

# Amendment

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Protocol Title: Pilot Study of Liposomal Doxorubicin Combined with Bevacizumab Followed by Bevacizumab Monotherapy in Adults with Advanced Kaposi's Sarcoma

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\*\* I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

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**Title:** Pilot Study of Liposomal Doxorubicin Combined with Bevacizumab Followed by Bevacizumab Monotherapy in Adults with Advanced Kaposi's Sarcoma

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**Commercial Agents:** Bevacizumab and Liposomal Doxorubicin

## PRÉCIS

### Background:

- Standard treatment for advanced Kaposi's sarcoma (KS) is a liposomal anthracycline, plus antiretroviral therapy (HAART) in patients with HIV.
- KS is not curable and relapses are common. Prolonged use of liposomal anthracyclines with cumulative dosing exceeding 550 mg/m<sup>2</sup> is frequently required.
- KS is notable for pathogenic autocrine and paracrine VEGF signaling. The monoclonal antibody, bevacizumab is a rational agent for the treatment of KS.
- Preliminary results from our phase II study of bevacizumab monotherapy, 03-C0110, suggest that bevacizumab has promising activity in the treatment of KS.
- The combination of anti-angiogenic therapy with cytotoxic chemotherapy has been a successful strategy in KS as well as other solid tumors.
- This pilot study will evaluate the activity and safety of liposomal doxorubicin combined with bevacizumab followed by bevacizumab maintenance in patients with advanced KS. A goal of this combination strategy is to develop a tolerable and highly active regimen that would limit the need for prolonged anthracycline use.

### Objectives:

- The primary objective is to estimate the overall response rate (ORR) of six cycles of liposomal doxorubicin combined with bevacizumab in patients with advanced KS.

### Eligibility:

#### Inclusion criteria:

- Age ≥18
- Biopsy proven KS
- Indication for chemotherapy
- Any HIV status
- Normal MUGA
- Able to tolerate aspirin 81 mg
- SBP <150, DBP <90
- Urine protein <1+ or 500mg/24hrs

#### Exclusion Criteria:

- Surgery within 4 weeks
- Thrombo-embolic disease
- Chemotherapy within 3 weeks
- Hemoptysis or severe gastrointestinal bleeding, unless caused by KS
- Pregnancy or breast feeding

### Design:

- This is an open label, single center pilot with 2 cohorts. **Cohort 1:** HIV negative, HIV infected with stable KS despite 1 year of HAART with HIV viremic control, or HIV infected with progressive KS despite 4 months of HAART with HIV viremic control. **Cohort 2:** All other patients with advanced AIDS-associated KS.

- Subjects will receive bevacizumab 15 mg/kg and liposomal doxorubicin 20 mg/m<sup>2</sup> every 3 weeks until complete response (CR) or a maximum of 6 cycles. Those with stable disease or better will continue on bevacizumab 15 mg/kg monotherapy every 3 weeks for 11 cycles. HIV infected subjects will receive HAART.
- ORR will be calculated with 80% CI for each cohort separately. If estimates in the two cohorts are similar ( $p > 0.30$  by a Fisher's exact test), they may be combined to form a somewhat more precise estimate of ORR after 6 cycles of treatment.
- A total of 10 evaluable patients will be accrued in each cohort.

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## 1. INTRODUCTION

### 1.1 STUDY OBJECTIVES

#### 1.1.1 Primary

- 1.1.1.1 Estimate the overall response rate of liposomal doxorubicin combined with bevacizumab after six 3-week cycles in patients with advanced KS.

#### 1.1.2 Secondary

- 1.1.2.1 Assess the safety and toxicities of liposomal doxorubicin combined with bevacizumab in patients with advanced KS
- 1.1.2.2 Estimate the complete response rate of liposomal doxorubicin combined with bevacizumab after six 3-week cycles in evaluable patients with advanced KS
- 1.1.2.3 Estimate the median number of cycles of liposomal doxorubicin combined with bevacizumab followed by bevacizumab alone required to achieve a partial response in evaluable patients with advanced KS
- 1.1.2.4 Estimate 12-month progression-free survival in evaluable patients with advanced KS treated with liposomal doxorubicin combined with bevacizumab followed by 11 cycles of bevacizumab
- 1.1.2.5 Evaluate the impact of liposomal doxorubicin combined with bevacizumab on CD4 counts
- 1.1.2.6 Evaluate the impact of liposomal doxorubicin combined with bevacizumab, followed by 11 cycles of bevacizumab maintenance, on peripheral blood mononuclear cell (PBMC) and saliva KSHV viral load
- 1.1.2.7 Explore the short-term effects of bevacizumab and intermediate and long term effects of the combination of bevacizumab and liposomal doxorubicin on blood flow to cutaneous KS lesions using non-invasive imaging techniques

### 1.2 BACKGROUND AND RATIONALE

Kaposi's Sarcoma (KS) is a multifocal angioproliferative malignancy histologically consisting of proliferative endothelial cells with enhanced vascular permeability, an inflammatory component, and a proliferation KSHV infected spindle cells. The  $\gamma$ -herpes virus, Kaposi's sarcoma-associated herpes virus (KSHV), also known as human herpesvirus-8 (HHV-8), is a necessary but insufficient cause of KS, while HIV is an important co-factor, which markedly increases the risk for the development of KS.

#### 1.2.1 Background on Liposomal Doxorubicin for the Treatment of KS

First line therapy for HIV-associated KS is combination anti-retroviral therapy (HAART). The effectiveness of HAART in the management of KS is likely due to control of HIV viremia and improved KSHV specific cellular immunity<sup>1,2</sup>. Regression of KS lesions has been reported with HAART. However, in patients with advanced or symptomatic disease, chemotherapy is indicated. Cytotoxic agents with modest efficacy in KS include bleomycin, vincristine, vinblastine, doxorubicin, and etoposide, whereas paclitaxel<sup>3,4</sup> and liposomal anthracyclines<sup>5,6</sup> have been demonstrated to have improved response rates over older agents. Liposomal preparation of doxorubicin may improve delivery of doxorubicin to the vascular lesions of KS<sup>7</sup>,

and the addition of liposomal doxorubicin has been demonstrated to be effective in leading to biopsy demonstrated regression of KS spindle-cells<sup>8</sup>. Currently, liposomal anthracyclines are the standard-of-care first line cytotoxic agent based on phase III studies (conducted prior to widespread availability of HAART) demonstrating overall response rates (ORR) of 46-59%, which were superior to combination chemotherapy. Liposomal anthracyclines also offer reduced toxicity<sup>5,6</sup> and improved quality-of-life compared to doxorubicin, bleomycin and vincristine (ABV)<sup>9</sup>. Studies of liposomal doxorubicin conducted after the introduction of HAART have demonstrated an ORR of 55-76%<sup>10-12</sup>, and liposomal doxorubicin has retained its high therapeutic index in long term follow-up of patients treated in the HAART era. However, KS is not curable, and one-year progression-free survival with either liposomal doxorubicin until best response (median number of cycles = 9) or 6 cycles of liposomal doxorubicin plus IL12 followed by IL-12 maintenance is approximately 70%<sup>11,13</sup>. Long-term administration of chemotherapy is often required. An approach to advanced KS that employs combination bio-chemotherapy followed by biologic therapy alone was established in our phase II study of liposomal doxorubicin combined with IL12 followed by IL12 alone. In this study, the median number of cycles required to obtain a partial response was 2 cycles, and seven of the nine complete responses observed in this study occurred while subjects were receiving IL12 monotherapy<sup>13</sup>.

Overall survival of patients with KS has improved dramatically since the introduction of HAART in 1996<sup>14</sup>. While death from KS is rare in HIV-infected individuals in North America and Europe<sup>15</sup>, patients with advanced KS, especially HIV-associated KS, are a population at risk for second malignancies or death. Based on SEER data from 1980-200, patients with HIV-associated KS have are 9 times more likely to develop a second cancer than the general population, and 75 times more likely to develop non-Hodgkin's lymphoma<sup>16</sup>. Estimates of death due to KS in the HAART era are available from recent KS treatment studies as well as from the Mortalité 2000 Study Group, which performed a national survey of causes of death among people with HIV performed in France in 2000<sup>17</sup>. For patients that require chemotherapy, approximately 3-8% of patients will die of KS within 12 months, despite liposomal doxorubicin based therapy.<sup>11,13</sup> Furthermore, an additional 9% died of second malignancies during phase IV evaluation of liposomal doxorubicin.<sup>11</sup> In the Mortalité 2000 Study of 964 deaths among people with HIV in France in 2000, 28% of all deaths were related to malignancy, and 15% of malignant deaths were due to KS, 39% were due to NHL, 1% was due to cervical cancer, and 45% were due to non-AIDS defining malignancies<sup>17</sup>.

Given the dramatically improved survival of patients with HIV after the introduction of HAART and the tendency for KS to recur, long-term toxicities must be considered. While cardiotoxicity associated with liposomal doxorubicin is less frequent than that with doxorubicin, long-term treatment with liposomal doxorubicin (at 20mg/m<sup>2</sup> per cycle) in the management of KS often leads to cumulative dosing greater than 550 mg/m<sup>2</sup><sup>11</sup>. The long-term cardiac toxicity of prolonged exposure to liposomal doxorubicin in this population is unknown. Symptomatic heart failure occurred in less than 1% of 754 patients with KS treated on clinical trials. (Doxil® prescribing information). However changes in echocardiograph findings have been noted in 11% of women with breast cancer treated with 50 mg/m<sup>2</sup> of liposomal doxorubicin to a cumulative dose of > 450 mg/m<sup>2</sup>, which has lead to a Black Box Warning for cumulative liposomal doxorubicin doses approaching 550mg/m<sup>2</sup>. (Doxil® prescribing information). Additionally, endomyocardial biopsies performed on patients who had received cumulative doses > 400 mg/m<sup>2</sup> of liposomal doxorubicin for the treatment of KS demonstrated early

(Billingham scale Grade 1) myocyte damage in 3 of 10 patients. Novel anthracycline sparing approaches are therefore needed.

### 1.2.2 Background on Targeting Angiogenesis in KS

Rational approaches to the targeted treatment of KS exploit pro-angiogenic signaling pathways that are central to the molecular pathogenesis of KSHV related malignancies. Multiple preclinical models and evaluation of KS biopsy samples have demonstrated that KS depends on autocrine and paracrine signaling through multiple pathways. Early work demonstrated that various KS cell lines expressed bFGF mRNA levels 6-8 times that of HUVEC cells. Cultured media from KS cell lines was able to stimulate both HUVEC and KS cell growth, while anti-bFGF Ab inhibited this paracrine signaling<sup>18</sup>. Shortly thereafter, it was noted that KS3 and KS4 cell lines, derived from pleural effusions, also expressed elevated levels VEGF<sub>121</sub> and VEGF<sub>165</sub>, two isoforms of VEGF-A derived from alternative splicing<sup>19</sup>. Using in-situ radiolabeled mRNA probes in biopsy samples from AIDS-KS cases, VEGF<sub>165</sub> mRNA was increased in spindle shaped cells (compared to granulation tissue) but not in tumor endothelial cells.

Immunohistochemistry for VEGF<sub>165</sub>, on the other hand, demonstrated high levels on the endothelial cells<sup>20</sup>. As the case with bFGF, cultured media of AIDS-KS cell lines also has high levels of VEGF-A, and anti-VEGF antibodies inhibit the HUVEC proliferation that occurs in the presence of AIDS-KS cell line cultured media. Furthermore, anti-bFGF Ab and anti-VEGF-A antibodies are synergistic in inhibiting paracrine signaling<sup>21</sup>. Evaluation of VEGFR-1 and VEGFR-2, the receptors for VEGF-A, in KS preclinical models and KS biopsies support the role of VEGF-A autocrine and paracrine signaling in the pathogenesis of KS. In immunohistochemical stains of KS biopsies, stromal vessels stain strongly for VEGFR-1 and VEGFR-2 while spindle cells had strong expression of VEGFR-2<sup>22</sup>. KS cell lines express elevated levels of VEGF-A mRNA, and both cell lines and KS tumor samples express increased VEGFR-1 and VEGFR-2 mRNA compared to normal skin biopsies<sup>23</sup>. In addition to up-regulation of VEGFR-2 in KS cell lines, the cultured media from KSHV infected BMEC lead to upregulation of VEGFR-2 in uninfected HUVEC<sup>24</sup>. VEGF-A siRNA inhibits KS cell proliferation<sup>23</sup>. Evaluation of various PEL cell lines confirms up-regulation of VEGF-A, VEGFR-1 and VEGFR-2 mRNA, and anti-VEGF-A antibodies inhibit the formation of PEL in a nude mouse xenograph model<sup>25</sup>. Antibodies against VEGFR-2, VEGF-A siRNA or VEGFR-2 siRNA inhibit cell proliferation, again supporting the importance of autocrine signaling<sup>26</sup>. Together, this data demonstrates the importance of paracrine and autocrine VEGF-A signaling, and provides evidence of upregulation of VEGF-A, VEGFR-1 and VEGFR-2 in both the spindle cell and endothelial cell compartments of KS lesions.

Additionally, VEGFR-3, a receptor whose expression is generally limited to lymphoid endothelial cells, has been evaluated in KS59 and KS35 cell lines. Immunohistochemistry and Western blot demonstrates robust VEGFR-3 expression in these cell lines<sup>27, 28</sup>.

Immunohistochemical and radiolabeled staining of KS biopsy samples confirmed VEGFR-3 expression on spindle cells but not endothelial cells.<sup>28-30</sup> When KSHV infected HUVEC cell cultures are compared to uninfected HUVEC cultures, increases in mRNA by RT-PCR for VEGF A, B, C, and D as well as VEGFR-1, VEGFR-2, and VEGFR-3 are noted, and IHC confirmed the strong expression of all three receptors in the KSHV infected HUVEC cells<sup>26</sup>. For the spindle cells in KS, additional proliferative pathways through VEGFR-3 appear to also be important.

Evaluation of KSHV lytic and latent genes suggests that the virus has developed redundant mechanisms for up-regulation of cellular VEGF. Evaluation of several viral genes, including v-GPCR, v-IL-6, LANA, and K1 offer some insight into the signaling pathways involved. The v-GPCR, encoded by ORF74, is a constitutively activated lytic gene most closely related to the chemokine receptor CXCR2. Transfection of NIH3T3 cells or a PEL cell line (BC3) with v-GPCR leads to cells that produce VEGF-A<sup>31,32</sup>. Cultured media from these transfected cells support HUVEC cell cultures, while anti-VEGF-A antibodies inhibit proliferation. Furthermore, inoculation of these transfected cells in nude mice leads to the formation of vascular tumors with histological features of KS. Evaluation of intracellular signaling cascades using kinase assays demonstrate that v-GPCR induces JNK and p38 but not MAPK<sup>31</sup>. A VEGF-A luciferase reporter in this model demonstrates that the upregulation of VEGF depends on a hypoxia response element (HRE) in the VEGF promoter. v-GPCR stimulated upregulation of VEGF is associated with p38 phosphorylation and stabilization of HIF-1<sup>33</sup>. HUVEC cell lines transfected with v-GPCR also maintain high mRNA expression of VEGFR-2, and these cells undergo apoptosis when exposed PTK787, a small molecule tyrosine kinase inhibitor that inhibits all VEGF receptors<sup>34</sup>.

v-IL6 is a second lytic gene product with 25% amino acid homology to human IL-6. Transfection of v-IL6 into NIH 3T3 also leads to 6-8 fold increases in VEGF-A mRNA. Subcutaneous injection of v-IL6 transfected NIH3T3 cells in nude mice also leads to spindle shaped tumors<sup>35</sup>.

The K1 gene of KSHV encodes a transmembrane glycoprotein related to the immunoglobulin receptor family with a constitutively active signaling through an intracellular immunoreceptor tyrosine-based activation motif (ITAM). K1 transgenic mice develop plasmacytoid and sarcomatoid tumors<sup>36</sup>, and the lymph nodes from the K1 transgenic mouse show high levels of VEGF-A by immunohistochemistry. A lymphoma cell line derived from tumors from the K1 transgenic mouse, KVL-1, produce high levels of VEGF in cultured supernatants. Transfection of K1 into various B-cell lymphoma cell lines also lead to increased VEGF compared to the same cell lines transfected with a K1-mutant that lacked the ITAM sequence<sup>37</sup>. In an endothelial cell model, K1 transduction into HUVECs leads to immortalized cell that produce high levels of VEGF-A through increased transcriptional activity. As in the KLV-1 model, increases in supernatant VEGF are only seen with transfection with wild-type K1 and not K1 mutation expression plasmids<sup>38</sup>. Co-immunoprecipitation and western blot studies suggest that K1 transfected endothelial cells activate VEGFR-2, with downstream signaling occurring through the PI3K/Akt pathway. KS tissue array from the AIDS and Cancer Specimen Resource was stained with polyclonal anti-K1 antibody. While some tumors only expressed LANA, others expressed both LANA and K1<sup>39</sup>, supporting the potential role of K1 in the pathogenesis of AIDS-associated KS.

LANA, which is a latent KSHV gene may also have an effect on VEGF transcription. 293T cells transduced with LANA have a dose dependent increase VEGF-A transcription and increased VEGF-A in cell culture supernatant<sup>40</sup>. The ability of LANA to affect VEGF-A expression may be due to effects on HIF-1. Co-immunoprecipitation assays demonstrate that LANA interacts with HIF-1 $\alpha$  in transduced 293T cells, and immunofluorescence studies show that LANA and HIF-1 co-localize in the nucleus of the PEL cell line, BCBL-1. In 293T cells transfected with LANA, HIF-1 promoter luciferase assays and RT-PCR demonstrate enhanced transcription of

HIF-1 $\alpha$  over vector alone 293T cells. These studies suggest that LANA is a regulator of HIF-1 $\alpha$ , and may enhance HIF-1 $\alpha$  transcriptional activating activity in the setting of hypoxia<sup>41</sup>.

Evaluation of these molecular pathways offer insight into the redundant mechanisms by which KSHV infection may lead to angiogenic and proliferative signaling through VEGF-A in KS, and provides rationale for targeting VEGF-A in the treatment of KS.

Anti-angiogenic approaches to the treatment of KS are also supported through early phase clinical studies conducted by the HIV and AIDS Malignancy Branch (HAMB). Phase I and II studies conducted by HAMB have evaluated monotherapy with thalidomide<sup>42</sup> and IL-12<sup>43</sup>, agents with pleiotropic effects that include potential anti-angiogenic mechanisms of action. In these studies, we demonstrated ORRs of 40-70%. More recently, we have been evaluating approaches employing anti-VEGF monoclonal antibodies in a Phase II study of bevacizumab (03-C-0110) in patients with classical or AIDS-associated KS that was stable or progressing on HAART. Bevacizumab (rhUMAb VEGF) is a recombinant humanized anti-VEGF monoclonal antibody composed of human IgG1 framework regions and antigen-binding complementarity-determining regions from a murine monoclonal antibody (muMAb VEGF A.4.6.1) that blocks binding of human VEGF-A to its receptors. Approximately 93% of the amino acid sequence, including most of the antibody framework, is derived from human IgG1, and ~7% of the sequence is derived from the murine antibody. In vitro binding studies have shown it to consistently and potently neutralize the biologic activities of human VEGF-A, including the endothelial cell mitogenic activity, the vascular permeability-enhancing activity, and the angiogenic properties in the chick chorioallantoic membrane<sup>44</sup>. This antibody has been shown to recognize all isoforms of VEGF-A, with a  $K_d$  of  $\sim 8 \times 10^{-10}$  M. muMAb VEGF A.4.6.1 is specific for VEGF-A; it fails to recognize other peptide growth factors tested (fibroblast growth factor, epidermal growth factor, hepatocyte growth factor, platelet-derived growth factor, and nerve growth factor).

### 1.2.3 Rationale for Combination of Liposomal Doxorubicin with Bevacizumab in Patients with Advanced KS

Based on the rationale of targeting VEGF in the treatment of KS, we have been conducting a phase II study of the humanized anti-VEGF monoclonal antibody, bevacizumab, in patients with KS. Study subjects are administered bevacizumab 15 mg/kg every 3 weeks. Interim review of our phase II study of single agent bevacizumab, which has enrolled 17 patients, demonstrates a somewhat promising ORR, preliminarily estimated at 22%, with 1-year progression free survival estimated at 40% (Unpublished data). As shown in Table 1, bevacizumab was reasonably well tolerated in this setting. While the response rate of bevacizumab monotherapy in KS is modest compared to cytotoxic therapy, this level of activity is comparable to that seen in other solid malignancies when used as monotherapy. Phase II studies that have evaluated bevacizumab monotherapy have demonstrated ORR of 16-21% in platinum resistant ovarian cancer<sup>45, 46</sup>, 10% in patients with renal cell carcinoma that progressed after IL-2<sup>47</sup>, and 7% in previously treated breast cancer<sup>48</sup>.

Table 1. Selected Toxicities of bevacizumab monotherapy in patients with Kaposi's sarcoma

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
HTN	6%		33%	
Proteinuria	56%	39%		
Epistaxis	44%			
Hematuria	67%			
Neutropenia		50%	33%	
Neutropenia +Infection			11%	
≥ 1 Other *			56%	11%

(Yarchoan, et. al., Unpublished)

\* Additional Grade 3 and 4 toxicities included: dizziness, CPK elevations, headache, rash, alkaline phosphatase elevations, hypophosphatemia, and hyperglycemia. One patient had multiple grade 4 toxicities related to a prolonged hospitalization for necrotizing fasciitis.

Despite the modest response rates when bevacizumab is used as monotherapy, the clinical benefit of bevacizumab when combined with cytotoxic agents, has been demonstrated in several other solid tumors. Bevacizumab increases progression-free survival when used alone or in combination with interferon- $\alpha$  in patients with renal cell carcinoma<sup>47, 49</sup> or in combination with paclitaxel (but not capecitabine<sup>50</sup>) in patients with previously treated breast cancer<sup>51</sup>. Furthermore, the addition of bevacizumab to standard cytotoxic regimens in both lung and colorectal cancer has led to improvements in progression-free and overall survival when compared to cytotoxic therapy alone<sup>52-55</sup>. Our previous phase II study of liposomal doxorubicin with IL-12<sup>13</sup> demonstrated an ORR of 83%, further supporting the rationale of combination anti-angiogenic and cytotoxic chemotherapy in advanced KS. Given a strong preclinical rationale, and the promising activity of bevacizumab as monotherapy in patients with KS that was stable or progressing on HAART, we hypothesize that a combination of liposomal doxorubicin, which has demonstrated efficacy in causing tumor regression, with bevacizumab that targets VEGF signaling, will provide a platform that may lead to improved overall response rates, time to response and progression free survival in patients. This combination could potentially offer an important therapeutic option for patients with advanced and difficult to control KS.

We further hypothesize that in addition to being a highly active combination, that liposomal doxorubicin combined with bevacizumab, at the dosing schedule used in our phase II study, will be safe and tolerable, and may potentially decrease anthracycline requirements in the management of patients with advanced KS.

Several phase II studies have evaluated the combination of doxorubicin with bevacizumab in other tumors. Ganjoo et. al. reported 13 patients with diffuse large B-cell lymphoma treated with bevacizumab 15mg/kg combined with the R-CHOP regimen (rituximab, cyclophosphamide, vincristine, doxorubicin and prednisone). Two of the 13 patients developed grade 3 hypertension, and 2 of 13 developed deep vein thromboses. No grade 3 or grade 4 proteinuria, heart failure or hemorrhage was observed with this regimen<sup>56</sup>. Wedham et. al treated 21 women with newly diagnosed inflammatory and local advanced breast cancer with neo-adjuvant bevacizumab 15 mg/kg in combination with doxorubicin 50 mg/m<sup>2</sup> and docetaxel 75 mg/m<sup>2</sup>. Grade 3 hypertension was observed in 8 patients, and 5 patients had surgical wound healing complications. Other toxicities were acceptable. Two patients had an asymptomatic decrease in left ventricular ejection fraction after a cumulative doxorubicin dose 300 mg/m<sup>2</sup>. Of the 2 patients, one had normalization of her ejection fraction 3 weeks after completing therapy, while the other had normalization in 6 months after discontinuing therapy and starting an angiotensin converting enzyme inhibitor<sup>57</sup>. Lastly, D'Amato et. al. evaluated the combination of bevacizumab 15 mg/kg combined with bolus doxorubicin 75 mg/m<sup>2</sup> as second-line therapy in 17 patients with metastatic sarcoma. Patients were evaluated by echocardiogram every 2 cycles, and 4 patients were removed from study due to asymptomatic Grade 2 decreases in ejection fraction, while 1 patient developed grade III congestive heart failure after 11 cycles and 1 patient grade IV congestive heart failure after 8 cycles of the combination. In 5 of these 6 patients, the ejection fraction improved after cessation of therapy. Overall, this data suggests that the combination may be associated with reversible asymptomatic decreases in ejection fraction at lower cumulative doses of anthracycline. Cardiac toxicity associated with cumulative dosing of bolus anthracyclines > 450 mg/m<sup>2</sup> and delayed wound healing must be considered in the evaluation of the combination of liposomal doxorubicin with bevacizumab. However, these phase II combination studies suggest an acceptable toxicity profile. Further combination regimens that include liposomal doxorubicin with bevacizumab are currently being evaluated in breast cancer (NCT00635050 and NCT00608972), and are planned for platinum sensitive ovarian cancer (NCT00698451).

#### 1.2.4 Bleeding and Thrombotic risk with bevacizumab in Relation to Patients with Kaposi Sarcoma and/or HIV

Bevacizumab has been associated with both bleeding and thrombosis in clinical studies. The risk of these appears to depend in part on tumor type and location, and the optimal strategy to mitigate these risks, including the possible role of thromboprophylaxis, remains unclear.

Overall, grade 3 and 4 events associated with bleeding or hemorrhage were observed in 4.0% of 1,132 patients treated with bevacizumab in a pooled database from eight phase 1, 2, and 3 clinical trials in multiple tumor types. The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage and minor mucocutaneous hemorrhage. Major or massive pulmonary hemorrhage/hemoptysis has been observed primarily in patients with squamous cell NSCLC, and the majority of these occurred in patients with additional bleeding risk factors such as tumor cavitation and/or necrosis<sup>53</sup>. Serious tumor associated bleedings have also been observed in patients with pancreatic cancer, gastric cancer, central nervous system (CNS) metastases, hepatoma, or varices treated with bevacizumab. GI hemorrhages, including rectal bleeding and melena have similarly been reported in patients with colorectal cancer<sup>52</sup>.

Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated. These were most commonly NCI-CTC Grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention, and did not require any changes in bevacizumab treatment regimen. There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

The risk of arterial thromboembolic events (ATE) is increased with bevacizumab therapy; such events included cerebral infarction, transient ischemic attack (TIA), myocardial infarction (MI), and other peripheral or visceral arterial thrombosis<sup>60</sup>. A pooled analysis of five randomized studies showed a two-fold increase in these events (3.8% vs. 1.7%)<sup>60</sup>. ATE led to a fatal outcome in 0.8% patients with bevacizumab (vs. 0.5% without bevacizumab). The rate of cerebrovascular accidents (including TIA) was 2.3% vs. 0.5%, and the rates of MI 1.7% vs. 0.7%. Certain baseline characteristics, such as age and prior arterial ischemic events, appear to confer additional risk. In patients >65 years treated with bevacizumab and chemotherapy, the rate of ATE was approximately 8.5%. Aspirin is a standard therapy for primary and secondary prophylaxis of ATE in patients at high risk of such events, and the use of aspirin  $\leq$ 325 mg daily was allowed in randomized studies of bevacizumab, though safety analyses specifically regarding aspirin use were not preplanned. Due to the relatively small numbers of aspirin users and ATE events, retrospective analyses of the ability of aspirin to affect the risk of ATE were inconclusive. Further analyses of the effects of concomitant use of bevacizumab and aspirin are ongoing.

In the phase 3 trial in metastatic colorectal cancer, there was also a slightly higher rate of venous thromboembolic events (VTE), including deep venous thrombosis, pulmonary embolism, and thrombophlebitis, in patients treated with chemotherapy plus bevacizumab compared with chemotherapy alone (19% vs. 16%)<sup>52</sup>. The incidence of NCI-CTC Grade  $\geq$ 3 VTEs in one NSCLC trial (E4599) was higher in the bevacizumab-containing arm compared to the chemotherapy control arm (5.6% vs. 3.2%). In clinical trials across all indications, the overall incidence of VTEs ranged from 2.8% to 17.3% in the bevacizumab-containing arms compared to 3.2% to 15.6% in the chemotherapy control arms. The use of bevacizumab with chemotherapy does not substantially increase the risk of VTE compared with chemotherapy alone. However, patients with metastatic colorectal cancer who receive bevacizumab and experienced VTE may be at higher risk for recurrence of VTE.

The baseline risks of bleeding and/or thrombosis associated with KS and HIV play an important part in estimating the potential risk of bleeding and/or thrombosis with bevacizumab therapy, but for KS these risks are not well established. HIV infection is established as a thrombotic risk factor (particularly when poorly controlled or associated with comorbidities), and an elevated risk of bleeding has not been reported in patients with HIV<sup>61-62</sup>. In KS, in our clinical practice mild bleeding from involved sites has been observed in patients with GI and/or pulmonary KS (Yarchoan, personal communication), but bleeding rates have not been estimated. Such bleeding is commonly asymptomatic (i.e. noted by stool guaic only), and usually responds promptly with institution of KS-directed therapy. Evidence regarding thrombotic risk in KS is mixed. One case series in the pre-HAART era, of patients with relatively severe KS, reported an increased incidence of thrombosis in limbs affected by KS<sup>63</sup>. This has not been confirmed, and in more recent studies of thrombotic risk in patients with HIV, KS has not emerged as an independent risk factor<sup>61,62,64</sup> (also, Musselwhite and Sereti, personal communication). One case-control study in post-transplant KS showed an increased occurrence of DVT in KS cases, but the small number

of cases and long time elapsed between KS and DVT (over 2 years) raise the possibility that this finding was coincidental<sup>65</sup>. Nonetheless, it would not be unexpected that the peripheral venous stasis and endothelial damage that may be seen in patients with extensive KS, combined with effects of HIV in those with HIV-associated disease, could elevate thrombotic risk. One superficial vein thrombosis was seen over 202 cycles of therapy in the HAMB phase 2 study of bevacizumab in KS (04-C-0110). Bleeding in that study included epistaxis (5%) and heavy menses (1 patient). Given the generally mild nature of observed bleeding, possible elevated thrombotic risk with HIV and KS, and potential severity of thrombotic complications, we will employ thromboprophylaxis with aspirin for all study subjects. This practice is common in current bevacizumab studies. Patients with recent bleeding not directly related to KS and/or a history of bleeding diatheses will not be eligible, nor will patients with known procoagulant disorders.

## **2. ELIGIBILITY ASSESSMENT AND ENROLLMENT**

### **2.1 ELIGIBILITY CRITERIA**

#### 2.1.1 Inclusion Criteria

- 2.1.1.1 Age  $\geq$  18 years
- 2.1.1.2 Kaposi's sarcoma pathologically confirmed by CCR pathology
- 2.1.1.3 Evaluable KS involving the skin and/or viscera, including at least one of the following:
  - 2.1.1.3.1 KS of the skin with  $\geq$  5 KS lesions that are evaluable by non-invasive methods that have not been treated with local therapeutic modalities
  - 2.1.1.3.2 Pulmonary KS evaluable by CT scan
  - 2.1.1.3.3 Gastrointestinal KS evaluable by direct visualization or fiberoptic instrumentation
  - 2.1.1.3.4 Biopsy proven lymph node involvement measurable by CT scan
- 2.1.1.4 ECOG performance status  $\leq$  2
- 2.1.1.5 Life expectancy  $>$  6 months
- 2.1.1.6 At least one of the following indications for therapy:
  - 2.1.1.6.1 Pulmonary involvement
  - 2.1.1.6.2 Visceral involvement
  - 2.1.1.6.3 Pain
  - 2.1.1.6.4 Edema
  - 2.1.1.6.5 Substantial lymph node involvement
  - 2.1.1.6.6 Ulcerating lesions
  - 2.1.1.6.7 Decreased range of joint motion due to KS
  - 2.1.1.6.8 Multiple lesions not amenable to local therapy
  - 2.1.1.6.9 Significant psychological impact leading to social withdrawal

- 2.1.1.7 Patients with HIV infection must be willing to comply with a regimen of highly active antiretroviral therapy (HAART).
  - 2.1.1.8 Patients may have received any number of prior therapies, including monotherapy with liposomal doxorubicin or bevacizumab
  - 2.1.1.9 Blood pressure
    - 2.1.1.9.1 SBP < 150 mm/Hg
    - 2.1.1.9.2 DBP < 90 mm/Hg
    - 2.1.1.9.3 Patients receiving anti-hypertensive medicines must be on a stable regimen for at least 1 month
  - 2.1.1.10 EF > 50% by MUGA
  - 2.1.1.11 The following hematologic parameters:
    - 2.1.1.11.1 Hemoglobin > 9 g/dl
    - 2.1.1.11.2 WBC > 1000/mm<sup>3</sup>
    - 2.1.1.11.3 ANC > 750/mm<sup>3</sup>
    - 2.1.1.11.4 Platelets > 75,000/mm<sup>3</sup>
    - 2.1.1.11.5 PT and PTT ≤ 120% of control, unless patient has the presence of a lupus anticoagulant
  - 2.1.1.12 The following hepatic parameters:
    - 2.1.1.12.1 Bilirubin ≤ 1.5 X ULN unless the patient is receiving protease inhibitor therapy (i.e. indinavir, ritonavir, nelfinavir, and atazanavir) known to be associated with increased bilirubin: in this case total bilirubin ≤ 7.5 mg/dl and the direct fraction is ≤ 0.7 mg/dl.
    - 2.1.1.12.2 AST/GOT ≤ 2.5 times the upper limit of normal
  - 2.1.1.13 Either serum creatinine ≤ 1.5 mg/dL or measured creatinine clearance ≥ 60 mL/min
  - 2.1.1.14 Either urine protein < 1+ or measured 24 hour urine protein < 500 milligram
  - 2.1.1.15 Able to take aspirin 81mg daily.
  - 2.1.1.16 Study participant must use birth control measure prior to study entry (during screening), during study participation, and for 12 weeks after bevacizumab is discontinued.
  - 2.1.1.17 Inclusion of women and minorities: Both men and women and members of all races and ethnic groups are eligible for this trial.
- 2.1.2 Exclusion Criteria
- 2.1.2.1 Inability to provide informed consent
  - 2.1.2.2 KS therapy other than HAART within 3 weeks
  - 2.1.2.3 History of cumulative doxorubicin or liposomal doxorubicin dose > 430 mg/m<sup>2</sup>

- 2.1.2.4 Supraphysiologic doses of corticosteroids within 3 weeks
- 2.1.2.5 Major surgical procedure (including periodontal) within 4 weeks
- 2.1.2.6 Surgical or other non-healing wounds, other than KS ulcers
- 2.1.2.7 Pregnancy (because of unknown potential for fetal malformation)
- 2.1.2.8 Breast feeding (because of unknown potential for adverse infant developmental consequences)
- 2.1.2.9 Has an uncontrolled illness including, but not limited to, ongoing or active infection requiring IV antibiotics, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, cirrhosis, or psychiatric illness/social situations that would limit adherence to study requirements
- 2.1.2.10 Past or present history of malignant tumors other than KS unless: a) in a complete remission for  $\geq 1$  year from the time a response was first documented; b) completely resected basal cell carcinoma; or c) in situ squamous cell carcinoma of the cervix or anus
- 2.1.2.11 Severe or life-threatening infection within 2 weeks of entry onto the study
- 2.1.2.12 History of deep venous or arterial thrombotic disease (including but not limited to, acute myocardial infarction due to coronary thrombosis, ischemic stroke, and peripheral arterial disease), unless:
  - 2.1.2.12.1 Line-related thrombosis without embolus
  - 2.1.2.12.2 Occurring  $\geq 1$  year prior to screening
- 2.1.2.13 Known 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.
- 2.1.2.14 Known bleeding diathesis
- 2.1.2.15 History of severe gastrointestinal bleeding within 6 months. Patients with gastrointestinal blood loss due to KS may be included. (see 2.1.1.3.3.)
- 2.1.2.16 Hemoptysis within 4 weeks
- 2.1.2.17 Substantial CNS disease including
  - 2.1.2.17.1 History of CNS bleeding
  - 2.1.2.17.2 Mass lesions in the brain
  - 2.1.2.17.3 Uncontrolled seizure disorder
  - 2.1.2.17.4 Recent history of CVA (e.g. within the past 6 months)
- 2.1.2.18 Proteinuria  $> 500$  mg/24hrs
- 2.1.2.19 Patients with any other abnormality that would be scored as a grade 3 or greater toxicity, except:

- 2.1.2.19.1 Lymphopenia
- 2.1.2.19.2 Direct manifestations of KS
- 2.1.2.19.3 Direct manifestation of HIV
- 2.1.2.19.4 Direct manifestation of HIV therapy (i.e. Hyperbilirubinemia associated with protease inhibitors)
- 2.1.2.19.5 Asymptomatic hyperuricemia
- 2.1.2.19.6 Hypophosphatemia
- 2.1.2.20 Previous bevacizumab within 6 weeks prior to enrollment
- 2.1.2.21 Known hypersensitivity to bevacizumab, Chinese hamster ovary cell products, or other recombinant human or humanized antibodies
- 2.1.2.22 Any condition, including the presence of laboratory abnormalities, which in the opinion of the Principal Investigator or Lead Associate Investigator places the subject at unacceptable risk if they were to participate in the study or confounds the ability to interpret data from the study.

## **2.2 RESEARCH ELIGIBILITY EVALUATION**

Potential subjects will be evaluated by a HAMB physician-investigator for protocol eligibility. Baseline pretreatment evaluation will include a complete medical history, review of systems, and physical examination with documentation of extent of KS and determination of target lesions. Screening is performed to determine eligibility and obtain certain baseline data. Screening studies, except for KS biopsy, HIV serology and cardiac studies (see section 3.5.1.4.) must be completed within 2 weeks of starting study.

### **2.2.1 Clinical Evaluation**

- 2.2.1.1 Complete medical history
- 2.2.1.2 Comprehensive physical examination

### **2.2.2 Pathology**

Biopsy demonstrating Kaposi's sarcoma, reviewed by NCI pathologists (See section 3.5.1.6. for punch biopsy sample collection instructions.)

### **2.2.3 Laboratory Data**

- 2.2.3.1 Acute care panel (Sodium, Potassium, Chloride, CO<sub>2</sub>, Creatinine, Glucose, and Urea Nitrogen)
- 2.2.3.2 Mineral panel (Phosphorus, Magnesium, Albumin, and Calcium)
- 2.2.3.3 Hepatic panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
- 2.2.3.4 Total protein, LDH, creatine kinase, uric Acid
- 2.2.3.5 C-reactive protein
- 2.2.3.6 APTT, PT, thrombin time (TT), fibrinogen, D-dimer

- 2.2.3.7 Hepatitis B S Ag, Hepatitis B S Ab, Hepatitis B core Ab.
- 2.2.3.8 Hepatitis C antibody
- 2.2.3.9 CBC, diff (automated lymphocyte count), reticulocyte count
- 2.2.3.10 HIV Western blot
- 2.2.3.11 HIV viral load if HIV seropositive by Western Blot
- 2.2.3.12 Lymphocyte phenotype TBNK. Simultaneous CBC and automated differential must be drawn.
- 2.2.3.13 Urinalysis
- 2.2.3.14 24-hour urine for protein if  $\geq 1+$  proteinuria on dipstick
  - 2.2.3.14.1 Urine  $\beta$ -hCG (women)
- 2.2.4 Cardiovascular Studies
  - 2.2.4.1 EKG
  - 2.2.4.2 Cardiac Multiple Uptake Gated Acquisition Scan (MUGA)
- 2.2.5 Imaging
  - 2.2.5.1 Chest x-ray
  - 2.2.5.2 Chest CT to assess abnormal chest x-ray, if indicated.
  - 2.2.5.3 Other studies when indicated to evaluate and measure internal tumor

### **2.3 REGISTRATION PROCEDURES**

Each potential subject will be discussed with the Principal Investigator or a physician Associate Investigator. Informed consent will be documented from eligible participating subjects using the NCI Institutional Review Board (IRB) approved Informed Consent form. Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail.

## **3. STUDY IMPLEMENTATION**

### **3.1 STUDY DESIGN**

This is an open label, single center pilot study that will enroll two cohorts of subjects.

- 3.1.1 Cohort 1: Patients who are either:
  - 3.1.1.1 HIV negative
  - 3.1.1.2 HIV infected with stable KS despite one year of HAART with HIV viremic control
  - 3.1.1.3 HIV infected with progressive KS despite four months of HAART with HIV viremic control

### 3.1.2 Cohort 2: All other patients with advanced AIDS-associated KS

The primary objective is to estimate the ORR of the combination of liposomal doxorubicin and bevacizumab after 6 cycles in subjects with advanced KS in each of these two cohorts. KS response in Cohort 1 subjects would not be considered as being influenced by the recent initiation of HAART, while KS response in Cohort 2 subjects may include a component of HAART effect. Subjects will be treated with bevacizumab combined with liposomal doxorubicin every three weeks until complete response or a maximum of 6 cycles. Subjects with a partial response or complete response will continue on bevacizumab monotherapy every 3 weeks for an additional 11 cycles. Information regarding efficacy and toxicity of the combination will be obtained. Duration of response will be assessed over 3-year follow-up.

## 3.2 DRUG ADMINISTRATION

Induction phase will consist of a loading dose of bevacizumab followed one week later by the combination of bevacizumab with liposomal doxorubicin administered every three weeks until complete response or a maximum of six cycles. Subjects with stable disease, partial response or complete response after the induction phase will be continued on maintenance bevacizumab for 11 cycles. See [Figure 1](#) for a schema of the dosing schedule.

### 3.2.1 Induction Phase

- 3.2.1.1 Bevacizumab 15 mg/kg will be infused over 90 minutes as a loading dose on Day 1 of Cycle 1, one week prior to initiation of the combination of bevacizumab and liposomal doxorubicin
- 3.2.1.2 On Day 8 of Cycle 1, bevacizumab 15 mg/kg will be infused over 60 minutes (as long as no adverse reactions incurred during the first infusion) prior to liposomal doxorubicin. See section 8.1.4. for dilution and mixing instructions.
- 3.2.1.3 On Day 1 of Cycles 2-6, bevacizumab 15 mg/kg will be infused over 30 minutes (as long as no adverse reactions incurred during the first two infusions) every 3 weeks prior to liposomal doxorubicin. See section 8.1.4. for dilution and mixing instructions.
- 3.2.1.4 Prior to each dose, blood pressure measurements should be obtained in the outpatient clinic and should not be repeated in the chemotherapy suite immediately prior to drug administration to avoid “white coat hypertension” that could be induced if measurements are taken in the chemotherapy administration suites immediately prior to drug administration. For SBP > 160 or DBP > 100 delay administration and repeat blood pressure measurement no sooner than 30 minutes later. If parameters remain elevated above these levels, bevacizumab will not be administered, and the responsible physician Investigator(s) will be notified.
- 3.2.1.5 Prior to each dose, urine dipstick for protein must be obtained. If  $\geq 2+$ , a 24-hour urine for protein must be obtained prior to dose administration.
- 3.2.1.6 Liposomal doxorubicin 20 mg/m<sup>2</sup> will be administered by intravenous bolus over 30 minutes every 3 weeks. See section 8.2.4. for dilution and mixing instructions.
- 3.2.1.7 Prior to liposomal doxorubicin administration, a CBC with differential should be obtained. Liposomal doxorubicin and bevacizumab should be held for an absolute neutrophil count (ANC) < 750 / $\mu$ L or a platelet count less than 75,000 / $\mu$ L.

### 3.2.2 Maintenance phase

Bevacizumab 15 mg/kg will be infused over 30 minutes every 3 weeks for a total of 11 cycles.

3.2.2.1 Prior to each dose, blood pressure measurements should be obtained in the outpatient clinic and should not be repeated in the chemotherapy suite immediately prior to drug administration to avoid “white coat hypertension” that could be induced if measurements are taken in the chemotherapy administration suites immediately prior to drug administration. For SBP > 160 or DBP > 100 delay administration and repeat blood pressure measurement no sooner than 30 minutes later. If parameters remain elevated above these levels, bevacizumab will not be administered, and the responsible physician Investigator(s) will be notified.

3.2.2.2 Prior to each dose, urine dipstick for protein must be obtained. If  $\geq 2+$ , a 24-hour urine for protein must be obtained prior to dose administration.

**Figure 1. Dosing Schedule**

<b>CYCLE</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	<b>17</b>
<b>WEEK</b>	<b>1</b>	<b>2</b>	<b>5</b>	<b>8</b>	<b>11</b>	<b>14</b>	<b>17</b>	<b>20</b>	<b>23</b>	<b>26</b>	<b>29</b>	<b>32</b>	<b>35</b>	<b>38</b>	<b>41</b>	<b>44</b>	<b>47</b>	<b>50</b>
<b>BEVACIZUMAB</b>	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
<b>LIPOSOMAL DOX</b>		↓	↓	↓	↓	↓	↓											

### 3.3 TREATMENT MODIFICATIONS

During the induction period, if there is an indication to hold bevacizumab, liposomal doxorubicin may be continued. If the bevacizumab is held for 1 week or less, the liposomal doxorubicin may be delayed until the bevacizumab is given. If there is indication to hold bevacizumab for more than one week, liposomal doxorubicin may be continued as monotherapy. Bevacizumab will be resumed on subsequent cycles if criteria for administration of bevacizumab are met.

If there is an indication to hold liposomal doxorubicin, both liposomal doxorubicin and bevacizumab will be held. Patients will be evaluated weekly until resolution of toxicities allow for continued administration of the combination of liposomal doxorubicin and bevacizumab.

In the event of unforeseen circumstances (e.g., travel difficulties) or Federal holidays, treatment may be rescheduled to the closest subsequent day possible, to a maximum of 1 week late, without constituting a protocol violation

Indications for treatment modification for each agent are outlined.

#### 3.3.1 Bevacizumab

##### 3.3.1.1 Hypertension

3.3.1.1.1 Bevacizumab will not be administered unless SBP < 160 and DBP < 100. Management of hypertension is outlined below.

3.3.1.1.2 Mild/Moderate Hypertension

For SBP > 140 and < 210 mm Hg or DBP > 90 and < 120 mm Hg that is sustained over at least a two week period, initiate or adjust anti-hypertensive therapy. Bevacizumab must be delayed until the blood SBP < 160 and DBP < 100.

#### 3.3.1.1.3 Severe Hypertension

For SBP  $\geq$  210 mm Hg or DBP  $\geq$  120 mm Hg but without end organ damage, begin anti-hypertensive therapy. Bevacizumab must be delayed until blood SBP < 150 and DBP < 90.

#### 3.3.1.1.4 Hypertensive Urgency or Emergency

Bevacizumab therapy will be permanently discontinued in the presence of hypertensive urgency (DBP > 120 with evidence of optic disc edema or progressive end-organ complications) or hypertensive emergency (SBP > 210 and DBP > 120 presenting with headaches, blurred vision, or focal neurological symptoms, or papilledema). Only grade 4 hypertension will require expedited reporting to the NCI-IRB.

#### 3.3.1.2 Thrombotic Events

##### 3.3.1.2.1 Deep Vein Thrombosis

3.3.1.2.2 Bevacizumab will not be administered in the presence of deep vein thrombosis or in the presence of therapeutic anticoagulation.

##### 3.3.1.2.3 Arterial Thromboembolic Events

3.3.1.2.4 Bevacizumab will not be administered in the presence of new arterial thrombotic disease, including but not limited to, myocardial infarct due to coronary thrombosis and ischemic stroke.

#### 3.3.1.3 Thrombocytopenia

Bevacizumab and thromboprophylaxis (aspirin 81 mg) will not be administered during the maintenance phase unless the platelets are  $\geq$  50,000 /  $\mu$ L.

#### 3.3.1.4 Neutropenia

Bevacizumab will be held if the absolute neutrophil count (ANC) is less than 750 cells/ $\mu$ L until ANC is again above 750 cells/ $\mu$ L. Filgrastim or pegfilgrastim may be scheduled to individual patient requirement in order to maintain a neutrophil count targeted to meet or exceed 750 cells/ $\mu$ L.

Subjects that develop neutropenic fever while receiving the combination of liposomal doxorubicin and bevacizumab will receive either filgrastim 5 $\mu$ g/kg (rounded to the nearest vial size of 300  $\mu$ g or 480  $\mu$ g) daily for 5 days or pegfilgrastim 6 mg once starting on day 2 of subsequent cycles that include liposomal doxorubicin.

Subjects that develop neutropenic fever while on bevacizumab monotherapy will receive either filgrastim 5 $\mu$ g/kg (rounded to the nearest vial size of 300  $\mu$ g or 480  $\mu$ g) daily for 5 days or pegfilgrastim 6 mg once, starting on day 2 of subsequent cycles.

### 3.3.1.5 Hemorrhage

#### 3.3.1.5.1 Life Threatening

In the case of hemoptysis, hematemesis, hematochezia, intracranial hemorrhage or any significant blood loss, bevacizumab will not be administered.

#### 3.3.1.5.2 Cutaneous KS

In the case of cutaneous bleeding at tumor site(s), bevacizumab may be delayed. If the bleeding is minor and appears to be due to tumor necrosis, bevacizumab may be administered without schedule or dose modification at the discretion of the Principal Investigator. Bevacizumab will be held for grade 3 tumor bleeding.

### 3.3.1.6 Proteinuria

If urine dipstick indicates  $\geq 2+$  proteinuria, bevacizumab administration will be delayed pending the results of a 24-hour urine collection for protein. If the 24-hour urine protein is  $\geq 2$  grams, bevacizumab will be delayed for up to 6 weeks, until the proteinuria is  $< 2+$  or the 24-hour urine protein is  $< 1$  grams/24 hr.

### 3.3.1.7 Liver Function Abnormalities

Bevacizumab should be withheld in the event of  $\geq$  Grade 3 LFT elevations and should not resume until the abnormalities have recovered to  $\leq$  Grade 1 (with the exception of hyperbilirubinemia attributable to protease inhibitor therapy, in which case the total bilirubin must recover to  $\leq 7.5$  mg/dL and the direct fraction  $\leq 0.7$  mg/dl.) If LFT elevations recur with re-treatment, bevacizumab should be permanently discontinued. Liver function tests included are the transaminases alanine aminotransferase (ALT, SGPT), aspartate aminotransferase (AST, SGOT), alkaline phosphatase, and bilirubin. Elevated bilirubin due to protease inhibitor therapy will not require treatment modification.

### 3.3.1.8 Allergic Reaction

In case of flushing, shortness of breath, facial edema, headache, chills, back pain, tightness of the chest and throat, and/or hypotension, fever or rash, the infusion should be suspended until the patient is assessed until the events have subsided. For grade 3 or 4 allergic reactions, bevacizumab will be permanently discontinued.

### 3.3.1.9 Surgical or periodontal procedures

If there is need for an elective major surgical or periodontal procedure, bevacizumab should be held beginning at least 6 weeks prior to the procedure and must not be resumed before 4 weeks after the surgical procedure. For urgent or emergent surgery or endoscopic procedures, bevacizumab will be held for 4 weeks after the procedure. Longer delays may be necessary, if clinically indicated, in order to ensure that adequate healing has taken place prior to bevacizumab resumption. For KS skin biopsies and other minor procedures (i.e. interventional radiology

diagnostic procedures), bevacizumab will be held only if there is evidence that healing is compromised.

#### 3.3.1.10 Reversible Posterior Leukoencephalopathy Syndrome (RPLS)

Bevacizumab should not be administered to patients with signs or symptoms of RPLS. Evaluation should include neurologic evaluation, ocular examination, head MRI, and blood pressure assessment. If these clinical criteria are consistent with the diagnosis of RPLS, bevacizumab will be permanently discontinued.

#### 3.3.1.11 Fistula Formation

Bevacizumab will be discontinued in the event of formation of a fistula.

#### 3.3.1.12 Other Grade 3 or 4 Toxicities

Bevacizumab will be held for any other Grade 3 or 4 toxicity thought to be at least possibly related to bevacizumab. Lymphopenia and other toxicities clearly related to HIV or its therapy or to the Kaposi's sarcoma itself will not require dose modification. Grade 3 or higher asymptomatic hyperuricemia, hypophosphatemia, elevated creatinine phosphokinase levels will not result in dose delay.

#### 3.3.1.13 Option of Deferring Bevacizumab Loading Dose

If deferral of cytotoxic chemotherapy for one week is deemed to be unsafe by the Principal Investigator or Lead Associate Investigator, the loading dose (Day 1) of bevacizumab may be omitted.

#### 3.3.1.14 Option of Additional Bevacizumab

For patients showing evidence of progressive improvement on bevacizumab after one year, therapy may be continued for up to an additional 12 months, for a maximum of up to 24 months of therapy from the date of enrollment on protocol. In addition, patients who receive additional liposomal doxorubicin after the initial 6 cycles (see Section 3.3.2.6.) may receive up to 12 months of bevacizumab, starting at the time of the re-initiation of the liposomal doxorubicin. Depending on when they start receiving their additional liposomal doxorubicin, such patients could thus receive up to 24 months total of therapy, starting from the date of enrollment on protocol. Subjects receiving bevacizumab beyond 12 months will be followed on-study for safety endpoints during therapy and 4 weeks after the last dose of therapy (see Section 3.10.1.1). Toxicity evaluation for patients receiving additional bevacizumab will follow the schedule outlined in Section 3.5.2. For patients receiving additional liposomal doxorubicin, repeat cardiac evaluation will occur 3 weeks (+/- 1 week) after the final dose of liposomal doxorubicin as outlined in Section 3.5.2.6. Patients will be followed on study for up to 2 years on study after their final dose of therapy (see Section 3.10.1.2.).

### 3.3.2 Liposomal Doxorubicin

#### 3.3.2.1 Infusion Related Events

In case of flushing, shortness of breath, facial edema, headache, chills, back pain, tightness of the chest and throat, and/or hypotension, the infusion should be suspended until the patient is assessed and the events have subsided. Unless

discontinuance of the drug is clinically indicated (due to repeated severe reactions and discussed with the Principal Investigator), the infusion rate should be resumed at one-half the previous rate.

### 3.3.2.2 Hematologic Toxicities

#### 3.3.2.2.1 Neutropenia

Liposomal doxorubicin will be held if the absolute neutrophil count (ANC) is less than 750 cells/ $\mu$ L on Day 1 of a cycle until ANC is again above 750 cells/ $\mu$ L. Liposomal doxorubicin will not be administered within 14 days following pegfilgrastim administration.

Filgrastim or pegfilgrastim may be scheduled to individual patient requirement in order to maintain a neutrophil count targeted to meet or exceed 750 cells/ $\mu$ L. Patients that develop neutropenic fever will receive either filgrastim 5 $\mu$ g/kg (rounded to the nearest vial size of 300  $\mu$ g or 480  $\mu$ g) daily for 5 days or pegfilgrastim 6 mg once, starting on day two of subsequent cycles that include liposomal doxorubicin.

#### 3.3.2.2.2 Thrombocytopenia

Liposomal doxorubicin will be held for up to 4 weeks if the platelet count is <75,000 /  $\mu$ L. Aspirin will also be held if the platelet count is <50,000 /  $\mu$ L. Liposomal doxorubicin dose will be reduced by 25% in subsequent cycles if the platelet count nadir is <25,000, or if there is > 1 week delay due to thrombocytopenia or if dose delays due to thrombocytopenia occur in > 1 cycle.

### 3.3.2.3 Palmer-Planter Erythrodysesthesia

3.3.2.3.1 Grade 1: Delay up to 2 weeks until substantial resolution.

3.3.2.3.2 Grade 2: Delay liposomal doxorubicin cycles up to two weeks until toxicity substantially resolves. If patient has experienced Grade 2 or higher hand-foot skin reaction, decrease dose by 25%.

3.3.2.3.3 Grade 3 or 4: Discontinue liposomal doxorubicin and begin maintenance phase of protocol after toxicity decreases to grade I or lower, provided this occurs within 4 weeks.

### 3.3.2.4 Stomatitis

3.3.2.4.1 Grade  $\geq$  2: Delay up to two weeks.

3.3.2.4.2 Grade  $\geq$  3: Delay up to two weeks and reduce dose by 25%.

### 3.3.2.5 Congestive Heart Failure

For CTCAEv3.0 Grade 2 or greater congestive heart failure and at least 10% decrease from baseline, discontinue liposomal doxorubicin and bevacizumab (including bevacizumab monotherapy).

### 3.3.2.6 Option of Additional Liposomal Doxorubicin

Up to 6 cycles of additional liposomal doxorubicin combined with bevacizumab may be prescribed for patients who demonstrate clinical improvement with

liposomal doxorubicin combined with bevacizumab, but later have progressive disease or disease requiring chemotherapy (as outlined in section 2.1.1.6) while receiving bevacizumab alone. Subjects requiring additional liposomal doxorubicin will be counted as progressive disease, but will continued to be followed for the safety and tolerability of the combination. There is no limit for maximum cumulative dose of liposomal doxorubicin.

### 3.4 PHARMACOKINETIC STUDIES

No pharmacokinetic studies are planned for this protocol.

### 3.5 PROTOCOL EVALUATION

The schedule for protocol evaluations, including baseline studies, on-treatment studies, and post-treatment studies are outlined below. Tests may be rescheduled to the closest day possible without constituting a protocol violation (e.g. for Federal holidays or unforeseen circumstances such as travel difficulties.)

#### 3.5.1 Baseline Studies

If the patient begins treatment within 48 hours of screening, the screening evaluations in section 2.2 do not need to be repeated.

##### 3.5.1.1 Clinical Evaluation

3.5.1.1.1 Complete Medical History, Vital Signs and Physical Exam

3.5.1.1.2 ECOG Performance Status

3.5.1.1.3 Review of symptoms, documenting baseline symptoms using CTCAE v3.0

##### 3.5.1.2 Clinical Kaposi's Sarcoma Evaluation

###### 3.5.1.2.1 Documentation of Extent of Disease

Baseline whole body photographs will be obtained upon entry into the study. At this time, 5 lesions (hereafter called **marker lesions**), representative of the patient'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. Detailed photographs of these lesions will be obtained with a metric rule beside them. The size, color and nodularity of these lesions will be recorded on Day 1 of each cycle. Documentation will depend on the number of lesions.

###### 3.5.1.2.1.1 50 or More Lesions

For patients 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. An attempt will be

made to distribute the “marker” lesions between the representative areas and the rest of the body.

3.5.1.2.1.2 Less than 50 Lesions

For patients 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.

3.5.1.2.2 Additional Studies for Visceral Disease

Additional studies, including but not limited to, gastrointestinal endoscopy, bronchoscopy, and CT scans, will be performed at entry where clinically indicated, based on clinical evaluation of the patient.

3.5.1.3 Laboratory Evaluation

3.5.1.3.1 Acute care panel (Sodium, Potassium, Chloride, CO<sub>2</sub>, Creatinine, Glucose, and Urea Nitrogen)

3.5.1.3.2 Mineral panel (Phosphorus, Magnesium, Albumin, and Calcium)

3.5.1.3.3 Hepatic panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)

3.5.1.3.4 C-reactive protein

3.5.1.3.5 Total protein, LDH, creatine kinase, uric Acid

3.5.1.3.6 CBC, diff (automated lymphocyte count), reticulocyte count

3.5.1.3.7 APTT, PT, fibrinogen, D-dimer

3.5.1.3.8 Hepatitis A IgG

3.5.1.3.9 VDRL

3.5.1.3.10 Thyroid function tests (TSH, Free T4)

3.5.1.3.11 Urinalysis, urine protein, urine creatinine

3.5.1.3.12 Pregnancy test (if female)

3.5.1.3.13 Lymphocyte phenotype TBNK. A simultaneous CBC and automated differential must be drawn.

3.5.1.3.14 HIV Viral load

3.5.1.3.15 Four 10cc red-topped tubes (send to SAIC, Frederick) for storage

3.5.1.3.15.1 Assays to include IL-6, viral IL-6, and CD105

3.5.1.3.15.2 One red top for KSHV Elisa or IFA (send to SAIC, Frederick) for storage

3.5.1.3.16 Up to 30 cc may be drawn for virologic testing, establishment of cell lines, evaluation of hematologic parameters, or other studies that become clinically important during conduct of the trial. If the investigators are interested in studying germline genotypic information other than VEGF or VEGF-R polymorphisms or HLA typing, they will return to the IRB with an amendment to the protocol in order to conduct this research.

- 3.5.1.3.17 Quantitative immunoglobulin levels, including IgE
- 3.5.1.3.18 Four yellow top tubes for storage for plasma and PBMC KSHV viral load levels (send to SAIC, Frederick)
- 3.5.1.3.19 Saliva for KSHV viral load to Dr. Denise Whitby (send to SAIC-Frederick Building 535, Room 428A)
- 3.5.1.4 Cardiology Evaluation
  - 3.5.1.4.1 EKG (must have EKG from within 6 weeks of starting therapy)
  - 3.5.1.4.2 MUGA (must have MUGA from within 6 weeks of starting therapy)
- 3.5.1.5 Radiology Evaluation
  - 3.5.1.5.1 CXR (must have within 2 weeks of starting therapy)
  - 3.5.1.5.2 Other imaging as clinically indicated (See section 3.5.1.2.2)
  - 3.5.1.5.3 Evaluation of the vascularity in KS will be performed using 3 non-invasive modalities: laser Doppler imaging, multi-spectral imaging, and infrared thermal imaging. Imaging will use the modalities explored in 01-C-0158. A target lesion of KS as well as normal skin will be identified and recorded for follow-up studies. Imaging should occur on Day 1, Cycle 1, prior to the loading dose of bevacizumab, or up to one 1 week prior to starting therapy. Use of these non-invasive modalities will be subject to technician and machine availability. Inability to schedule non-invasive imaging should not alter planned therapy, and will not result in a protocol violation.
- 3.5.1.6 Pathologic Kaposi's Sarcoma Evaluation

If adequate research tissue was obtained during screening, do not repeat baseline pathology evaluation. Otherwise, obtain one to two 4-6 mm cutaneous KS "punch" biopsies for the following studies. Divide the tissue into approximately equal 3mm pieces. If only limited tissue is available, the priority for laboratories is to submit the material in the order listed below:

  - 3.5.1.6.1 CCR Pathology for histology and LANA IHC
  - 3.5.1.6.2 If adequate tissue is available, a sample will be sent to the lab of Dr. Giovanna Tosato for IHC of DII4, PDGF-C, G-CSF and other potential markers of resistance to bevacizumab (Building 37, Room 4134B)
  - 3.5.1.6.3 Molecular pathology: if there is sufficient tissue, an aliquot will be sent to the laboratory of Dr. Robert Yarchoan (Building 10, Room 5A25) for storage for molecular pathology studies (dry, transported in a tube placed on ice).
- 3.5.2 Evaluation During Treatment Phase
  - 3.5.2.1 Evaluation Schedule
    - 3.5.2.1.1 Cycle 1: Subjects will be evaluated on Day 1, Day 8 and Day 15
    - 3.5.2.1.2 Cycle 2 – 17: Subjects will be evaluated on Day 1
  - 3.5.2.2 General Clinical Evaluation

- 3.5.2.2.1 Vital signs and focused physical exam, with documentation of pertinent positive and negative findings.

The Principal Investigator or an Associate Investigator will be notified if the SBP >140 or DBP >90 (See section 3.3.1.1).
- 3.5.2.2.2 ECOG Performance Status
- 3.5.2.2.3 Evaluation of subjective symptoms, documenting symptoms using CTCAE v3.0
- 3.5.2.3 Laboratory Studies
  - 3.5.2.3.1 Tests Obtained on Day 1 of Every Cycle
    - 3.5.2.3.1.1 CBC with differential, reticulocyte count, ESR
    - 3.5.2.3.1.2 Acute care panel (Sodium, Potassium, Chloride, CO<sub>2</sub>, Creatinine, Glucose, and Urea Nitrogen)
    - 3.5.2.3.1.3 Mineral panel (Phosphorus, Magnesium, Albumin, and Calcium)
    - 3.5.2.3.1.4 Hepatic panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
    - 3.5.2.3.1.5 Total protein, LDH, creatine kinase, uric Acid
    - 3.5.2.3.1.6 APTT, PT,
    - 3.5.2.3.1.7 Urinalysis
    - 3.5.2.3.1.8 Two Red top tubes to storage (send to SAIC, Frederick), assays to include IL6, viral IL-6, and CD105
    - 3.5.2.3.1.9 Up to 30 cc of blood may optionally be drawn for virologic testing, establishment of cell lines, evaluation of hematologic parameters, or other studies that become clinically important during conduct of the trial. For patients co-enrolled in 01-C-0038, this will routinely include 2 green top tubes and 2 ACD yellow top tubes for storage of plasma and cells for future studies. If the investigators are interested in studying patient germline genotypic information other than VEGF or VEGF-R polymorphisms or HLA typing, they will return to the IRB with an amendment to the protocol in order to conduct this research.
  - 3.5.2.3.2 Tests Obtained on (Day 21 +/- 1 week) of Every 3rd Cycle
    - 3.5.2.3.2.1 HIV viral load if subject was HIV infected at entry
    - 3.5.2.3.2.2 Lymphocyte phenotype TBNK. A simultaneous CBC and automated differential must be drawn
    - 3.5.2.3.2.3 4 yellow top tubes for storage for plasma and PBMC KSHV viral loads
    - 3.5.2.3.2.4 Saliva for KSHV viral Load to Dr. Denise Whitby (send to SAIC-Frederick Building 535, Room 428A)
- 3.5.2.4 Evaluation of Extent of Kaposi's Sarcoma
  - 3.5.2.4.1 Day 1 of Every Cycle

The marker lesions will be measured, and a record made of their size, color, and nodularity, and the total number of lesions (if the patient, at baseline, had less than 50 lesions) or the lesions within previously defined representative areas (if the patient had more than 50 lesions) will be counted at the completion of each cycle as described in Section 3.5.1.4.1.

3.5.2.4.2 Day 21 (+/- 7 days) of Cycle 6 and Cycle 17

Whole body and marker lesion photographs will be obtained.

3.5.2.5 Pathologic Kaposi's Sarcoma Evaluation

3.5.2.5.1 Scheduled Biopsies

One to two 4-6 mm cutaneous KS "Punch" Biopsies will be performed to confirm clinical complete response, or to document progressive disease if there is clinical uncertainty about new lesions. Divide the tissue into approximately equal 3mm pieces. If only limited tissue is available, the priority for laboratories is to submit the material in the order listed below:

3.5.2.5.1.1 CCR Pathology histology and LANA IHC

3.5.2.5.1.2 If adequate tissue is available, a sample will be sent to the lab of Dr. Giovanna Tosato for IHC of Dll4, PDGF-C, G-CSF and other potential markers of resistance to bevacizumab (Building 37, Room 4134B)

3.5.2.5.1.3 Molecular pathology: if there is sufficient tissue, an aliquot will be sent to the laboratory of Dr. Robert Yarchoan (Building 10, Room 5A25) for storage for molecular pathology studies (dry, transported in a tube placed on ice).

3.5.2.5.2 Research Biopsies

Paired tissue biopsies may be obtained (including one sample at baseline) for correlative biomarker studies evaluating the effects of therapy on Kaposi's sarcoma. If the investigators are interested in studying germline genotypic information other than VEGF or VEGF-R polymorphisms or HLA typing, they will return to the IRB with an amendment to the protocol in order to conduct this research. Research biopsies are not required for participation in this study.

3.5.2.6 Cardiology Evaluation

3.5.2.6.1 Repeat MUGA will be performed on cycle 7 day 1 (+/- 7 days), after the completion of the combination of liposomal doxorubicin and bevacizumab.

3.5.2.6.2 If additional liposomal doxorubicin is given, MUGA will be repeated on day 21 (+/- 7 days) after the 6th or last dose of additional liposomal doxorubicin.

3.5.2.6.3 Patients with an ejection fraction less than <50% ( $\geq$ CTCAEv3.0 Grade 2 toxicity) and at least 10% decrease from baseline on follow-up will have a repeat MUGA 3 months (+/- 7days) after the abnormal evaluation.

3.5.2.6.4 Patients with a continued ejection fraction less than <50% ( $\geq$ CTCAEv3.0 Grade 2 toxicity) and at least 10% decrease from baseline on 3-month follow-up will have a repeat MUGA at the end of the follow-up period.

- 3.5.2.6.5 Additional diagnostic cardiology evaluations will be performed only as clinically indicated
- 3.5.2.7 Radiology evaluation
  - 3.5.2.7.1 Abnormal non-invasive imaging from baseline studies should be repeated Day 21 (+/-7 days) of every 3rd cycle until complete response.
  - 3.5.2.7.2 Abnormal imaging from invasive studies should be repeated once a subject achieves a clinical complete response.
  - 3.5.2.7.3 Evaluation of the vascularity in KS will be performed using 3 non-invasive modalities: laser Doppler imaging, multi-spectral imaging, and infrared thermal imaging. Imaging will use the modalities explored in 01-C-0158. The target lesion and target normal skin established at baseline will be used as the imaged site. Use of these non-invasive modalities will be subject to technician and machine availability. Inability to schedule non-invasive imaging should not alter planned therapy, and will not result in a protocol violation. Follow-up imaging will be repeated as follows:
    - 3.5.2.7.3.1 Day 8, cycle 1, prior to the administration of the combination of bevacizumab with liposomal doxorubicin.
    - 3.5.2.7.3.2 Day 1, cycle 7 (+/- 7 days), after completing 6 cycles of the combination of liposomal doxorubicin with bevacizumab.
    - 3.5.2.7.3.3 Day 21 cycle 17 (+/- 7 days), after completing bevacizumab monotherapy (if there are persistent lesions).
- 3.5.3 Off-Study Evaluation
  - 3.5.3.1 General Evaluation

General evaluation will consist of history with review of symptoms and physical with vital signs. Adverse events reported during a review of systems will be evaluated using CTCAEv3.
  - 3.5.3.2 Laboratory Studies
    - 3.5.3.2.1 CBC with differential, reticulocyte count
    - 3.5.3.2.2 Acute care panel (Sodium, Potassium, Chloride, CO<sub>2</sub>, Creatinine, Glucose, and Urea Nitrogen)
    - 3.5.3.2.3 Mineral panel (Phosphorus, Magnesium, Albumin, and Calcium)
    - 3.5.3.2.4 Hepatic panel (Alkaline Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin)
    - 3.5.3.2.5 C-reactive protein
    - 3.5.3.2.6 Total protein, creatine kinase, uric acid, LDH
    - 3.5.3.2.7 APTT, PT, thrombin time (TT), fibrinogen, D-dimer Urinalysis, urine protein, urine creatinine

- 3.5.3.2.8 Four Red top tubes to storage (send to SAIC, Frederick), assays to include IL6 CD105, and possibly KSHV viral IL-6.
- 3.5.3.2.9 HIV viral load if subject was HIV infected at entry
- 3.5.3.2.10 FACS: including CD4 and CD8 T-cells. A simultaneous CBC and automated differential must be drawn
- 3.5.3.2.11 4 yellow to tubes for storage for plasma and PBMC KSHV viral loads
- 3.5.3.2.12 Saliva for KSHV viral Load to Dr. Denise Whitby (send to SAIC-Frederick Building 535, Room 428A)
- 3.5.3.2.13 Up to 30 cc of blood may optionally be drawn for virologic testing, establishment of cell lines, evaluation of hematologic parameters, or other studies that become clinically important during conduct of the trial. If the investigators are interested in studying patient germline genotypic information, they will return to the IRB with an amendment to the protocol in order to conduct this research.

### **3.6 CONCURRENT THERAPIES**

#### **3.6.1 Anti-retroviral Therapy**

Patients will receive antiretroviral therapy if it is indicated. Combination therapy will be generally based on Department of Health and Human Services Guidelines for treatment of HIV infection, available at <http://www.aidsinfo.nih.gov/guidelines/>. However, patients may have extenuating circumstances requiring deviation from these guidelines. Whenever possible, changing antiretroviral therapy should be avoided unless needed for optimal patient care. Additionally, referring physicians may manage this component of patient care.

#### **3.6.2 Pneumocystis jiroveci Prophylaxis**

For HIV-infected patients with history of PCP or with CD4 cells  $\leq 200/\text{mm}^3$  should receive Bactrim DS PO 3x/week. Alternatives include but are not limited Bactrim DS PO daily, Dapsone 100 mg PO qd, and monthly aerosolized pentamidine.

#### **3.6.3 MAC Prophylaxis**

Consider for all HIV-infected patients with a historic CD4 nadir less than 75 cells/ $\text{mm}^3$ , or for patients whose CD4 cells fall below this level while on study. Recommend azithromycin 1200 mg once weekly, but other agents are acceptable.

#### **3.6.4 Antihypertensive Therapy**

Blood pressure will be monitored at baseline and throughout follow-up. Blood pressure diagnosis and management will follow the general principals of the Seventh Report of the Joint Committee on the Prevention, Detection, Evaluation, and Treatment of high Blood pressure (JNC-7)<sup>58</sup>. All patients who are diagnosed with hypertension during screening or follow-up will be treated for hypertension.

##### **3.6.4.1 Blood Pressure Definitions**

- 3.6.4.1.1 Blood pressure will be measured with the subject sitting in a supine position for 5 minutes, with the arm supported at heart level, using a bladder cuff that covers at least 80% of the subjects upper arm
- 3.6.4.1.2 Pre-Hypertension: Systolic Blood Pressure (SBP) 120-139 or Diastolic Blood Pressure 80-89
- 3.6.4.1.3 Stage 1 Hypertension: SBP 140-159 or DBP 90-99
- 3.6.4.1.4 Stage 2 Hypertension: SBP  $\geq$  160 or DBP  $\geq$  100
- 3.6.4.1.5 Goal Blood pressure for patients receiving anti-hypertensive agents <140/90 or <130/85 if subject has diabetes or chronic renal insufficiency

#### 3.6.4.2 Other Cardiovascular Risk Factors

Subjects noted to have hypertension at baseline will be evaluated through medical history for other cardiovascular risk factors, including age (>55 in men or >65 in women), family history of premature cardiovascular disease in first degree relatives (< 55 in men or < 65 in women), smoking history, obesity, diabetes, chronic renal insufficiency and dyslipidemia. Patients with >2 cardiovascular risk factors should be evaluated and monitored by either the subject's primary internist or a cardiologist.

#### 3.6.4.3 Treatment of Hypertension

##### 3.6.4.3.1 Initiation of Anti-hypertensive therapy

Antihypertensive therapy will be started for all subjects who are diagnosed with  $\geq$  Stage 1 (see Section 3.6.4.1.3.) hypertension on two separate days.

##### 3.6.4.3.2 Antihypertensive Agents

Patient treatment will be individualized using standard anti-hypertensive therapy, including but not limited to the following classes of drugs: thiazide diuretics, beta-blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and calcium channel blockers. Priority will be given to angiotensin receptor blockade when feasible<sup>59</sup>, due to potential protection against proteinuria.

#### 3.6.5 Thromboprophylaxis

- 3.6.5.1 Subjects will receive thromboprophylaxis with aspirin 81mg orally once daily for the duration of study therapy, ceasing 3 months following the last cycle of therapy if continued aspirin is not otherwise indicated.

- 3.6.5.2 Aspirin will not be administered when platelet count is  $\leq$  50,000/ $\mu$ L.

#### 3.6.6 Contraindicated Therapies

- 3.6.6.1 It is important to avoid systemic glucocorticoid steroid administration if at all possible, as glucocorticoids may exacerbate KS. Short courses of corticosteroids (<10 days) are not strictly contraindicated.
- 3.6.6.2 Specific therapy for KS (e.g. cytotoxic chemotherapy agents, radiation therapy, topical anti-KS medications) is not allowed during the treatment phase of the protocol.

- 3.6.6.3 Patients will not receive any immunomodulatory agents or, as stated above, therapies for their KS. Patients may receive erythropoietin or filgrastim (G-CSF) as per standard medical practice. The use of other cytokines will not be allowed.
- 3.6.6.4 Intravenous antibiotics may not be administered concurrently with bevacizumab and/or liposomal doxorubicin. In the setting of infections requiring IV antibiotics, the guidelines for treatment modifications (Section 3.3.) and off study criteria (Section 3.9.) will be followed.

### **3.7 SURGICAL GUIDELINES**

Elective surgery is contraindicated in patients receiving bevacizumab due to potential wound healing complications. If there is need for an elective major surgical or periodontal procedure, bevacizumab should be held beginning at least 6 weeks prior to the procedure and must not be resumed before 4 weeks after the surgical procedure. For urgent or emergent surgery or endoscopic procedures, bevacizumab will be held for 4 weeks after the procedure. Longer delays may be necessary, if clinically indicated, in order to ensure that adequate healing has taken place prior to bevacizumab resumption. For KS skin biopsies and other minor procedures (i.e. interventional radiology diagnostic procedures), bevacizumab will be held only if there is evidence that healing is compromised. For eligibility criteria for enrollment after surgery, see sections 2.1.2.4. and 2.1.2.5.

### **3.8 RADIATION THERAPY GUIDELINES**

Radiation therapy is contraindicated for patients receiving therapy on protocol, as the effects of radiation therapy in combination with bevacizumab in patients with KS are unknown.

### **3.9 OFF TREATMENT AND OFF STUDY CRITERIA**

Subjects will be monitored each cycle for toxicity, co-morbidities and evidence of disease progression on physical exam. Treatment will be discontinued for any of the reasons listed below. Subjects removed from treatment for criteria 3.9.1 through 3.9.7 will be followed on-study in post-treatment evaluation as outlined in section 3.10. Patients will be taken off-study based on criteria 3.9.8 through 3.9.12.

- 3.9.1 Grade 3 or 4 allergy attributed to bevacizumab.
- 3.9.2 Grade 3 or 4 toxicity possibly, likely or definitely attributable to liposomal doxorubicin combined with bevacizumab or bevacizumab alone that does not resolve to grade 1 or baseline (whichever is higher) within 6 weeks, with the following exceptions (consistent with Section 2.1.2.19):
  - 3.9.2.1 Lymphopenia
  - 3.9.2.2 Asymptomatic hyperuricemia
  - 3.9.2.3 Asymptomatic hyperbilirubinemia
  - 3.9.2.4 Hypophosphatemia
  - 3.9.2.5 Hypermagnesemia or hypomagnesemia
  - 3.9.2.6 Asymptomatic elevations in creatine phosphokinase (CPK)
  - 3.9.2.7 Direct manifestations of KS, HIV, or HIV therapy

- 3.9.3 Development of venous or arterial thrombosis
- 3.9.4 Proteinuria >2 gram/24 hours that does not resolve to  $\leq$  1.5 grams within 4 weeks.
- 3.9.5 Severe illness lasting > 6 weeks that precludes administration of liposomal doxorubicin in combination with bevacizumab or bevacizumab alone.
- 3.9.6 Pregnancy
- 3.9.7 Institution of a non-protocol treatment for KS
- 3.9.8 Disease progression as compared to baseline and lasting at least 6 weeks (see section 5.2 for definitions of response criteria) not attributed to immune reconstitution syndrome. As KS may wax and wane, and improvements may be seen despite an initial period of progression, physician investigators may continue therapy through the first 6 cycles, despite meeting formal criteria for progressive disease, as long as disease progression is not clinically significant or life threatening. Such cases must be discussed with the Principal Investigator and study subject. Furthermore, patients may be eligible for additional liposomal doxorubicin and/or bevacizumab, as outlined in Sections 3.3.1.13 and 3.3.2.6.
- 3.9.9 Subject requests to discontinue treatment and/or continued evaluation.
- 3.9.10 Subjects may be taken off-study for lack of adherence to protocol therapies (including HAART) and the assessment that continuing on the study would compromise subject safety and interfere with protocol integrity. Additionally, the Principal Investigator may take a subject off-study for adverse events deemed sufficiently serious to warrant discontinuation of therapy.  
Subjects may be taken off study at the discretion of the Principal Investigator

### **3.10 OFF-STUDY PROCEDURE**

Authorized staff must notify Central Registration Office (CRO) when a patient is taken off-study. An off-study form from the web site (<http://home.ccr.cancer.gov/intra/eligibility/welcome.htm>) main page must be completed and faxed to 301-480-0757.

- 3.10.1 Unacceptable toxicities that have not resolved at time of “off treatment” or “off study” must be followed until stabilization or resolution.

### **3.11 POST TREATMENT EVALUATION (FOLLOW-UP)**

#### **3.11.1 Clinical**

- 3.11.1.1 Subjects will be evaluated for short-term toxicity at a follow up appointment scheduled 4 weeks (+/- 1 week) after completion of treatment. If any drug toxicity remains at the follow-up evaluation, patients will be followed as medically indicated until the toxicity stabilizes.
  - 3.11.1.1.1 Review of symptoms will be performed, and toxicity will be graded using the NCI – CTCAE v3.0.
  - 3.11.1.1.2 Objective signs will include a complete physical examination (with recording of abnormalities), blood pressure weight changes, and fever. Temperatures and weights will be evaluated at each clinic visit.

- 3.11.1.2 Subjects will be evaluated in clinic every 3 months or as clinically indicated for up to 2 years after completion of therapy to evaluate for disease progression. This follow up will end when patients meet criteria for disease progression.

### 3.11.2 Laboratory studies

The following laboratory studies will be drawn 4 weeks (+/- 1 week) after completion of therapy.

- 3.11.2.1 CBC with differential, ESR, reticulocyte count
- 3.11.2.2 APTT, PT, fibrinogen
- 3.11.2.3 Acute panel, hepatic panel, mineral panel
- 3.11.2.4 Thyroid function tests (TSH, Free T4)
- 3.11.2.5 Creatine kinase, total protein, amylase, LDH, uric acid
- 3.11.2.6 Urinalysis,
- 3.11.2.7 Four Red top tubes to storage (send to SAIC, Frederick), assays to include IL6, viral IL-6 and CD105
- 3.11.2.8 Lymphocyte phenotype TBNK. A simultaneous CBC and automated differential must be drawn
- 3.11.2.9 HIV viral load (if HIV positive at entry)
- 3.11.2.10 Up to 30 cc of blood may optionally be drawn for virologic testing, establishment of cell lines, evaluation of hematologic parameters, or other studies that become clinically important during conduct of the trial.
- 3.11.2.11 Four yellow top tubes for storage for plasma and PBMC KSHV viral load (send to SAIC, Frederick)

### 3.11.3 Extent of Kaposi's Sarcoma

- 3.11.3.1 The marker lesions will be measured, and a record made of their size, color, and nodularity, and the total number of lesions (if the patient, at baseline, had less than 50 lesions) or the lesions within previously defined representative areas (if the patient had more than 50 lesions) will be counted at the completion of each cycle as described in Section 3.5.2.4.1.

- 3.11.3.2 Whole body and marker lesion photographs will be obtained.

### 3.11.4 Pathologic Kaposi's Sarcoma Evaluation

One to two 4-6 mm cutaneous KS "Punch" Biopsies will be performed, if indicated, to confirm clinical complete response, or to document progressive disease if there is clinical uncertainty about new lesions. Tissue handling is outlined in Section 3.5.2.5.

## 4. SUPPORTIVE CARE

### 4.1 GENERAL KS AND HIV RELATED

Medications may be administered as clinically indicated, or at the discretion of the Principal Investigator, with the following exceptions:

- 4.1.1 Other specific therapies for KS

4.1.2 Any medications noted in the exclusion criteria or Section 3.6.5. with the exception of short-term courses of corticosteroids.

#### **4.2 OPPORTUNISTIC INFECTIONS**

Subjects who develop opportunistic infections, including but not limited to pneumocystis jiroveci 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 mandatory for subjects diagnosed with mycobacterium tuberculosis.

#### **4.3 ANEMIA**

If subject develops symptomatic anemia, or if the hemoglobin falls below 8 mg/dl erythropoietin may be considered. Appropriate evaluation for etiology of the anemia should be initiated.

#### **4.4 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. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count  $> 50,000/\text{mm}^3$ . Liposomal doxorubicin in combination with bevacizumab will not be administered unless the platelets are  $\geq 75,000/\text{mm}^3$ , and bevacizumab monotherapy will not be administered unless the platelets are  $\geq 50,000/\text{mm}^3$ .

#### **4.5 FEBRILE NEUTROPENIA**

Patients who develop febrile neutropenia will be hospitalized and treated with intravenous antibiotics. See sections 3.3.1.4. and 3.3.2.2.1 for Treatment Modifications and filgrastim use pertaining to neutropenia. The use of filgrastim will be based on whether the development of neutropenia or neutropenic fever develops while the patient is receiving the combination of liposomal doxorubicin with bevacizumab or bevacizumab monotherapy.

#### **4.6 HYPERTENSION**

Blood pressure will be monitored at baseline and throughout treatment and follow-up. Antihypertensive medications will be introduced early in subjects that develop hypertension. The procedures for monitoring blood pressure and treating hypertension are fully outlined in Section 3.6.4.

### **5. DATA COLLECTION AND EVALUATION**

#### **5.1 DATA COLLECTION**

Members of the HIV and AIDS Malignancy Branch clinical research team will collect data on study subjects according to the Schedule of Evaluations outlined in [Appendix 1](#). Complete records must be maintained on each patient including supplementary information obtained from outside laboratories, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. The primary source documentation will assure the following:

5.1.1 The patient satisfied each eligibility criterion.

5.1.2 Signed informed consent was obtained prior to registration and treatment.

5.1.3 Treatment was given according to protocol or any protocol deviations or non-compliance is documented and justified.

5.1.4 Toxicity and response were assessed according to protocol.

5.1.5 Drug accountability records were kept on each patient.

Clinical data will be coded for database entry. Data will be stored in the CCR C3D clinical trials database. Dr. Robert Yarchoan, the Principal Investigator, will be responsible for the protocol.

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. Patients will be followed for adverse events for 4 weeks after removal from study treatment or until off-study, whichever comes first.

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 patient's outcome.

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

**Loss or destruction of data:** Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

## 5.2 RESPONSE CRITERIA

The evaluation of the response of KS to an agent or regimen is difficult to grade by means of commonly used oncologic definitions. However, in an effort to standardize the evaluation of therapy against KS, the AIDS Clinical Trial Group Oncology Committee has devised a set of staging and response definitions for KS. We will use a modification of these criteria to assess responses, which is consistent with the criteria used in our previous KS studies. 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 patients 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).

### 5.2.1 Complete Response

- 5.2.1.1 The absence of any detectable residual disease, including tumor-associated edema, persisting for at least 4 weeks.
- 5.2.1.2 In patients in whom pigmented macular skin lesions persist after apparent CR, biopsy of at least one representative lesion is required to document the absence of malignant cells.
- 5.2.1.3 In patients known to have had visceral disease, an attempt at restaging with appropriate endoscopic or radiographic procedures should be made. If such procedures are medically contraindicated, the patient may be classified as having a clinical CR.

#### 5.2.2 Clinical Complete Response

- 5.2.2.1 The absence of any detectable residual disease, including tumor associated edema, persisting for at least 4 weeks.
- 5.2.2.2 For patients with pigmented macular skin lesions persisting after apparent complete response, a representative lesion has not been biopsied.
- 5.2.2.3 For patients 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 patient has no clinical evidence of visceral disease, the patient may be classified as having a clinical CR.

#### 5.2.3 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:

- 5.2.3.1 A 50% or greater decrease in the number and/or size of previously existing lesions lasting for at least 4 weeks **or**
- 5.2.3.2 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**
- 5.2.3.3 A 50% decrease in the sum of the products of the largest perpendicular diameters of the marker lesions lasting for at least 4 weeks **or**
- 5.2.3.4 A 50% decrease in radiologically measurable visceral lesions sustained without evidence of re-growth for at least 4 weeks **or**
- 5.2.3.5 Patients who otherwise meet the criteria for a CR but still have residual tumor-associated edema or effusions will be classified as having a PR.

#### 5.2.4 Progressive Disease

- 5.2.4.1 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.

- 5.2.4.2 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**
- 5.2.4.3 A change in character from macular to plaque-like or nodular of at least 25% of the lesions **or**
- 5.2.4.4 New visceral sites of involvement or progression of visceral disease **or**
- 5.2.4.5 The development of new or increasing tumor-associated edema or effusion that lasts at least 1 week and interferes with the patient's normal activities.

#### 5.2.5 Stable Disease

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

#### 5.2.6 Overall Response

Overall Response = Complete Response + Clinical Complete Response + Partial Response. The Overall Response Rate (ORR) is the fraction of subjects with an Overall Response after 6 cycles of liposomal doxorubicin in combination with bevacizumab.

#### 5.2.7 Progression-free survival

As Kaposi's Sarcoma is a disease in which recurrence is common, progression will be determined based on progression from best response. As in section 5.2.4., progression must be documented on two visits at least 1 week apart. The criteria for progression from best response include:

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

Progression-free survival is defined as the time interval from the date of enrolment to the date of progression from best response.

### 5.3 TOXICITY CRITERIA

#### 5.3.1 Toxicity Grading

This study will utilize the NCI CTC version 3.0 for toxicity and adverse event reporting. (See: <http://ctep.info.nih.gov>). All appropriate treatment areas should have access to a copy of the CTCAE version 3.0.

### 5.3.2 Toxicity Attribution

The Principal Investigator or physician Associate Investigator will document the relationship of the protocol intervention to each adverse event by assigning attribution per adverse event at time of clinical evaluation. Attribution will be rated as follows:

- 5.3.2.1 Unrelated
- 5.3.2.2 The adverse event is clearly not related to the investigational agents.
- 5.3.2.3 Unlikely Related
- 5.3.2.4 The adverse event is doubtfully related to the investigational agents.
- 5.3.2.5 Possibly Related
- 5.3.2.6 The adverse event may be related to the investigational agents.
- 5.3.2.7 Probably Related
- 5.3.2.8 The adverse event is likely related to the investigational agents.
- 5.3.2.9 Definitely Related
- 5.3.2.10 The adverse event is clearly related to the investigational agents.

## 5.4 STATISTICAL CONSIDERATIONS

### 5.4.1 Racial/gender make-up

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria outlined in section 2.1. Outreach efforts will be made to extend accrual to a representative population. As a practical matter, it should be noted that historically in the US, KS has been relatively rare in females with HIV infection. However, our more recent studies have increasingly accrued female immigrants from Africa.

### 5.4.2 Age exclusion

Patients under the age of 18 will be excluded. This is a pilot clinical study of an agent with unknown safety profile in children and adolescents in a disease that is rare in children, and for which standard therapies are available. Anti-angiogenesis therapy theoretically could interfere with growth in children and adolescents. Additionally, since the protocol requires several skin biopsies, patient-volunteers should enter the protocol with full consent, rather than just assent.

### 5.4.3 Sample size and accrual limit

#### 5.4.3.1 Primary Outcome

This is a pilot study. The primary objective is to estimate of the overall response rate of the combination of liposomal doxorubicin and bevacizumab after 6 cycles in patients with advanced Kaposi's sarcoma. The ORR will be evaluated in two cohorts of patients.

**Cohort 1:** Patients who are either:

- 5.4.3.1.1 HIV negative
- 5.4.3.1.2 HIV infected with stable KS despite one year of HAART with HIV viremic control
- 5.4.3.1.3 HIV infected with progressive KS despite four months of HAART with HIV viremic control

**Cohort 2:** All other patients with advanced AIDS-associated KS

Ten evaluable patients will be enrolled in each cohort (20 total). Patients are considered evaluable if they (1) have received at least 2 cycles of liposomal doxorubicin, OR (2) have come off the protocol for reasons of treatment toxicity. Up to 3 patients in each cohort may be replaced if they come off the study before being considered evaluable. Thus, the accrual ceiling will be 26 patients. With 10 evaluable patients in each cohort, the 80% two sided confidence intervals about the fraction with any responses are as follows:

<u>Number of responses</u>	<u>80% CI</u>
1.	.01-.34
2.	.05-.45
3.	.12-.55
4.	.19-.65
5.	.27-.73
6.	.35-.81
7.	.45-.88
8.	.55-.95
9.	.66-.99

Thus, the evaluation of 10 patients in each cohort may allow an estimate with an 80% CI width of approximately .33 to .45. The estimates in the two cohorts will be compared and if they are similar ( $p > 0.30$  by a Fisher's exact test), they may be combined to form a somewhat more precise estimate of the fraction that respond within 6 cycles of treatment.

5.4.3.2 Secondary Outcomes

The following secondary outcomes will be evaluated with the following statistical considerations.

- 5.4.3.2.1 **Assess the toxicity of liposomal doxorubicin combined with bevacizumab in patients with advanced KS.**

Toxicities (including those possibly, probably, and definitely attributed to study treatment) will be assessed by grade. Toxicities will be evaluated both per cycle and per patient. All patients will be considered evaluable for toxicity.

5.4.3.2.2 **Estimate the complete response rate after 6 cycles of the combination of liposomal doxorubicin in combination with bevacizumab in evaluable patients with advanced KS.**

The evaluable patients from each cohort will be evaluated separately as described in section 5.4.3.1. The 80% confidence intervals of the estimates of complete response rates and the corresponding to the number of response are listed in Section 5.4.3.1.

The estimates in the two cohorts will be compared and if they are similar ( $p > 0.30$  by a Fisher's exact test), they may be combined to form a somewhat more precise estimate of the fraction that have a complete response within 6 cycles of treatment.

5.4.3.2.3 **Estimate the median number of cycles of liposomal doxorubicin in combination with bevacizumab required to achieve a partial response in patients with advanced KS.**

The median number of cycles required to achieve a partial response will be described for evaluable patients in each cohort separately, along with a range of number of cycles required. Patients with progressive disease will be excluded from this analysis.

5.4.3.2.4 **Estimate 12-month progression free survival in evaluable patients with advanced KS treated with the combination of liposomal doxorubicin and bevacizumab followed by 11 cycles of bevacizumab.**

Progression-free survival will be calculated using Kaplan-Meier methodology. Separate curves will be formed for evaluable patients in each cohort, and a combined curve may also be formed if the two cohorts have similar PFS (which will be indicated by having a  $p$ -value  $> 0.30$  by a two-tailed log rank test). Since the number of patients will be small, these results will be considered to provide approximate estimates of PFS. The two-sided 80% confidence interval around the PFS estimate at one year will be computed, and it will be of interest to determine if this interval includes 70%, the result that was found in our previous Doxil/IL-12 study. In view of the pilot nature and limited size of this study, this will only be done to determine the approximate impact of this treatment with regard to PFS in an informal manner.

5.4.3.2.5 **Evaluate the impact of the combination of liposomal doxorubicin in combination with bevacizumab, as well as bevacizumab maintenance on CD4 counts.**

The effect that the combination of liposomal doxorubicin and bevacizumab followed by maintenance bevacizumab have on CD4 counts will be evaluated separately in each cohort, as patients in Cohort 2 would be expected to have increases in their CD4 counts once on an optimized HAART regimen. CD4 counts will be evaluated at baseline, on Day 1 of cycles 4,7,11, and 15, as well

as 6 months after completing therapy. Changes in CD4 count at each of these time points will be calculated by subtracting the baseline value from the subsequent time points, and tested for being different than zero using the Wilcoxon signed rank test. As these will be considered entirely exploratory analyses, there will not be any formal adjustment for multiple comparisons, although the findings will be interpreted informally in the context of the number of tests performed.

**5.4.3.2.6 Evaluate the effect of liposomal doxorubicin combined with bevacizumab followed by bevacizumab maintenance, on PBMC and saliva KSHV viral load.**

We will perform an exploratory analysis of the effect of the combination of liposomal doxorubicin and bevacizumab on log<sub>10</sub> saliva and PBMC KSHV viral load in all patients in Cohort 1 and Cohort 2 combined.

**5.4.3.2.7 Explore, in a preliminary manner, the short-term effects of bevacizumab and intermediate and long term effects of the combination of bevacizumab and liposomal doxorubicin on blood flow to cutaneous KS lesions using non-invasive imaging techniques.**

Descriptive changes in imaging results from each of the three modalities of target lesions will be detailed for each study subject longitudinally at time points Cycle 1 Day 1, Cycle 1 Day 8, Cycle 7 Day 1, and Cycle 17 Day 21 (if there are persistent lesions).

**5.4.4 Projected Accrual Timeline**

It is anticipated that up to 3 years may be required to accrue 20 patients to enroll into this pilot trial, with the ceiling set at 26 to allow for replacement of patients.

**5.5 MULTI-INSTITUTIONAL GUIDELINES**

Not applicable.

**5.6 DATA AND SAFETY MONITORING PLAN**

Patient data will be collected in a timely manner and reviewed by a physician Associate Investigator and/or the Principal Investigator, Robert Yarchoan, for toxicity. Any toxicity  $\geq$  Grade 3 will be reviewed by the Principal Investigator. In the event that an unacceptable toxicity occurs, the IRB will be informed and appropriate measures, as outlined in Section 7, will be taken. The Principal Investigator will do the monitoring of this pilot study in an ongoing manner, and a Data and Safety Monitoring Board will not be used.

**6. HUMAN SUBJECTS PROTECTIONS**

**6.1 RATIONALE FOR SUBJECT SELECTION**

This protocol is designed for the treatment of all adult subjects with advanced KS. The main epidemiologic groups that would be potential subjects are those with HIV-associated KS, transplant recipients, and those with classic or endemic KS, who are generally older individuals from areas where the prevalence of KSHV infection is high. KS disproportionately affects men, with a male:female ratio that varies between epidemiologic groups. At the HAMB, most women who have participated in our previous KS studies have been immigrants from Africa.

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

## **6.2 PARTICIPATION OF CHILDREN**

KS is an extremely rare tumor in children, with a different presentation than that seen in adults. KS in children generally presents with lymphadenopathy, which likely represents a biologically different disease than that seen in adults. As such, subjects <18 will be excluded from this protocol.

## **6.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS**

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

### **6.3.1 Potential Benefits**

The potential benefit to individual patient-volunteers is that the protocol therapy may result in rapid improvement of advanced KS. It may also provide long-term control of KS without the high potential cumulative toxicity due to complications associated with chronic administration of standard cytotoxic therapy. Participation in a clinical study will help researchers better understand the pathophysiology of KS, as well as allow for an evaluation of liposomal doxorubicin in combination bevacizumab in patients with advanced KS.

### **6.3.2 Potential Risks and Discomforts**

There may be no direct benefit to the patient-volunteers on this study. Potential risks include the potential toxicity of the agents. Additionally, the safety of the agents has not been evaluated when used in combination have not been defined in this population. See Section 8 for potential toxicities. Additional risks include the small risk of complications related to cutaneous "punch" biopsies, which include infection and pain.

## **6.4 RISK/BENEFITS ANALYSIS**

The risks to individual study subjects are reasonable in relation to the anticipated benefits. This protocol combines a standard FDA approved agent for the treatment of KS with bevacizumab, which has strong preclinical rationale, an acceptable toxicity profile and promising clinical activity in our previous phase II study, 03-C-0110. Potential therapeutic benefits include limitation of cumulative anthracycline dosing, more rapid tumor regression, and additional therapeutic options for patients for difficult to manage KS. The risks include potential additive toxicities and additional studies associated with participation in a clinical study. The major alternative for patients with advanced KS is to receive FDA approved agents outside of a clinical study. The comparative risks and benefits are acceptable for an early phase clinical study, when compared to the alternatives for patients with advanced KS.

## **6.5 SPECIMEN HANDLING GUIDELINES**

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.

#### 6.5.1 AIDS Monitoring Laboratory

Many samples on this study will be processed and stored in the AIDS Monitoring Laboratory (AML) run by Science Applications International Corporation (SAIC) in the NCI-Frederick facility located with Fort Detrick. The samples are stored under code, and the information linking these unique codes to the patients 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). Clinical testing of all samples will be one in accordance to the protocol. 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.

#### 6.5.2 Tracking Samples

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.

#### 6.5.3 Samples for Planned Collaboration

6.5.3.1 **KSHV PCR and serology:** Some of the specimens are sent to the laboratory of Dr. Denise Whitby, also in Science Applications International Corporation (SAIC) in the NCI-Frederick facility located with Fort Detrick. The samples sent are coded by the protocol research team and have no patient identifiers. They are logged in by Dr. Whitby's laboratory and kept in a locked facility. They are run in batch when enough specimens are collected. Records are kept when the specimens are used for analysis.

6.5.3.2 **CD103 and VEGF polymorphisms:** Specimens for these correlative studies will be processed and stored in the AML as outlined in section 6.5.1. Coded samples with no patient identifiers will be analyzed in batches once enough specimens are collected. Assays will be performed in the laboratory of Dr. William Figg

6.5.3.3 **DII4, PDGF-C and G-CSF IHC:** Specimens for these correlative studies will be processed and stored in the lab of Dr. Giovanna Tosato, (Building 37, Room 4134B). The samples sent are coded by the protocol research team and have no patient identifiers. They are logged in by Dr. Tosato's laboratory and kept in a

locked facility. They are run in batch when enough specimens are collected. Records are kept when the specimens are used for analysis.

#### 6.5.4 Dr. Yarchoan's 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.

#### 6.5.5 Routine Samples

Many routine samples and a sample of the biopsy specimens are sent to the Clinical Pathology or Pathology Departments of the NIH Clinical Center. These samples will be handled according to the procedures of these departments.

#### 6.5.6 Co-enrollment in 01-C-0038

If patients have 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 the samples may also be tested under the specifications of that study. Similarly, if patients have co-enrolled on other studies approved by the NCI IRB that call for maintaining and testing the samples, then they may be transferred to those studies.

#### 6.5.7 Unused Samples

At the termination of the study, if patients have 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 the samples will be transferred to that study unless the patients request that this not occur. Also, if patients have co-enrolled on other studies approved by the NCI IRB that call for maintaining the samples, then they will be maintained on those protocols. Otherwise, the unused samples will be destroyed.

#### 6.5.8 Lost or Destroyed Samples

The PI will report any loss or destruction of the samples to the IRB, and any new use of the samples, specimens, or data will require prospective IRB approval.

#### 6.5.9 Non-Invasive Imaging

Dr. Amir Gandjbakhche will maintain non-invasive images. Images will be coded with no name identification. Records will be maintained in a locked facility.

### **6.6 CONSENT AND ASSENT PROCESS AND DOCUMENTATION**

Informed written consent will be obtained in all patients on this trial. There will be no minors enrolled < 18 years old, so assent is unnecessary. All potential participants will be provided an informed consent form to review prior to their screening visit. The Principal Investigator or a physician Associate Investigator will discuss the risks, benefits and alternatives to participation in this study, and answer all patient questions regarding study participation. Informed consent will be obtained on an IRB approved informed consent form, with signatures obtained from the consenting physician, a witness, and the study subject. The original informed consent will be stored in the patient's official hospital records, with a copy stored in the patient's research chart, and a copy provided to the study subject.

## **7. SAFETY REPORTING REQUIREMENTS/ DATA AND SAFETY MONITORING PLAN**

### **7.1 DEFINITIONS**

#### 7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

#### 7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

#### 7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

"Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

#### 7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

#### 7.1.5 Serious Adverse Event

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 drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- 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.

#### 7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

#### 7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

#### 7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

#### 7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

#### 7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to
  - (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  - (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

## 7.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

### 7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

### 7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.

2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
  - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
  - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
  - All Grade 5 events regardless of attribution;
  - All Serious Events regardless of attribution.

**NOTE:** Grade 1 events are not required to be reported.

### **7.3 DATA AND SAFETY MONITORING PLAN**

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

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

The principal investigator will review adverse event and response data on each patient 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. PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION**

### **8.1 BEVACIZUMAB**

#### 8.1.1 Study Drug Supply

##### 8.1.1.1 CCR/CRADA

8.1.1.2 The agent (hereinafter referred to as Agent), bevacizumab, used in this protocol is/are provided to the NCI under Cooperative Research and Development Agreement (CRADA) 2407 between the Pharmaceutical Company, Genentech, (hereinafter referred to as Collaborator(s)) and the NCI HIV and AIDS Malignancy Branch. Therefore, the following obligations/guidelines, in addition to the provisions in the “Intellectual Property Option to Collaborator” (<http://ctep.cancer.gov/industry>) apply to the use of the Agent in this study:

8.1.1.2.1 Agent may not be used outside the scope of this protocol, nor can Agent be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent are confidential and proprietary to Collaborator(s) and should be maintained as such by the investigators.

8.1.1.2.2 For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different CTAs or CRADAs, the access to and use of data by each Collaborator

shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data.):

- 8.1.1.2.2.1 NCI will provide all Collaborators with written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations, which would tend to restrict NCI's participation in the proposed combination protocol.
  - 8.1.1.2.2.2 Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.
  - 8.1.1.2.2.3 Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.
  - 8.1.1.2.3 Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the Standards for Privacy of Individually Identifiable Health Information set forth in 45 C.F.R. Part 164.
  - 8.1.1.2.4 When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators of Collaborator's wish to contact them.
- 8.1.2 Supplier
- 8.1.2.1 Genentech, Inc.
  - 8.1.2.2 Manufacturer – Genentech, Inc.
  - 8.1.2.3 Chemical name
    - 8.1.2.3.1 Bevacizumab (RhuMAb VEGF; Avastin®)
    - 8.1.2.3.2 Recombinant humanized anti-vascular endothelial growth factor monoclonal antibody
    - 8.1.2.3.3 The bevacizumab to be supplied for this protocol is intended for clinical trial use only and is not the commercially available Avastin. Investigational bevacizumab and commercially available Avastin may be produced at separate facilities and some difference may exist between the two products, although both are required to meet similar product testing criteria and are expected to be very similar in safety and activity. For further details and molecule characterization, see the updated bevacizumab Investigator Brochure.
  - 8.1.2.4 Mechanism of action: Binds to VEGF

### 8.1.3 Toxicity

A summary of previously reported adverse events is listed below.

#### 8.1.3.1 **Infusion related events**

Flushing, tachycardia, hypotension, hypertension, rash, fever, chills.

#### 8.1.3.2 **Cardiovascular**

Pericardial effusion, hypertension (including hypertensive crisis), hypotension, and decrease in cardiac function.

#### 8.1.3.3 **Hematologic**

Arterial and venous thrombosis/embolism (including pulmonary embolism, mesenteric vein thrombosis, ischemic bowel, cerebral vascular accident); hemorrhage (including epistaxis, CNS hemorrhage/bleeding, hematemesis, hemoptysis, GI bleeding, pulmonary hemorrhage); hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, leukopenia. **Thrombosis and hemorrhage could be life threatening and fatal.**

#### 8.1.3.4 **Constitutional**

Fever, rigors, chills, headache, infection without neutropenia, asthenia.

#### 8.1.3.5 **Skin/integument**

Rash/desquamation; delay in wound healing.

#### 8.1.3.6 **Gastrointestinal**

Nausea, colitis, stomatitis/pharyngitis, intestinal obstruction, vomiting. Rare adverse events include bowel perforation, bowel anastomotic dehiscence and the possibility of delay in wound healing. These events have been reported in clinical trials using bevacizumab alone or in combination with chemotherapy, and were likely related to co-existing factors such as tumor involvement, chemotherapy, recent invasive procedures or bowel inflammation. However, it cannot be excluded that bevacizumab contributed to these events.

#### 8.1.3.7 **Hepatic (rare)**

Reversible and marked elevations of liver function tests (total bilirubin and /or transaminase and alkaline phosphatase) have been rarely reported when bevacizumab is used in combination with chemotherapy or concurrently with other drugs that are potentially hepatotoxic. The mechanism of such hepatic toxicities is unclear. It is possible that in rare occasion, bevacizumab may potentiate the liver side effects of a concurrent medication, although it is unclear at this time whether bevacizumab alone can cause LFT derangement.

#### 8.1.3.8 **Pulmonary**

Pulmonary infiltration, dyspnea

#### 8.1.3.9 **Renal/Genitourinary**

Proteinuria, nephrotic syndrome

#### 8.1.3.10 **Musculoskeletal**

Arthralgia, chest pain

#### 8.1.3.11 **Central Nervous System**

Reversible Posterior Leukoencephalopathy Syndrome (RPLS) or similar leukoencephalopathy syndrome related to vasogenic edema of the white matter have been rarely reported in association with bevacizumab therapy (<1%). Clinical presentations are variable and may include altered mental status, seizure and cortical visual deficit. HTN is a common risk factor and was present in most (though not all) patients on bevacizumab who developed RPLS. MRI scans are key to diagnosis and typically demonstrate vasogenic edema (hyperintensity in T2 and FLAIR images and hypointensity in T1 images) predominantly in the white matter of the posterior parietal and occipital lobes; less frequently, the anterior distributions and the gray matter may also be involved. RPLS should be in the differential diagnosis in patients presenting with unexplained mental status change, visual disturbance, seizure or other CNS findings. RPLS is potentially reversible, but timely correction of the underlying causes, including control of blood pressure and interruption of the offending drug, is important in order to prevent progression to irreversible tissue damage.

#### 8.1.4 Formulation and Preparation

8.1.4.1 **How supplied:** Bevacizumab is supplied as a clear to slightly opalescent, sterile liquid ready for parenteral administration in vials of two sizes:

8.1.4.1.1 Each 100mg (25 mg/mL – 4 mL fill) glass vial contains bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection, USP or

8.1.4.1.2 Each 1000 mg (25 mg/ml- 40 ml fill) glass vial contains bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection, USP.

8.1.4.2 **Preparation:** Opened vials must be used within 8 hours. Vials contain no preservative and are intended for single use only. The calculated dose should be placed in a sterile, empty IV bag and diluted with a sufficient amount of 0.9% Sodium Chloride Injection to obtain a final volume of 100 mL. Once the bevacizumab has been added to the bag with 0.9% Sodium Chloride Injection, the solution must be administered within 8 hours. When the rhuMAb VEGF drug product container is empty, 0.9% NS from the primary line should be used to flush the secondary set to complete rhuMAb VEGF delivery. 0.9% NS infusion should be continued to flush the primary IV set with a volume of fluid at least equal to the tubing priming volume, thus insuring complete drug delivery. Note that this flush is not included in the infusion times below."

#### 8.1.5 Stability and Storage

Bevacizumab is shipped on blue ice by overnight delivery. On receipt, bevacizumab should be stored in the refrigerator (2° to 8° C) and should remain refrigerated until just prior to use. Do not freeze. Do not shake. Shelf-life studies of bevacizumab are continuing. Opened vials must be used within 8 hours. Vials contain no preservative

and are intended for single use only. Once the bevacizumab has been added to a bag of sterile saline, the solution must be administered within 8 hours.

#### 8.1.6 Administration Procedures

##### 8.1.6.1 Route: Intravenous

8.1.6.2 The initial dose should be administered over a minimum of 90 minutes. If no adverse reactions occur, the second dose should be administered over a minimum of 60 minutes. Again, if no adverse reactions occur, the third and subsequent doses should be administered over a minimum of 30 minutes. If infusion-related adverse reactions occur, subsequent infusions should be administered over the shortest period that is well tolerated.

#### 8.1.7 Incompatibilities

Unknown incompatibilities

## 8.2 LIPOSOMAL DOXORUBICIN

#### 8.2.1 Commercially available

8.2.1.1 FDA indication for use in AIDS-associated KS

8.2.1.2 Manufacturer: Ortho Biotech Products, L.P. (Doxil) or Sun Pharmaceutical Industries, Ltd. (Lipodox). Lipodox may be used in place of Doxil if Doxil is not available.

#### 8.2.2 Supplier

Clinical Center Pharmacy will obtain liposomal doxorubicin from commercial sources.

#### 8.2.3 Toxicity

##### 8.2.3.1 **Hematologic effects**

8.2.3.1.1 Neutropenia is the predominant manifestation of hematologic toxicity.

8.2.3.1.2 Thrombocytopenia and anemia occur less frequently.

##### 8.2.3.2 **Cardiac Effects**

###### 8.2.3.2.1 **Conduction Disorders**

Acute transient abnormal ECG findings (ST-T wave changes, prolongation of QT interval, arrhythmias)

###### 8.2.3.2.2 **Cardiomyopathy**

Subacute chronic cardiotoxicity, resulting in cardiomyopathy, usually occurs within 1 year after discontinuance of anthracycline treatment, but experience with liposomal doxorubicin at high cumulative doses currently is too limited to establish its cardiotoxic potential. Late onset cardiotoxicity occurs with high cumulative doses of anthracycline therapy, mainly in those who received the drugs as children. The potential for this complication in the liposomal preparation is not known.

##### 8.2.3.3 Gastrointestinal effects

Stomatitis, esophagitis (mucositis)

#### 8.2.3.4 Dermatologic Effects

8.2.3.4.1 Alopecia (less with liposomal preparation than with the conventional formulation)

8.2.3.4.2 Palmar-plantar erythrodysesthesia

8.2.3.4.3 Radiation recall injury

#### 8.2.3.5 Infusion-related Reactions

Flushing, shortness of breath, facial edema, headache, chills, back pain, chest and throat tightness, hypotension.

#### 8.2.3.6 Local Effects

Extravasation produces local tissue necrosis, cellulitis, vesication, thrombophlebitis, lymphangitis or painful induration.

#### 8.2.3.7 Pregnancy

Liposomal doxorubicin can cause fetal harm.

### 8.2.4 Formulation and Preparation

8.2.4.1 Formulated in long-circulating pegylated liposomes. The concentrated product is supplied in 10 cc vials containing 2/mg/mL of liposomal doxorubicin.

8.2.4.2 The concentrate must be diluted in 250 mL 5% dextrose prior to IV infusion. Diluents containing preservatives should not be used, and other drugs should not be added to the solution. If a precipitant forms, the solution should not be used.

### 8.2.5 Stability and Storage

8.2.5.1 Refrigerate the concentrate at 2-8° C; do not freeze. The diluted solution should also be refrigerated at 2-8° C and used within 24 hours.

### 8.2.6 Administration procedures

8.2.6.1 The diluted formulation should be administered intravenously, without use of inline filters and should not be mixed with other drugs.

8.2.6.2 Only use 5% dextrose as diluent and no preservatives.

8.2.6.3 Initial infusion rate should be over 30 minutes.

### 8.2.7 Incompatibilities

8.2.7.1 Liposomal doxorubicin has not been formally evaluated for drug interactions. Other myelosuppressive agents may potentiate the myelotoxicity of liposomal doxorubicin.

8.2.7.2 Phenobarbital may increase elimination of liposomal doxorubicin

8.2.7.3 Liposomal doxorubicin may decrease serum phenytoin concentrations

8.2.7.4 Streptozocin may inhibit hepatic metabolism of liposomal doxorubicin.

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## 10. APPENDICES

### 10.1 APPENDIX 1: SCHEDULE OF EVALUATION

Study Evaluation	Screening <sup>1</sup>	Cycle 1 D1, 8, 15	Subsequent Cycles, D1	Follow-up q3m	Off Study
Eligibility Checklist	X				
KS Biopsy	X <sup>2</sup>	X <sup>2</sup>			
Document Extent of Disease		X	X	X	X
Photography, Target Lesions		X <sup>3</sup>	X <sup>4</sup>		
History and Physical <sup>5</sup>	X	X	X	X	X
Toxicity Assessment	X	X	X	X	X
C-reactive protein	X				X
Acute care, hepatic and mineral panels, TP, uric acid, CK, LDH	X	X	X	X	X
CBC with Differential, Retic	X	X	X	X	X
PT/PTT	X				
FACS (CD4 and CD8)	X		X <sup>6</sup>		
HIV Western Blot	X				
HIV Viral Load (HIV infected)	X		X <sup>6</sup>		
Hepatitis Serology <sup>7</sup>	X				
VDRL	X				
TFTs					
Urine Studies <sup>8</sup>	X	X	X	X	X
Urine β-hCG (women)	X	X <sup>9</sup>	X		
EKG	X				
MUGA	X		X (cycle7) <sup>10</sup>		
CXR	X		X <sup>11</sup>		

Study Evaluation	Screening <sup>1</sup>	Cycle 1 D1, 8, 15	Subsequent Cycles, D1	Follow-up q3m	Off Study
Additional Imaging as Indicated for Staging	X				
4 Red Top Tubes <sup>12</sup> (SAIC)		X	X	X	
4 Yellow Top <sup>13</sup> (SAIC)		X	X (q3 cycles)	X	
Saliva (DW lab, SAIC)		X	X (q3cycles)	X	
Non-invasive imaging <sup>14</sup>		X	X		

1. Results must be completed within 2 weeks of starting study, with the exception of: a) EKG, MUGA must be within 6 weeks of study, b) a positive HIV Western Blot may be documented anytime prior to enrollment, an negative HIV western blot must be documented within 2 weeks of starting study
2. In addition to screening biopsy, additional punch biopsies may be required to confirm clinical complete responses, document progression on uncertain cases. Optional biopsies may be performed for biomarker studies on Day 8.
3. Day 1 only
4. Day 1 cycle 7 (completion of liposomal doxorubicin + bevacizumab) and Day 21 cycle 17 (completion of bevacizumab)
5. Includes vital signs, current medications, and Performance Status
6. FACS for CD4/CD8 and HIV viral load on Cycles 4, 7, 11, 15, 18
7. Hepatitis B S Ag, Hepatitis B S Ab, Hepatitis B core Ab., HAV total, HCV Ab
8. Urinalysis, urine protein, urine creatinine. If  $\geq 1+$  proteinuria on dipstick, then 24 hour urine collection
9. Day 1 only
10. MUGA on Day 1 cycle 7 (+/- 7 days) after completing scheduled 6 cycles of liposomal doxorubicin. Subjects with EF <50% should have a follow-up MUGA 3 months later. Repeat MUGA will be performed if additional liposomal doxorubicin is administered in combination with bevacizumab. This will be scheduled 21 (+/- 7) days after 6<sup>th</sup> or final dose of liposomal doxorubicin
11. Repeat abnormal imaging q 3 cycles until documentation of complete response.
12. Red top tubes for cytokine studies, human endoglin/Cd105, and KSHV serology
13. Yellow-top tubes for PBMC KSHV viral loads
14. Day 1 Cycle 1, Day 8 Cycle 1, Day 1 cycle 7 (completion of liposomal doxorubicin + bevacizumab) and Day 21 cycle 17 (completion of bevacizumab) if there are persistent lesions.

## 10.2 APPENDIX 2: ECOG PERFORMANCE 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.

### **10.3 APPENDIX 3: DESCRIPTION OF NON-INVASIVE IMAGING DEVICES**

#### **1. Laser Doppler Imaging Device**

A Moor Instrument moorLDI-2l-simultaneous two wavelength scan will be used. The scan images at 685-690 and 780 nm. This imager scans a low power laser beam (5mW, 800 micron diameter) in a raster pattern over the skin. There is no contact with the skin. The scanner is produced by Moor Instruments Ltd., 501 Silverside Rd., Suite 66, Wilmington, DE 19803. The scanner is held on a special stand (Moor MS2 stand) made specifically for such devices.

A very closely related instrument, the moor LDI-VR scanner is approved by the FDA by patient use. This scanner just utilizes one wavelength of light (633 nm). The device that will be used in the present study differs from the FDA-approved device in that it also assesses the lesions using a second wavelength and that the first wavelength is slightly longer. The other potential safety issue is that the 780 nm wavelength is in the infrared spectrum and would not be visible. Thus patients and users would not necessarily blink if it shined into their eyes. However, both lights shine on the same spot so patients or users would blink upon seeing the 685 nm wavelength (which is visible). Also, the machine is constructed to minimize the chance of anyone (patient or user) looking into the laser light.

#### **2. Multispectral Imaging**

For multispectral imaging, a charge coupled device (CCD) handheld camera will be used. This uses CCD technology similar to that utilized in most digital cameras. The lesions will be illuminated uniformly by a white light held approximately 30 cm from the patient's skin. Using optical filters, those wavelengths associated with oxyhemoglobin and deoxyhemoglobin absorption spectra will be selected and CCD images will be made. The camera is a cooled Princeton CCD Camera interfaced by cable with a personal computer with associated data analysis software. The camera is low voltage. The camera will not directly contact the patient and will be held 5 cm or more from the patient's skin (approximately 30 to 50).

#### **3. Thermal Imaging**

For thermal imaging, infrared-sensitive handheld cameras will be used. We plan to use a camera modified from the BioEar PRISM 2000 Thermal Metabolic Imaging System (10618 Rockley Rd., Houston, TX). The name of the camera is the ThermoVision™ Alert, FLIR Systems, USA. This camera is at ambient temperature. If it becomes available, we will also test a cooled camera modified from the ThermaCAM SC3000 (FLIR Systems, 16 Esquire rd., Boston, MA). Cooling has the potential of giving more accurate measurements with less background noise. If this camera is found to be superior and can be made available on a long-term basis, we will switch over to its use.

These cameras use low voltage electricity (12- 13 volts) provided by an AC adapter. They do not touch the patient and will be held more than 5 cm from the patient's skin (approximately 50 cm).