectable HIV-1 molecular evolution during suppressive antiretroviral therapy**. *PLoS pathogens* 2014, **10**(3):e1004010.

88. de Sanjose S, Marshall V, Sola J, Palacio V, Almirall R, Goedert JJ, Bosch FX, Whitby D: **Prevalence of Kaposi's sarcoma-associated herpesvirus infection in sex workers and women from the general population in Spain**. *Int J Cancer* 2002, **98**(1):155-158.

89. Yuan CC, Miley W, Waters D: **A quantification of human cells using an ERV-3 real time PCR assay**. *J Virol Methods* 2001, **91**(2):109-117.

90. Whitby D, Marshall VA, Bagni RK, Miley WJ, McCloud TG, Hines-Boykin R, Goedert JJ, Conde BA, Nagashima K, Mikovits J *et al*: **Reactivation of Kaposi's sarcoma-associated herpesvirus by natural products from Kaposi's sarcoma endemic regions**. *Int J Cancer* 2007, **120**(2):321-328.

91. Engels EA, Biggar RJ, Marshall VA, Walters MA, Gamache CJ, Whitby D, Goedert JJ: **Detection and quantification of Kaposi's sarcoma-associated herpesvirus to predict AIDS-associated Kaposi's sarcoma**. *Aids* 2003, **17**(12):1847-1851.

92. Jasper GA, Arun I, Venzon D, Kreitman RJ, Wayne AS, Yuan CM, Marti GE, Stetler-Stevenson M: **Variables affecting the quantitation of CD22 in neoplastic B cells**. *Cytometry Part B: Clinical Cytometry*:n/a-n/a.

93. Ramasamy I, Brisco M, Morley A: **Improved PCR method for detecting monoclonal immunoglobulin heavy chain rearrangement in B cell neoplasms**. *J Clin Pathol* 1992, **45**(9):770-775.

94. van Dongen JJ, Langerak AW, Bruggemann M, Evans PA, Hummel M, Lavender FL, Delabesse E, Davi F, Schuuring E, Garcia-Sanz R *et al*: **Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936**. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, UK* 2003, **17**(12):2257-2317.

95. Krown SE, Metroka C, Wernz JC: **Kaposi's sarcoma in the acquired immune deficiency syndrome: a proposal for uniform evaluation, response, and staging criteria. AIDS Clinical Trials Group Oncology Committee**. *J Clin Oncol* 1989, **7**(9):1201-1207.

96. Brookmeyer R, Crowley JJ: **A confidence interval for the median survival time.** *Biometrics* 1982, **38**:29-41.

97. de Sanjose S, Mbisa G, Perez-Alvarez S, Benavente Y, Sukvirach S, Hieu NT, Shin HR, Anh PT, Thomas J, Lazcano E *et al*: **Geographic variation in the prevalence of Kaposi sarcoma-associated herpesvirus and risk factors for transmission**. *The Journal of infectious diseases* 2009, **199**(10):1449-1456.

98. Engels EA, Atkinson JO, Graubard BI, McQuillan GM, Gamache C, Mbisa G, Cohn S, Whitby D, Goedert JJ: **Risk factors for human herpesvirus 8 infection among adults in the United States and evidence for sexual transmission**. *J Infect Dis* 2007, **196**(2):199-207.

99. Mbulaiteye SM, Atkinson JO, Whitby D, Wohl DA, Gallant JE, Royal S, Goedert JJ, Rabkin CS: **Risk factors for human herpesvirus 8 seropositivity in the AIDS Cancer Cohort Study**. *J Clin Virol* 2006, **35**(4):442-449.

100. Grulich AE, Cunningham P, Munier ML, Prestage G, Amin J, Ringland C, Whitby D, Kippax S, Kaldor JM, Rawlinson W: **Sexual behaviour and human herpesvirus 8 infection in homosexual men in Australia**. *Sexual health* 2005, **2**(1):13-18.

16 APPENDICES

16.1 APPENDIX A:PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

16.2 APPENDIX B: CALCULATION OF CREATININE CLEARANCE

1. Calculation: Cockcroft-Gault formula for creatinine clearance estimation:

Males: CrCl (estimated) =(140-age) * (weight)/(sCr * 72)

Females: CrCl (estimated) =(140-age) * (weight * 0.85)/(sCr * 72)

Notes: CrCl (estimated): estimated creatinine clearance rate in mL/min

Age in years

Weight in kilograms (lean or actual body weight, whichever is less)

sCr:serum creatinine in mg/dL

Estimated creatinine clearance should not be used in the following circumstances:

- altered protein intake (vegetarian diet, creatine supplements)
- altered muscle mass (malnutrition, wasting, amputation)
- in such cases, a 24-hour urine collection for measured creatinine clearance should be obtained

---

Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41

2. Calculation: 24 hour Creatinine Clearance

Males and Females: CrCl=(uCr * uV) / (sCr *1440)

Notes: CrCl: Creatinine clearance in mL/min

sCr: serum creatinine in mg/dL

uCr: urine creatinine in mg/dL

uV: 24 hour urine volume in mL

1440: number of minutes in 24 hours

3. Calculation: Lean Body Weight

Males: Lean Body Weight = $\left(1.1 \times \text{TBW } [\text{kg}]\right) - \left(0.0128 \times \text{BMI } \left[\text{kg/m}^2\right] \times \text{TBW } [\text{kg}]\right)$

Females: Lean Body Weight = $\left(1.07 \times \text{TBW } [\text{kg}]\right) - \left(0.0148 \times \text{BMI } \left[\text{kg/m}^2\right] \times \text{TBW } [\text{kg}]\right)$

TBW: total (*or* actual) body weight
BMI: body mass index (weight in kilograms / (height [m])^2

Green B, Duffull SB. What is the best size descriptor to use for pharmacokinetic studies in the obese? Br J Clin Pharmacol 2004;58:119-33

16.3 APPENDIX C: LENALIDOMIDE RISKS OF FETAL EXPOSURE, PREGNANCY TESTING GUIDELINES AND ACCEPTABLE BIRTH CONTROL METHODS

Risks Associated with Pregnancy

Lenalidomide was found to be teratogenic in a developmental study in rabbits. Lenalidomide is an analogue of thalidomide. Thalidomide is a known human teratogen that causes severe life-threatening human birth defects. If lenalidomide is taken during pregnancy, it may cause birth defects or death to an unborn baby.

Criteria for females of childbearing potential (FCBP)

This protocol defines a female of childbearing potential as a sexually mature woman who:

1. has not undergone a hysterectomy or bilateral oophorectomy or
2. has not been naturally postmenopausal (amenorrhea following cancer therapy does not rule out childbearing potential) for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

Counseling

For a female of childbearing potential, lenalidomide is contraindicated unless all of the following are met (ie, all females of childbearing potential must be counseled concerning the following risks and requirements prior to the start of lenalidomide study therapy):

- She understands the potential teratogenic risk to the unborn child
- She understands the need for effective contraception, without interruption, 28 days before starting study treatment, throughout the entire duration of study treatment, dose interruption and 28 days after the end of study treatment
- She should be capable of complying with effective contraceptive measures
- She is informed and understands the potential consequences of pregnancy and the need to notify her study doctor immediately if there is a risk of pregnancy
- She understands the need to commence the study treatment as soon as study drug is dispensed following a negative pregnancy test
- She understands the need and accepts to undergo pregnancy testing based on the frequency outlined in this protocol
- She acknowledges that she understands the hazards and necessary precautions associated with the use of lenalidomide

The investigator must ensure that females of childbearing potential:

- Comply with the conditions for pregnancy risk minimization, including confirmation that she has an adequate level of understanding
- Acknowledge the aforementioned requirements

For a female NOT of childbearing potential, lenalidomide is contraindicated unless all of the following are met (i.e., all females NOT of childbearing potential must be counseled concerning the following risks and requirements prior to the start of lenalidomide study therapy):

- She acknowledges that she understands the hazards and necessary precautions associated with the use of lenalidomide

The effect of lenalidomide on spermatogenesis is not known and has not been studied. Therefore, male participants taking lenalidomide must meet the following conditions (ie, all males must be counseled concerning the following risks and requirements prior to the start of lenalidomide study therapy):

- Understand the potential teratogenic risk if engaged in sexual activity with a pregnant female or a female of childbearing potential
- Understand the need for the use of a condom even if he has had a vasectomy, if engaged in sexual activity with a pregnant female or a female of childbearing potential.

Contraception

Females of childbearing potential (FCBP) enrolled in this protocol must agree to use two reliable forms of contraception simultaneously or to practice complete abstinence from heterosexual contact during the following time periods related to this study: 1) for at least 28 days before starting study drug; 2) while participating in the study; 3) dose interruptions; and 4) for at least 28 days after study treatment discontinuation.

The two methods of reliable contraception must include one highly effective method and one additional effective (barrier) method. FCBP must be referred to a qualified provider of contraceptive methods if needed. The following are examples of highly effective and additional effective methods of contraception:

Highly effective methods:

- Intrauterine device (IUD)
- Hormonal (birth control pills, injections, implants)
- Tubal ligation
- Partner's vasectomy

Additional effective methods:

- Male condom
- Diaphragm
- Cervical Cap

Implants and levonorgestrel-releasing intrauterine systems are associated with an increased risk of infection at the time of insertion and irregular vaginal bleeding. Prophylactic antibiotics should be considered particularly in participants with neutropenia.

Pregnancy testing

Medically supervised pregnancy tests with a minimum sensitivity of 25 mIU/mL must be performed for females of childbearing potential, including females of childbearing potential who commit to complete abstinence, as outlined below.

Before starting study drug

Female Participants:

FCBP must have two negative pregnancy tests (sensitivity of at least 25 mIU/mL) prior to starting study drug. The first pregnancy test must be performed within 10-14 days prior to the start of study drug and the second pregnancy test must be performed within 24 hours prior to the start of study drug. The participant may not receive study drug until the study doctor has verified that the results of these pregnancy tests are negative.

Male Participants:

Must practice complete abstinence or agree to use a condom during sexual contact with a pregnant female or a female of childbearing potential while participating in the study, during dose interruptions and for at least 28 days following study drug discontinuation, even if he has undergone a successful vasectomy.

During study participation and for 28 days following study drug discontinuation

Female Participants:

- FCBP with regular or no menstrual cycles must agree to have pregnancy tests weekly for the first 28 days of study participation and then every 28 days while on study, at study discontinuation, and at day 28 following study drug discontinuation. If menstrual cycles are irregular, the pregnancy testing must occur weekly for the first 28 days and then every 14 days while on study, at study discontinuation, and at days 14 and 28 following study drug discontinuation.
- At each visit, the Investigator must confirm with the FCBP that she is continuing to use two reliable methods of birth control.
- Counseling about pregnancy precautions and the potential risks of fetal exposure must be conducted at a minimum of every 28 days.
- If pregnancy or a positive pregnancy test does occur in a study participant, study drug must be immediately discontinued.
- Pregnancy testing and counseling must be performed if a participant misses her period or if her pregnancy test or her menstrual bleeding is abnormal. Study drug treatment must be discontinued during this evaluation.
- Females must agree to abstain from breastfeeding during study participation and for at least 28 days after study drug discontinuation.

Male Participants:

- Counseling about the requirement for complete abstinence or condom use during sexual contact with a pregnant female or a female of childbearing potential and the potential risks of fetal exposure to lenalidomide must be conducted at a minimum of every 28 days.
- If pregnancy or a positive pregnancy test does occur in the partner of a male study participant during study participation, the investigator must be notified immediately.

Additional precautions

- Participants should be instructed never to give this medicinal product to another person and to return any unused capsules to the study doctor at the end of treatment.
- Participants should not donate blood during therapy and for at least 28 days following discontinuation of study drug.

- Male participants should not donate semen or sperm during therapy or for at least 28 days following discontinuation of study drug.
- **Only enough study drug for one cycle of therapy may be dispensed with each cycle of therapy.**

16.4 APPENDIX D: LENALIDOMIDE INFORMATION SHEET FOR PATIENTS ENROLLED IN CLINICAL RESEARCH STUDIES

Please read this lenalidomide Information Sheet before you start taking lenalidomide and each time you get a new supply, since there may be new information. This lenalidomide Information Sheet does not take the place of an informed consent to participate in clinical research or talking to your study doctor or healthcare provider about your medical condition or your treatment.

What is the most important information I should know about lenalidomide?

Lenalidomide may cause birth defects (deformed babies) or death of an unborn baby. Lenalidomide is similar to the medicine thalidomide. It is known that thalidomide causes life-threatening birth defects. Lenalidomide has not been tested in pregnant women but may also cause birth defects. Lenalidomide was found to cause birth defects when tested in pregnant rabbits.

If you are a female who is able to become pregnant:

Do not take lenalidomide if you are pregnant or plan to become pregnant

- for 28 days before starting lenalidomide
- while taking lenalidomide
- during dose interruptions of lenalidomide
- for 28 days after stopping lenalidomide

Stop taking lenalidomide if you become pregnant during lenalidomide treatment

Do not breastfeed while taking lenalidomide

You must have pregnancy testing done at the following times:

- within 10 – 14 days and again 24 hours prior to the first dose of lenalidomide
- weekly for the first 28 days
- every 28 days after the first month or every 14 days if you have irregular menstrual periods
- if you miss your period or have unusual menstrual bleeding
- 28 days after the last dose of lenalidomide (14 and 28 days after the last dose if menstrual periods are irregular)

You must either not have any sexual relations with a man or use two reliable, separate forms of effective birth control at the same time:

- for 28 days before starting lenalidomide
- while taking lenalidomide
- during dose interruptions of lenalidomide
- and for 28 days after stopping lenalidomide

The study doctor will be able to advise you where to get additional advice on contraception.

If you suspect you are pregnant at any time during the study, you must stop lenalidomide immediately and immediately inform your study doctor. The study doctor will report all cases of pregnancy to Celgene Corporation.

If you are a female not of childbearing potential:

In order to ensure that an unborn baby is not exposed to lenalidomide, your study doctor will confirm that you are not able to become pregnant.

If you are a male:

The effect of lenalidomide on sperm development is not known and has not been studied. The risk to the fetus in females of child bearing potential whose male partner is receiving lenalidomide is unknown at this time.

Male participants must either **not have any sexual relations with a female who can become pregnant or a pregnant female or must use a condom during sexual intercourse with a pregnant female or a female that can become pregnant** (including those who have had a vasectomy):

- While you are taking lenalidomide
- During dose interruptions of lenalidomide
- For 28 days after you stop taking lenalidomide

Male participants should not donate sperm or semen while taking lenalidomide and for 28 days after stopping lenalidomide.

If you suspect that your partner is pregnant any time during the study, you must immediately inform your study doctor. The study doctor will report all cases of pregnancy to Celgene Corporation.

Lenalidomide restrictions in sharing lenalidomide and donating blood:

- **Do not share lenalidomide with other people.** It must be kept out of the reach of children and should never be given to any other person.
- **Do not give blood** while you take lenalidomide and for 28 days after stopping lenalidomide.
- **Do not break, chew, or open lenalidomide capsules.**
- You will be supplied with no more than one cycle of lenalidomide
- Return unused lenalidomide capsules to your study doctor.

Additional information is provided in the informed consent form and you can ask your study doctor for more information.

16.5 APPENDIX E: PARTICIPANT DRUG ADMINISTRATION DIARY

Participant Name _____ Study ID _____

Please complete this form and return to the research nurse or doctor every cycle

You will take: **Lenalidomide** Dose: _____

DAY	DATE	TIME TAKEN	COMMENTS (side effects or missed doses)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

21			
22		Rest Day	
23		Rest Day	
24		Rest Day	
25		Rest Day	
26		Rest Day	
27		Rest Day	
28		Rest Day	

Participant Signature:_____

16.6 APPENDIX F: SELECT STRONG INDUCERS AND INHIBITORS OF CYP450 3A4

Strong Inducers of CYP3A4		
Carbamazepine	Nevirapine	Primidone
Dexamethasone	Oxcarbazepine	Rifabutin
Efavirenz	Phenobarbital	Rifampin
Ethosuximide	Phenylbutazone	Ritonavir
Glucocorticoids	Phenytoin	St. John's Wort
Griseofulvin	Prednisone	Topiramate

Strong Inhibitors of CYP3A4		
Atazanavir	Enoxacin	Nefazodone
Anastrozole	Erythromycin	Nelfinavir
Clarithromycin	Furafylline	Propoxyphene
Clotrimazole	Gestodene	Quinidine
Cobicstat	Grapefruit Juice	Quinupristin
Cyclosporine	Indinavir	Ritonavir
Dalfopristin	Isoniazid	Saquinavir
Delavirdine	Itraconazole	Tacrolimus
Diltiazem	Ketoconazole	Telithromycin
Efavirenz	Midazolam	Voriconazole

16.7 APPENDIX G: EPOCH ADMIXTURES: PREPARATION AND ADMINISTRATION NCI CC ONLY

Preparation

All 3-in-1 admixtures dispensed from the Pharmacy will contain a 24-hour supply of etoposide, doxorubicin, and vincristine, PLUS 40 mL overfill (excess) fluid and a proportional amount of drug to compensate for volume lost in parenteral product containers and administration set tubing.

Etoposide Dose	Volume of Fluid Containing a Daily Dose	Volume of Overfill (fluid + drug)	Total Volume in the Product (including overfill)
≤ 130 mg	528 mL	40 mL	568 mL
> 130 mg	1056 mL	40 mL	1096 mL

Before dispensing 3-in-1 admixtures, Pharmacy staff will:

[1] Attach an administration set appropriate for use with a portable pump,

[2] Purge all air from the drug product container and administration set tubing,

[3] The administration set will be primed close to its distal tip, and

[4] The administration set tubing will be capped with a Luer-locking closed system transfer device cap (e.g., Spinning Spiros® Closed Male Luer Red Cap) before the product is dispensed form the Pharmacy.

Pre-printed product labeling will identify the 'Total Volume To Infuse' and the 'Volume of Overfill (fluid + drug)'.

Completed drug supplies will be replaced daily for four consecutive days to complete a 96-hour drug infusion (unless treatment is interrupted or discontinued due to unanticipated events).

Administration

Portable pumps used to administer etoposide + doxorubicin + vincristine admixtures will be programmed to deliver one of two fixed volumes at one of two corresponding fixed rates based on the amount of etoposide and fluid that is ordered (see the table, below).

Etoposide Dose	Total Volume to Infuse per 24 hours	Volume of Overfill (drug-containing fluid)*	Administration Rate
≤ 130 mg	528 mL	40 mL	22 mL/hour
> 130 mg	1056 mL	40 mL	44 mL/hour

* DO NOT attempt to infuse the overfill.

At the end of an infusion, some residual fluid is expected because overfill (excess fluid and drug) was added; however, nurses are asked to return to the Pharmacy for measurement any drug containers that appear to contain a greater amount of residual drug than expected.

Example at right: The amount of fluid remaining in a bag after completing a 24-hour infusion (1056 mL delivered).