



**AIDS MALIGNANCY  
CLINICAL TRIALS CONSORTIUM**

**AMC PROTOCOL #059:  
A Phase 1/2 Trial of PTC299 in Patients with  
HIV-Related Kaposi's Sarcoma**

**A Multi-Center Trial of the AIDS Malignancy  
Clinical Trials Consortium (AMC)**

**Sponsored by:** National Cancer Institute  
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**Protocol Chair:** Susan E. Krown, MD

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**AMC PROTOCOL SIGNATURE PAGE**

I, \_\_\_\_\_, Principal Investigator at site \_\_\_\_\_, agree to conduct and follow this protocol: **AMC Protocol #059 - A Phase 1/2 Trial of PTC299 in Patients with HIV-related Kaposi's Sarcoma (Version 9.0, 10/22/2010)**, as written according to AMC, NCI and FDA guidelines. I understand that no deviations from the above protocol may be made without written permission from the Protocol Chair(s).

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date (mm/dd/yyyy)

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## PROTOCOL ROSTER

### AMC Protocol #059 A Phase 1/2 Trial of PTC299 in Patients with HIV-Related Kaposi's Sarcoma

#### **Protocol Chair:**

Susan E. Krown, MD  
Memorial Sloan-Kettering Cancer Center  
1275 York Avenue  
New York, NY 10065  
Tel: (212) 639-7426  
Fax: (212) 717-3342  
E-mail: [krowns@mskcc.org](mailto:krowns@mskcc.org)

#### **Protocol Statistician:**

Jeannette Y. Lee, PhD  
AMC Statistical Center  
University of Arkansas for Medical Sciences  
4301 W. Markham, #781  
Little Rock, AK 72205-7199  
Tel: (501) 526-6712  
Fax: (501) 526-6729  
E-mail: [jylee@uams.edu](mailto:jylee@uams.edu)

#### **PTC Therapeutics Medical Monitor:**

Angela Ogden, MD  
PTC Therapeutics, Inc.  
100 Corporate Court  
South Plainfield, NJ 07080  
Tel: (908) 912-9226  
Fax: (908) 222-1669  
E-mail: [aogden@ptcbio.com](mailto:aogden@ptcbio.com)  
[ptc299saereport@ptcbio.com](mailto:ptc299saereport@ptcbio.com)

#### **Data Management/Operations:**

AMC Operations Center  
The EMMES Corporation  
401 N. Washington Street, Suite 700  
Rockville, MD 20850  
Tel: (301) 251-1161  
Fax: (240) 238-2842  
E-mail: [amcpm@emmes.com](mailto:amcpm@emmes.com)

**SITES PARTICIPATING IN AMC #059**

This trial is open to all AMC sites for subject accrual.

## SCHEMA

**TITLE:** Phase 1/2 Trial of PTC299 in Patients with HIV-Associated Kaposi's Sarcoma (KS)

**DESIGN:** Open-label, escalating-dose Phase 1-2 trial with expansion of the cohort at the maximum safely tolerated dose.

**SAMPLE SIZE:** 3 to 38 evaluable subjects (up to 44 subjects may be required to reach a total of 38 evaluable subjects).

**POPULATION:** HIV-infected subjects with biopsy proven KS.

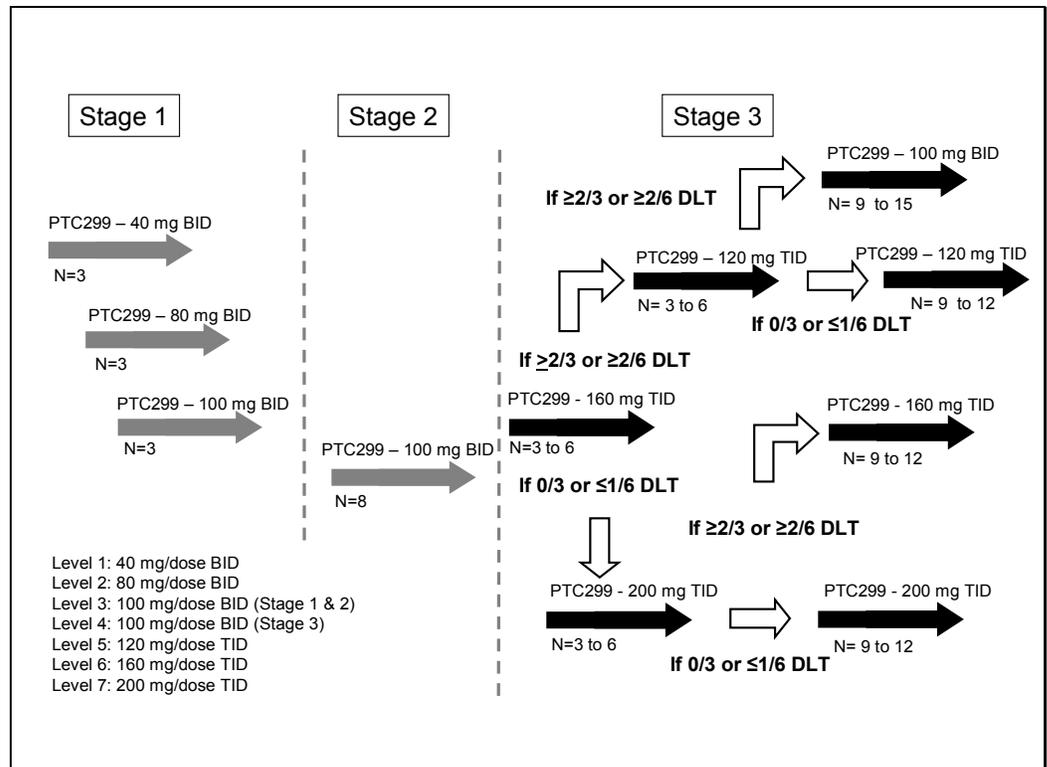
**REGIMEN:** In Stage 1, subjects will be sequentially enrolled onto one of three dose levels. The initial dose level will be 40 mg/dose by mouth BID, with escalation in subsequent subject cohorts to 80 mg/dose BID and 100 mg/dose BID. Subjects will receive PTC299 in consecutive 28-day cycles for a maximum of 12 cycles.

Dose escalations in Stage 1 will occur after a minimum of three subjects have completed 28 days of therapy without dose-limiting toxicity. No inpatient dose escalations will be allowed.

In Stage 2, additional subjects will be entered at the highest dose and schedule of PTC299 determined to be safe and well tolerated in Stage 1 (eg, 100 mg/dose BID).

In Stage 3, further dose escalation will continue to 160 mg/dose TID and 200 mg/dose TID. Additional subjects will be accrued at the highest dose level of PTC299 (eg, 200 mg/dose TID) determined to be safe and well tolerated. Dose levels of 120 mg/dose TID and 100 mg/dose BID are made available should the dose levels of 160 mg/dose TID or 120 mg/dose TID (respectively) not be well tolerated.

The study schema is shown in the diagram below.



**DURATION:**

Subjects will continue on study protocol for up to 12 treatment cycles (48 weeks). Subjects who do not show objective response of their KS lesions will be removed after 6 treatment cycles (24 weeks). Protocol treatment will be discontinued if a subject develops tumor progression, unacceptable toxicity or develops one of the protocol-defined reasons for treatment discontinuation.

**PRIMARY OBJECTIVES:**

- To define the safety and toxicity of PTC299 in patients with HIV-associated KS.
- To establish the maximum safely tolerated dose of PTC299 in patients with HIV-associated KS.
- To estimate the response rate of HIV-associated KS to treatment with PTC299.

**SECONDARY OBJECTIVES:**

- To describe the pharmacokinetics of PTC299 in patients with HIV-associated KS.
- To describe the effects of PTC299 on circulating VEGF, VEGFR and cytokine levels in patients with HIV-associated KS.
- To describe the effects of PTC299 on HIV and KSHV viral loads.
- To describe the effects of PTC299 on T-lymphocyte subsets (CD4 and CD8).
- To describe the effects of PTC299 on KS tumor biopsies with respect to expression of VEGF, the VEGFR-2 and -3, phospho-Akt, p53, HIF-1 $\alpha$  and proliferation, measured by Ki-67 staining.
- To describe the effects of PTC299 on viral gene expression and cellular gene transcription in KS tumor biopsies using real-time QPCR-based profiling.

## 1.0 INTRODUCTION

### 1.1 Background: Kaposi's Sarcoma and Vascular Endothelial Growth Factor

Kaposi's sarcoma (KS), the most common malignancy associated with human immune deficiency virus (HIV) infection, is a virus-induced angiogenic neoplasm of endothelial origin. The neoangiogenesis that characterizes KS is driven by vascular endothelial growth factor (VEGF) and inhibiting VEGF expression or VEGF signaling has been found to inhibit tumor growth in clinical and experimental studies. VEGF is strongly overexpressed in KS lesions and contributes to the angiogenic phenotype of KS in experimental models [1-5]. Tissue culture studies have implicated the Kaposi's sarcoma herpesvirus (KSHV) genes K1 and vGPCR in the induction of VEGF and VEGF receptors (VEGFRs) [1, 5-7]. Since KSHV infection causes wide-ranging reprogramming of infected endothelial cells, VEGF/VEGFR induction by other viral genes needs to be considered as well [8-10]. In addition, significantly higher levels of serum VEGF have been seen in HIV-1-infected persons with KS compared with HIV-1-infected persons without KS [11]. These findings provide a rationale for therapeutic trials in KS of agents that inhibit VEGF production or VEGFR signaling.

In addition to a direct contribution of VEGF to KS tumor cell growth, there may also be a contribution of non-transformed, non-KSHV-infected capillary endothelial cells, which constitute the vascular supply of KS tumors. Reduction of blood flow induces a reduction of oxygen, which induces tumor hypoxia. Hypoxia causes p53-dependent apoptosis, but also, via induction of HIF-1 $\alpha$ , induces VEGF production. HIF-1 $\alpha$  and the cellular response to hypoxia are modulated by KSHV [12-16]. Therefore, p53, HIF-1 $\alpha$  and some of their representative targets will also be characterized as secondary biological endpoints in this study.

### 1.2 Background Related to Experimental Therapy

#### 1.2.1 Role of Vascular Endothelial Growth Factor in Cancer

In order to initiate and maintain tumor growth, primary and metastatic tumors must recruit and sustain a blood supply through the process of angiogenesis [17]. Among the tumor-derived paracrine growth factors that stimulate endothelial cell proliferation and migration to the tumor bed, VEGF-A plays a central role [18]. Tumor-derived VEGF is one of a family of angiogenic glycoproteins that share 30 to 60% amino acid sequence identity. Other members of this family include VEGF-B (a stimulus for physiological angiogenesis in muscle), VEGF-C, and VEGF-D (regulators of lymphangiogenesis), and placenta growth factor (PlGF) [19]. Through alternative splicing during transcription, 4 primary VEGF-A isoforms (VEGF121, VEGF165, VEGF189, and VEGF206), having 121, 165, 189, and 206 amino acids, respectively, are produced [20,21]. Of these, VEGF165 is secreted as the primary functional isoform [19]. VEGF121 is also secreted and is a major regulator of vascular permeability. The VEGF189 and VEGF206 isoforms act more locally due to sequestration within the extracellular matrix. Recently, an alternatively spliced set of VEGF isoforms has been identified [22]. These splice variants of VEGF (e.g., VEGF165b) vary in the carboxyl terminus and act to inhibit aberrant angiogenesis [23-27]. VEGF effects are mediated primarily through two tyrosine kinase receptors known as VEGFRs, i.e., VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), found on

endothelial cells [18,19,28]. A third receptor, neuropilin, may play a direct autocrine role in mediating antiapoptotic VEGF signals on tumor cells [29].

VEGF acts as a mitogen for endothelial cell proliferation [30] and as a survival factor that prevents endothelial cell apoptosis [31-33]. VEGF-mediated vascular leakage encourages extravascular matrix formation in support of endothelial cells and the incursion of stromal cells into tumor sites [34,35]. The central role of VEGF in tumor-related pathophysiology is demonstrated by its adverse prognostic significance for tumor growth and progression [25,35]. For certain cancers, elevated tumor VEGF levels have been linked to unfavorable histochemical features, stage, and prognosis [36-39]. The adverse significance of persistence of VEGF in residual locally advanced breast cancers following neoadjuvant chemotherapy has been documented [40]. VEGF elaboration into the blood has been observed in animals and in cancer patients, particularly in association with the development of progressive disease [41-43]. Endothelial cell leakage induced by VEGF has been implicated as the basis of malignant pleural and peritoneal effusions that can occur in several types of cancers [44,45].

Studies of VEGF pathway inhibition have confirmed the critical function of VEGF in tumorigenesis [25]. VEGF inhibition markedly reduces the density of capillary vessels and vascular permeability in breast and other tumors [45-48]. Treatment of tumor-bearing animals with monoclonal antibodies to VEGF or with inhibitors of VEGFR has demonstrated tumor growth inhibition in a variety of tumor types, including carcinomas, sarcomas, glioblastomas, and melanomas [25,49-57]. The addition of VEGF inhibitors to chemotherapeutic agents in animal models has shown improved control of tumor growth compared to that seen with use of cytotoxic agents alone [59-60]. In experimental systems, scheduling of anti-VEGF therapy relative to cytotoxic therapy may have implications for maximizing response to treatment [58,61,62].

### 1.2.2 Existing Clinical Methods for VEGF Inhibition

Pharmacological methods for VEGF inhibition have almost exclusively focused on development of agents that block the distal portion of the VEGF signaling pathway, i.e., through inhibition of VEGF binding to its receptors or by blockade of the tyrosine kinase activity of the VEGFRs. Four of these agents have been sufficiently developed to have demonstrated clinical proof of concept in randomized clinical trials in various cancers:

- Bevacizumab (Avastin<sup>®</sup> – Genentech) [60, 63-71], a monoclonal antibody to VEGF that blocks binding to VEGFR
- Sunitinib (Sutent<sup>®</sup> – Pfizer) [52, 53, 59, 72-78], a receptor tyrosine kinase inhibitor
- Sorafenib (Nexavar<sup>®</sup> – Bayer) [81-89], a receptor tyrosine kinase inhibitor
- Pazopanib (Votrient<sup>®</sup> – GlaxoSmithKline) [139], a receptor tyrosine kinase inhibitor

The principal on-target adverse effects observed with chronic VEGF inhibition may be best discerned with highly specific monoclonal antibody treatment (e.g., bevacizumab). These effects have included bleeding (usually transient, low-grade

epistaxis), arterial and venous thrombosis (when given together with chemotherapy; probably secondary to thrombin-VEGF-VEGFR interactions [90, 91], hypertension (potentially due to secondary inhibition of endothelial nitric oxide production), delayed wound healing, and asymptomatic proteinuria (resulting from disruption of normal glomerular filtration) [64-67, 69-71, 91-96]. Recently reported bevacizumab-related adverse events have included nasal septal perforation [97, 98] and reversible posterior leukoencephalopathy syndrome in association with hypertension [99]. Inhibitors of VEGFR, such as sunitinib, sorafenib, pazopanib, and similar drugs still in development, have also induced on-target effects related to VEGF inhibition (e.g., hypertension) [88, 100, 139].

The primary off-target effect of bevacizumab treatment is the cytokine-release phenomenon that is characteristic of clinical administration of monoclonal antibodies [91,101]. VEGFR inhibitors have induced a number of off-target adverse events that appear to be due to varying levels of nonspecific inhibition of tyrosine kinase receptors other than VEGFR. Such effects include light-headedness, ataxia, headache, hoarseness, nausea, vomiting, diarrhea, rash, subungual hemorrhage, myelosuppression, fatigue, hypothyroidism, QT interval prolongation, and heart failure [73, 75, 76, 81-84, 86, 87, 102-116].

Collectively, these data provide clinical validation of VEGF inhibition as a therapeutic approach in many malignancies, but also offer insights into the potential limitations of existing drugs. Novel approaches to VEGF down-regulation may offer the opportunity to minimize the liabilities of current methods, while taking advantage of the extensive body of existing knowledge regarding VEGF biology and VEGF inhibition.

### 1.2.3 Post-transcriptional Regulation of VEGF Production

Within tumors, there are several potential stimuli for VEGF production. In some tumor types, cellular hypoxia is a primary stress stimulus for tumor VEGF production. In others, there is excessive production of VEGF because of oncogene (e.g., ras) activation or tumor suppressor (e.g., p53 or Von Hippel Lindau) disruption [117-119], or due to proinflammatory factors (e.g., prostaglandin E2, interleukin 1 [IL-1], or interleukin 6 [IL-6] [120-123]). Collectively, the presence of one or more of these stimuli provides an explanation for the nearly universal occurrence of VEGF overexpression in almost all cancer types.

Some mechanisms of enhanced VEGF production are mediated at the transcriptional level. For example, mutation of the Von Hippel Lindau tumor suppressor provokes constitutive overexpression of hypoxia inducible factor 1 (HIF-1) that, in turn, enhances VEGF gene transcription [119]. However, post-transcriptional regulation plays a major and critical role in VEGF production. Initiation of translation of most transcripts is dependent upon the interaction of ribosomes with a molecular “cap” at the 5' end of the UTR of the mRNA. Such cap-dependent translation is largely suppressed under conditions of cell stress, such as hypoxia. By contrast, the 5'-UTR of VEGF mRNA contains a guanine-cytosine-rich sequence known as an internal ribosomal entry site (IRES) that initiates synthesis of the VEGF protein independently of normal cap-dependent translation [124-127]. In this case, IRES-

dependent translation is actually increased in the presence of hypoxia [125,128]. In addition, the 3'-UTR of VEGF mRNA harbors an adenosine-uracil-rich stability determinant that regulates mRNA turnover rates [127]. Under the hypoxic conditions commonly found in tumor tissue, augmented translation mediated by the IRES, coupled with enhanced stabilization of VEGF mRNA, results in an increase in VEGF production and subsequent angiogenesis that can support tumor growth. Of interest, all 4 primary VEGF isoforms (VEGF121, VEGF165, VEGF189, and VEGF206) share the same 5'-UTR regulatory elements and 3'-UTR stability determinants [27].

#### 1.2.4 Development of PTC299

Knowledge of the importance of post-transcriptional regulation in VEGF biology offers a potential alternative to existing clinical methods for modulating the VEGF pathway and supported the development of small, orally bioavailable molecules to inhibit VEGF production within tumors rather than interfering with VEGF signaling at endothelial cells. Employing cell-based in vitro reporter assays, a set of chemical scaffolds was isolated by PTC Therapeutics that selectively inhibit VEGF production by tumors. Chemical structure optimization, pharmacological characterization, and toxicological evaluation led to identification of PTC299, an orally bioavailable, new chemical entity that impairs tumor VEGF production and angiogenesis.

#### 1.2.5 Nonclinical Characterization of PTC299

##### 1.2.5.1 Drug Substance and Formulation

PTC299 is a 6-chloro- $\beta$ -carboline derivative with 1 stereogenic center. PTC299 is the S-enantiomer. The compound has no known structural similarity to existing drugs. The drug substance is a white to off-white, crystalline powder with a molecular weight of ~467 Daltons and low aqueous solubility. PTC299 is orally bioavailable. For toxicological and clinical testing, PTC299 has been manufactured according to current Good Manufacturing Practices (cGMP). For clinical use, it is being formulated under cGMP and is provided in lipid-filled gelatin capsules.

##### 1.2.5.2 Nonclinical In Vitro Activity

Building on the well-established use of firefly luciferase as a biological reporter system, PTC Therapeutics has developed functional cell-based translation assays to identify compounds that can inhibit VEGF protein production. These assays have documented that PTC299 acts specifically through IRES-dependent mechanisms to inhibit production of the luciferase reporter. Assessments of stereoselectivity demonstrate that PTC299, as the S enantiomer, is responsible for these effects on the reporter; the R enantiomer is essentially inactive in suppressing reporter production.

These experiments with the reporter system have been extended to human tumor cell lines to determine the ability of PTC299 to inhibit production of native VEGF. In these cell lines, PTC299 shows potent VEGF inhibition

of all 4 VEGF isoforms (VEGF121, VEGF165, VEGF189, and VEGF206). The R enantiomer is inactive.

In vitro, PTC299 preferentially inhibits VEGF produced in cells stimulated by stressors such as hypoxia or oncogenic transformation, largely sparing VEGF production in unperturbed cells. For example, in HeLa cells and normal keratinocytes, VEGF generated by cells under hypoxia (1% oxygen) is much more sensitive to PTC299 inhibition than that generated under normoxia (21% oxygen). If translated to the clinic, this action of PTC299 to selectively suppress pathological VEGF overproduction while sparing physiological VEGF homeostasis may offer an improved therapeutic ratio relative to existing VEGF inhibitors that block all VEGF action at the level of the endothelial receptor.

VEGF suppression has been examined in multiple human tumor cell lines grown in vitro. PTC299 is able to modulate VEGF production across a spectrum of tumor types. PTC299 is potent; effective drug concentrations achieving 50% reductions (IC50) in VEGF levels are generally  $\leq 50$  nM.

The specific mechanism of action accounting for the PTC299 effect on VEGF remains an area of ongoing investigation. Available data suggest that the presence of the 5'-UTR in the VEGF mRNA is required to observe the PTC299 inhibitory actions on luciferase or VEGF protein production. Activity is clearly post-transcriptional; real-time quantitative polymerase chain reaction (QPCR) assessments of mRNA have shown that PTC299 does not alter the levels of VEGF message. Analyses of its effects on ribosome association with VEGF transcripts indicate that PTC299 does not impede initiation of VEGF translation or promote disassociation of ribosomes from VEGF mRNA.

PTC299 also provokes a late G1/early S-phase cell cycle delay (i.e., between the late resting or pre-DNA synthesis phase, and the early DNA synthesis phase) in those tumor cell lines in which VEGF generation is decreased by the drug. Further characterization indicates that this effect is concentration dependent, occurring at low-nanomolar EC50 values similar to those associated with reducing VEGF production. The effect is seen by 14 to 18 hours after PTC299 treatment, with reversion to normal cell cycle progression by ~24 hours after cessation of drug exposure. The cell cycle delay and inhibition of VEGF protein production occur in concert, linking these phenotypes in tumors. Interestingly, inhibition of VEGF production in these same tumor cells with small interfering RNA (siRNA) does not induce a cell cycle delay. Conversely, mimosine, a DNA synthesis inhibitor that halts cell cycle progression at the G1/S interface, does not reduce VEGF production. Thus, current evidence indicates that the effects of PTC299 on the tumor cell cycle occur in parallel with its actions on VEGF production in tumors.

Additional studies are in progress to determine the molecular target of PTC299 and to determine how the drug acts to inhibit tumor VEGF production and delay tumor cell cycling.

#### 1.2.5.3 Nonclinical In Vivo Activity

PTC299 demonstrates potent in vivo biological activity as measured by inhibition of tumor-generated VEGF, suppression of tumor-related angiogenesis, and delay of tumor growth.

The stereospecificity of PTC299 activity seen in vitro is also observed in vivo. Treatment with PTC299, the S enantiomer, results in significant inhibition of tumor VEGF production in nude mice bearing HT1080 human fibrosarcoma xenografts. By contrast, the R enantiomer is inactive in this model system. While substantially decreasing intratumoral levels of VEGF-A, and PlGF, and impeding tumor growth, PTC299 does not significantly alter intratumoral concentrations of other tumor associated proteins (e.g., fibroblast growth factor-2, survivin, platelet-derived growth factor, and endostatin), confirming its specificity for VEGF family proteins. Analysis of vessels in HT1080 fibrosarcoma xenografts by immunohistochemistry indicates that PTC299 actions to reduce tumor VEGF concentrations result in an expected decrease in tumor vessel density.

PTC299 treatment of HT1080 fibrosarcoma xenografts over a range of 1 to 10 mg/kg given once per day (QD) or twice per day (BID) for 18 days achieve >85% reductions in mean tumor VEGF concentrations relative to control values. Reductions in tumor VEGF concentrations are associated with parallel reductions in circulating human plasma VEGF levels by  $\geq 95\%$  relative to controls. Evaluation of tumor size demonstrates substantial tumor growth delay in PTC299-treated animals. Activity is maximal at doses  $\geq 1$  mg/kg BID in mouse models. The dose of 1 mg/kg BID is associated with mean Day 1 trough plasma concentrations of  $\sim 0.10$  to  $0.15$   $\mu\text{g/mL}$  ( $0.21$  to  $0.32$   $\mu\text{M}$ ). Time-course experiments indicate that PTC299 exerts its effects rapidly, with significant pharmacological effects – reduced tumor-derived plasma VEGF concentrations, delayed tumor cell cycling through S-phase, and decreased tumor growth – occurring within the first 3 days of dosing.

PTC299-mediated inhibition of in vivo VEGF production has also been documented in models of human cancers grown as xenografts in nude mice (including breast cancer, melanoma, non-small-cell lung cancer, and neuroblastoma), confirming that the effect can be seen across multiple tumor types. In concert with the decreases in tumor VEGF levels, single-agent PTC299 treatment results in significant tumor regression or delay of tumor growth in these same xenograft models. In orthotopic nude mouse models involving treatment of SY5Y neuroblastoma or SKNEP Ewing sarcoma growing under the renal capsule, >95% decreases in tumor size are observed.

When given alone or in combination with tamoxifen in an MCF-7 breast tumor xenograft, with 5-fluorouracil (5-FU) in an HCT116 colon tumor xenograft, or with paclitaxel in an A431 epidermoid vulvar tumor xenograft, PTC299 induces cytoreduction and tumor growth delay, effecting cures in some animals. In these combination therapy studies, single-agent PTC299 appears to be well tolerated and when given in combination, does not increase the toxicity of the other agent.

Similarly, when combined with the anti-VEGF antibody, bevacizumab, in an HT1080 fibrosarcoma xenograft model, or with the VEGFR-2 tyrosine kinase inhibitor, sunitinib, in a Calu-6 NSCLC xenograft model, PTC299 enhances antitumor activity; these data confirm the novel mechanism of action of PTC299 relative to other VEGF inhibitors, indicate that targeting two different points in the VEGF signaling pathway may be advantageous, and suggest that PTC299 may offer activity even in subjects who have already received these other types of antiangiogenic agents.

Collectively, the preclinical in vivo data confirm that PTC299-mediated VEGF suppression is accompanied by functional antiangiogenic consequences and that PTC299 has substantial antitumor activity when given alone or in combination with chemotherapeutic agents or antiangiogenic agents that are commonly employed in the clinic. In addition, the preclinical findings suggest that reductions in tumor-derived VEGF circulating in plasma or serum may offer mechanism-specific evidence of PTC299 activity in the clinic.

#### 1.2.5.4 Nonclinical Safety Pharmacology

In vitro and in vivo safety pharmacology studies with PTC299 demonstrate a favorable safety profile. These studies provide evidence that the drug is unlikely to cause serious off-target effects or adverse effects on critical organ systems.

The cytotoxic effects of PTC299 on nontransformed primary human cells have been evaluated in cultured human bone marrow progenitor cells at concentrations up to 3  $\mu\text{M}$  (1.4  $\mu\text{g}/\text{mL}$ ); the data from these experiments demonstrate that PTC299 does not inhibit erythroid or myeloid progenitor proliferation. When taken together with the nonclinical efficacy findings and toxicology data, these results support the selectivity of PTC299.

In an in vitro screen against 62 clinically relevant enzymatic and receptor targets (NovaScreen General Side Effect Profile), PTC299 at a concentration of 5  $\mu\text{M}$  (2.3  $\mu\text{g}/\text{mL}$ ) shows no evidence of meaningful interaction. Similarly, in testing at a pharmacologically active concentration of 100 nM (0.047  $\mu\text{g}/\text{mL}$ ), PTC299 demonstrates no inhibition of enzyme activity in a panel of 208 biologically important kinases and 16 phosphatases. In vitro assessment in an electrophysiological assay at

concentrations up to 5  $\mu\text{M}$  (2.3  $\mu\text{g/mL}$ ) indicates that PTC299 does not inhibit human ether-à-go-go-related gene (hERG) channel current and thus would not be expected to induce clinical QT prolongation. All of these results suggest that PTC299 is unlikely to induce adverse, off-target pharmacological effects as predicted by these assays.

PTC299 has also proved safe in standard in vivo Good Laboratory Practices (GLP) studies of pharmacological safety. A functional observation battery in Sprague-Dawley rats dosed daily for 7 days by oral gavage at dose levels of 40, 120, and 400 mg/kg has revealed no adverse behavioral or neurological effects at any dose level. In a cardiopulmonary function study in awake telemeterized male beagle dogs, single doses of PTC299 by oral gavage at dose levels of 30, 60, and 120 mg/kg induced no meaningful changes in pulmonary, cardiovascular, arterial blood gas, or electrocardiographic (ECG) (including QT interval) parameters.

#### 1.2.5.5 Nonclinical Pharmacokinetics and Drug Metabolism

Nonclinical studies have demonstrated that PTC299 is orally bioavailable in mice, rats, and dogs. Plasma concentration-time data indicate rapid uptake of drug but a relative long mean time of maximum concentration ( $T_{\text{max}}$ ), generally ranging between 6 and 12 hours postdose. In all three species, the relationship between PTC299 dose and plasma exposure describes a “bell-shaped curve”, i.e., plasma exposures initially rise with dose to a peak and then decrease despite further increases in dose. These bell-shaped dose-exposure relationships are consistent with saturation of absorption and/or possible precipitation of drug within the gastrointestinal tract at higher dose levels. Maximal exposure in the dog is less than the maximal exposure in rodents. This difference is likely explained by more rapid PTC299 metabolism in the dog; the half-life ( $t_{1/2}$ ) values for PTC299 in dogs (3 to 8 hours) are shorter than in rodents (14 to 20 hours), and there is more rapid biotransformation of PTC299 in dogs than in rats, both in vitro in hepatocytes and in vivo.

The absorption, distribution, and excretion of PTC299 have been evaluated in vivo in a GLP study of oral administration of 50 mg/kg of  $^{14}\text{C}$ -labeled PTC299 in rats. In this study, absorbed radioactivity was rapidly distributed into the whole body with the  $T_{\text{max}}$  in blood and plasma occurring at 4 hours postdose in both sexes. The mean  $t_{1/2}$  in blood and plasma was long, at >60 hours for both sexes. Excluding the gastrointestinal tract, maximum concentration ( $C_{\text{max}}$ ) values in most tissues occurred at 6 to 12 hours postdose, with the highest values occurring in adipose tissues such as adrenal gland, brown fat, and liver. By 72 hours postdose, discernable residual radioactivity remained concentrated in fatty tissues in both sexes. Approximately 65 to 70% of the administered radioactivity was recovered in feces, with essentially no excretion in urine.

As expected given its low solubility, the in vitro protein binding of PTC299 in plasma is high (>99.5%) in all species tested (mouse, rat, dog, monkey,

and human). Given the similarities in protein binding across species, the data suggest that cross-species exposure comparisons do not need to be adjusted to take protein binding into account.

Incubation of <sup>14</sup>C-PTC299 in primary hepatocytes results in production of 2 major metabolites in all four species tested (rat, dog, monkey, and human). Biotransformation leads to O-desmethyl-<sup>14</sup>C-PTC299 as a primary metabolite and O-desmethyl-<sup>14</sup>C-PTC299-glucuronide as a secondary metabolite. Metabolism is most extensive in dog and monkey hepatocytes, occurs to an intermediate degree in human hepatocytes, and is minimal in rat hepatocytes. No metabolites are produced in human hepatocytes that are not observed in the species (rat and dog) used to evaluate the nonclinical toxicology of PTC299. The primary metabolite, O-desmethyl-PTC299, is ~4 to 6-fold less active than PTC299 in inhibiting tumor VEGF production when tested by enzyme-linked immunosorbent assay (ELISA). The collective data suggest that substantial metabolism of PTC299 to a highly active metabolite in humans is unlikely.

When evaluated at concentrations through 3  $\mu$ M (1.4  $\mu$ g/mL) in human hepatic microsomes or in assays using purified human recombinant cytochrome P450 (CYP) enzymes, PTC299 appears to inhibit the activity of the CYP2D6 enzyme. Thus, PTC299 may enhance or prolong exposure to drugs that are primarily metabolized by this enzyme.

#### 1.2.5.6 Nonclinical Toxicology

Comprehensive nonclinical toxicology testing to support initiation of the Phase 1a single-dose clinical study has shown that PTC299 is very well tolerated at exposure levels greater than those expected to provide therapeutic activity. Completed GLP toxicology studies include 1-day, 7-day, and 28-day studies of oral gavage administration in rats, and 7- and 28-day studies of oral capsule administration in dogs.

In rats given single oral gavage doses of PTC299 at doses of 100, 200, or 400 mg/kg, no notable clinical or clinical pathological toxicities were observed at any dose level. Because maximal exposure occurred at 100 mg/kg, this dose is considered the NOAEL for 1 day of dosing.

In the subsequent 7-day study, rats were administered oral gavage PTC299 doses of 40, 120, and 400 mg/kg/day. Maximal exposures occurred at a dose of 120 mg/kg/day. At this dose, notable changes included increases in mean prothrombin time (PT) and mean activated partial thromboplastin time (aPTT) in males but not in females. These changes may be consistent with a sensitivity of male rats to reductions in vitamin K absorption [129-133]. Elevations of ~2.5- to 3-fold in mean cholesterol levels and 1.3-fold in mean glucose levels were also noted in males and females receiving PTC299. Based on the collective toxicity and toxicokinetic findings, the NOAEL for 7 days of PTC299 administration for male rats is 40 mg/kg/day and for female rats is 120 mg/kg/day.

In the 28-day study (with a 14-day recovery period), rats received oral gavage PTC299 doses of 12, 40, and 120 mg/kg/day. Exposures were maximal at 120 mg/kg/day. Consistent with the 7-day study, the 28-day study showed reversible increases in mean PT and aPTT in males but not in females. Other chemistry changes included 2- to 3-fold elevations in mean cholesterol levels in all PTC299 dose groups, and minimally increased glucose and alkaline phosphatase values in females and minimally increased chloride and minimally decreased potassium values in males dosed with PTC299 at 40 and 120 mg/kg/day. Increased adrenal weights were observed at all dose levels and did not reverse within the 14-day recovery period; these changes correlated with minimal to mild hypertrophy without evidence of degenerative changes or necrosis in the glucocorticoid-producing region of the adrenal glands in male and female animals.

In dogs given PTC299 at doses of 10, 30, or 60 mg/kg/dose BID (20, 60, and 120 mg/kg/day) orally in gelatin capsules for 7 consecutive days, exposures were maximal at 30 mg/kg/dose BID. Animals receiving PTC299 had an increased incidence and frequency of soft stools in both males and females but no other notable drug-related effects. Considering exposure values, the NOAEL for 7 days is considered 30 mg/kg/dose BID (60 mg/kg/day).

In the 28-day study (with a 15-day recovery period), dogs were administered PTC299 doses of 5, 15, and 30 mg/kg/dose BID (10, 30, or 60 mg/kg/day) in gelatin capsules. Maximal exposures occurred at 30 mg/kg/dose BID (60 mg/kg/day). PTC299 was clinically well tolerated in male and female dogs at the low- and mid-dose levels but at the high dose, clinical adverse findings included decreased food consumption resulting in decreased body weights. The target organ of toxicity was the small intestine. Microscopic findings of erosion, necrosis and/or ulceration of the mucosa, submucosal inflammation, epithelial hyperplasia of the mucosa of the crypts and/or congestion of the Peyer's patches in the small intestine were seen in several dogs at the high dose levels. Based on the findings, the NOAEL for 28 days of PTC299 administration in dogs is considered 15 mg/kg/dose BID (30 mg/kg/day).

Genotoxicity was assessed in a battery of in vitro and in vivo studies that included a bacterial reverse mutation study, a chromosome aberration study in Chinese hamster ovary (CHO) cells, and a micronucleus study in rats by the oral route. The in vitro studies were performed in the presence and absence of an exogenous metabolic activation system. There was no evidence of genotoxic effects with PTC299 in these studies.

## 1.2.6 Prior Clinical Experience

### 1.2.6.1 Phase 1 Single-Dose Study in Healthy Volunteers

PTC299 has been evaluated in a Phase 1, randomized, placebo-controlled, escalating, single-dose, safety, tolerability, pharmacokinetic (PK), and food effect study in healthy adult volunteers (Protocol PTC299-ONC-001-HV). The primary objective of the study was to determine a dose range for PTC299 that safely achieved pharmacologically active target plasma concentrations (as determined from xenograft studies) and that was appropriate for use in a subsequent multiple-dose study.

Subjects in the study were enrolled in two stages. In Stage 1, 40 subjects were accrued in five cohorts of eight subjects with each cohort receiving a sequentially higher single dose of PTC299 at body-weight-adjusted (mean total) dose levels of 0.03 mg/kg (2 mg), 0.1 mg/kg (7 mg), 0.3 mg/kg (24 mg), 1 mg/kg (75 mg), and 3 mg/kg (203 mg). Within a cohort, six subjects (three males and three females) received PTC299 and two subjects (one male and one female) received placebo. An additional 12 subjects (six males and six females) were enrolled in Stage 2 to evaluate the effect of food on the PK of PTC299 when given at a body-weight adjusted (mean total) dose of 1 mg/kg (75 mg).

During both Stages 1 and 2 of the study, data regarding adverse events, vital signs, blood counts, coagulation assessments, blood chemistry determinations, urinalyses, and ECGs were collected at baseline and repeatedly over 72 hours after administration of the study medication, and again at a follow-up visit 7 days after the last study treatment. In both Stages 1 and 2, blood samples for assessment of plasma PTC299 concentrations were collected at multiple timepoints. PTC299 concentrations were analyzed using liquid chromatography with tandem mass spectroscopy (LC-MS/MS), validated for human plasma. Similarly, blood for measurement of plasma VEGF-A levels was collected at multiple time points. Plasma VEGF-A concentrations were analyzed using a commercially available ELISA (R&D Systems), validated for human plasma.

As planned, 40 subjects (20 males and 20 females) completed their participation in Stage 1 of the study, and 12 subjects (six males and six females) completed their participation in Stage 2 of the study. Subject ages ranged from 20 to 55 years (Stage 1) and 18 to 52 years (Stage 2). Their body weights ranged from 51 to 98 kg (Stage 1) and 59 to 85 kg (Stage 2).

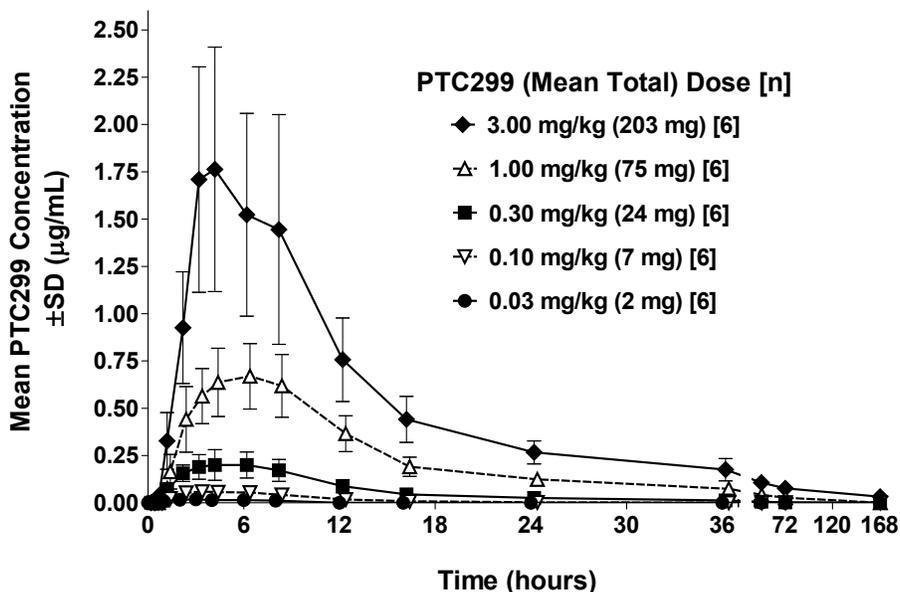
PTC299 was well tolerated and there were no serious drug-related adverse events. Among the 40 subjects dosed in Stage 1, the most frequent treatment-emergent adverse events were headache (nine episodes in eight subjects, all receiving PTC299) and nausea (five episodes in five subjects, four receiving PTC299 and one receiving placebo). Other types of adverse events occurred in fewer than five subjects (10%). During Stage 2, the most frequent adverse events were headaches (three episodes in three subjects)

and back pain (two episodes in two subjects); other adverse events were noted only in single subjects. All adverse events were Grade 1 in severity, except one case of Grade 2 diarrhea in a subject receiving 1 mg/kg of PTC299 in the fasted state in Stage 2. The incidence, relationship to study drug, and severity of adverse events were not clearly dose dependent, although the number of headaches may have increased slightly with dose. No deaths or serious adverse events occurred during the study. No subject prematurely terminated the study for safety reasons.

In both stages, there were no safety concerns based on subjects' physical examinations, vital sign measurements, or ECGs. No clinically significant changes in hematology, coagulation, or chemistry parameters were observed. Similarly, no clinically meaningful urinalysis abnormalities were seen.

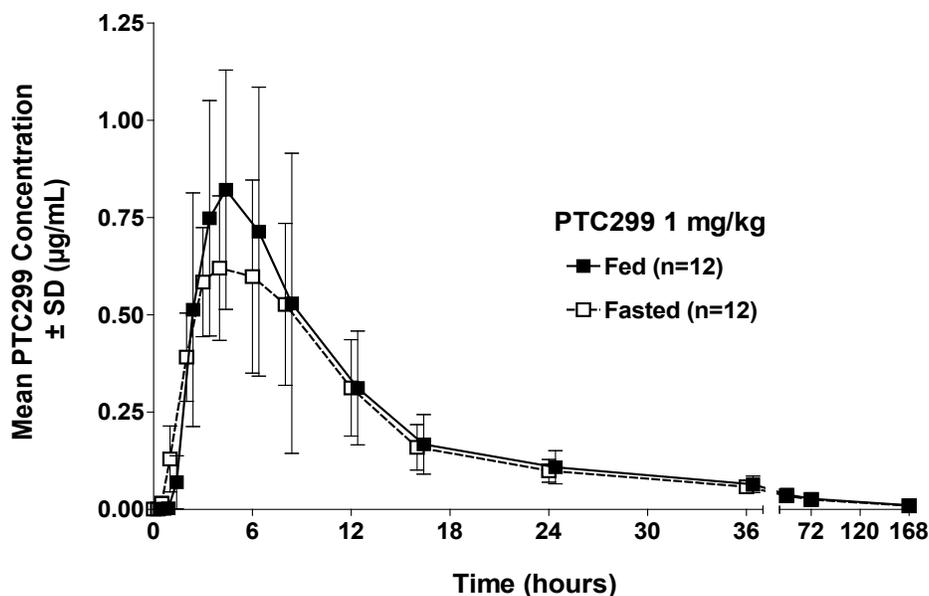
Mean plasma concentration-time profiles for PTC299 during Stage 1 are shown in Figure 1 below. Mean plasma concentration-time profiles for PTC299 according to fed or fasted status of subjects are shown in Figure 2 below. PTC299 appeared in plasma after a lag time of ~30 minutes. At body-weight-adjusted (mean total) doses  $\geq 0.30$  (24 mg) mg/kg, PTC299 concentrations persisted in plasma through 72 hours and, at the 3.0-mg/kg (203 mg) dose, low concentrations of PTC299 were still evident at 168 hours after drug administration. The mean  $C_{max}$  was increased in subjects when they received the drug after a high-fat, high-calorie meal. With or without food, the target plasma concentration of 0.10 to 0.15  $\mu\text{g/mL}$  established in animal tumor models was safely achieved.

**Figure 1. Plasma Concentrations of PTC299 by Dose after a Single Dose of PTC299 in Healthy Volunteers**



Abbreviation: SD = standard deviation

**Figure 2. Plasma Concentrations of PTC299 by Fed/Fasted Status after a 1.0-mg/kg Dose of PTC299 in Healthy Volunteers**



Abbreviation: SD = standard deviation

PK parameters for PTC299 in plasma are shown in Table 1. The mean  $T_{max}$  was in the range of 3 to 6 hours. During Stage 1, mean values for  $C_{max}$  and AUC rose steadily with dose. Increases in mean  $C_{max}$  values were generally dose proportional. Increases in mean  $AUC_{0-24}$  values were somewhat greater than dose proportional through the body-weight-adjusted (mean total) 1.00-mg/kg (75 mg) dose level and then less than dose proportional in the transition from the 1.00-mg/kg (75 mg) to the 3.00-mg/kg (203 mg) dose levels. The PTC299 plasma-concentration time course was well described by a 2-compartment model. Fitting of individual subject data resulted in a mean half-life during the distribution phase ( $t_{1/2\alpha}$ ) of ~3 hours and a terminal half-life ( $t_{1/2\beta}$ ) in the range of 28 to 56 hours.

As also shown in Table 1, ingestion of a high-fat, high-calorie meal just prior to administration of a body-weight-adjusted (mean total) 1mg/kg (75 mg) of PTC299 in Stage 2 increased the mean  $C_{max}$  by ~40% but did not materially change other PK parameters.

**Table 1. Mean (SD) PK Parameters by Stage and Dose after a Single Dose of PTC299 in Healthy Volunteers**

Parameter, units	PTC299 (Mean Total) Dose, mg/kg (mg) [n]						
	Stage 1					Stage 2	
	0.03 (2) [6]	0.10 (7) [6]	0.30 (24) [6]	1.00 (75) [6]	3.00 (203) [6]	1.0 (75) Fasted [12]	1.0 (75) Fed [12]
T <sub>max,a</sub> hours	4.34 (2.17)	3.84 (2.13)	5.17 (1.33)	5.33 (1.03)	3.50 (0.55)	4.34 (1.57)	3.58 (1.38)
C <sub>max,a</sub> µg/mL	0.0195 (0.004)	0.0632 (0.016)	0.214 (0.078)	0.682 (0.166)	1.87 (0.64)	0.663 (0.223)	0.933 (0.348)
C <sub>24h,a</sub> µg/mL	0.0 (0.0)	0.002 (0.004)	0.03 (0.01)	0.12 (0.03)	0.27 (0.06)	0.10 (0.03)	0.11 (0.04)
AUC <sub>0-24,a</sub> µg•hr/mL	0.132 (0.044)	0.574 (0.131)	2.33 (0.814)	8.39 (2.11)	19.7 (6.16)	7.39 (2.39)	8.25 (3.56)
AUC <sub>0-t,a</sub> µg•hr/mL	0.116 (0.045)	0.569 (0.135)	2.75 (1.11)	12.9 (3.29)	31.4 (7.17)	11.4 (3.66)	12.3 (4.86)
Dose-normalized C <sub>max,a</sub> µg/mL/mg/kg	0.65 (0.13)	0.63 (0.16)	0.71 (0.26)	0.68 (0.17)	0.62 (0.21)	0.66 (0.22)	0.93 (0.35)
Dose-normalized AUC <sub>0-24,a</sub> µg•hr/mL/mg/kg	4.4 (1.5)	5.7 (1.3)	7.8 (2.7)	8.4 (2.1)	6.6 (2.1)	7.4 (2.4)	8.2 (3.6)
t <sub>1/2, α,b</sub> hours	NE (NE)	NE (NE)	2.96 (0.72)	3.33 (0.67)	3.50 (1.27)	3.37 (1.13)	3.18 (1.13)
t <sub>1/2, β,b</sub> hours	NE (NE)	NE (NE)	28.3 (15.1)	42.2 (17.6)	55.8 (16.9)	53.4 (27.0)	43.4 (25.8)

a. Noncompartmental

b. Based on 2-compartment model fit with time lag and weighted with 1/concentration

Abbreviations: AUC = area under the concentration-time curve, C<sub>max</sub> = maximum concentration, t<sub>1/2</sub>=half-life, T<sub>max</sub> = time of maximum concentration; NE = not estimable

During Stage 1, C<sub>max</sub> values were marginally higher (p=0.043, ANOVA) for females relative to males, but AUC<sub>0-24</sub> values were not significantly different. During Stage 2, C<sub>max</sub> and AUC<sub>0-24</sub> values were higher for females than for males (p<0.01 for both comparisons, ANOVA). The relevance of these differences in this study is not clear given that similar gender-related differences were not observed in the subsequent Phase 1 multiple-dose study.

VEGF-A was evaluated in the plasma samples from subjects enrolled in the body-weight-adjusted (mean total) 3-mg/kg (203 mg) dose group (in Stage 1). The mean changes from baseline in the PTC299 group were similar to those in the placebo group over the course of the sampling period.

### 1.2.6.2 Phase 1 Multiple-Dose Study in Healthy Volunteers

PTC299 has also been evaluated in a Phase 1, randomized, placebo-controlled, escalating, multiple-dose, safety, tolerability, and PK study in healthy adult volunteers (Protocol PTC299-ONC-002-HV). The primary objective of the study was to determine a dose range for PTC299 that safely achieves pharmacologically active target plasma concentrations (as determined from xenograft studies) and that is appropriate for use in subsequent studies in patients with cancer.

Subjects in the study were enrolled in two stages. In Stage 1, 24 subjects were accrued in three cohorts of eight subjects with each cohort receiving a sequentially higher doses of PTC299 at body-weight-adjusted (mean total) doses of 0.3 mg/kg (21 mg/dose), 0.6 mg/kg (44 mg/dose), and 1.2 mg/kg/dose (78 mg/dose) BID for 7 days. In Stage 2, 8 subjects were accrued to receive PTC299 at a body-weight-adjusted (mean total) dose of 1.6 mg/kg/dose TID (103 mg/dose) for 7 days. Within each cohort, 6 subjects (3 males and 3 females) received PTC299 and two subjects (one male and one female) received placebo.

During both Stages 1 and 2 of the study, data regarding adverse events (AEs), vital signs, blood counts, coagulation assessments, blood chemistry determinations, urinalyses, and ECGs were collected at baseline and repeatedly over 9 days during the in-patient observation period (7 days of drug administration and 2 days after the last dose), and again at follow-up visits 7 and 14 days after the last study treatment. In both Stages 1 and 2, blood samples for assessment of plasma PTC299 concentrations were collected at multiple timepoints. PTC299 concentrations in plasma were analyzed using a validated LC-MS/MS method. Similarly, blood for measurement of plasma and serum VEGF-A levels was collected at multiple time points. Plasma and serum VEGF-A concentrations were analyzed using a commercially available, validated ELISA (R&D Systems).

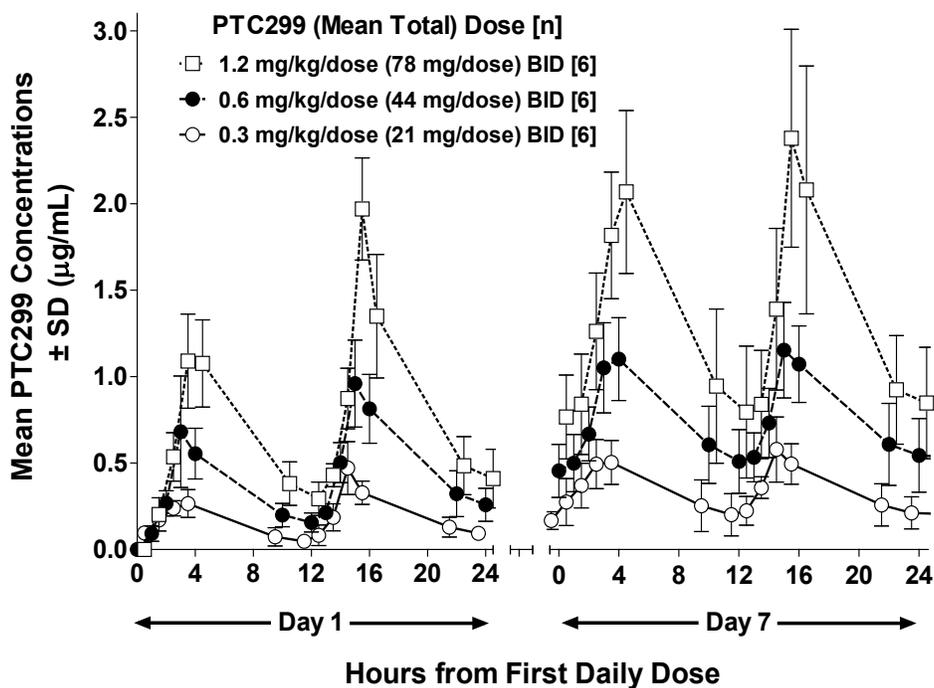
As planned, 24 subjects (12 males and 12 females) completed their participation in Stage 1 of the study, and 8 subjects (4 males and 4 females) completed their participation in Stage 2 of the study. Subject ages ranged from 21 to 64 years (Stage 1) and 31 to 65 years (Stage 2). Their body weights ranged from 46 to 90 kg (Stage 1) and 51 to 78 kg (Stage 2).

PTC299 was generally well tolerated. Sporadic episodes of diarrhea, constipation, nausea, eye pruritus, back pain, productive cough, and insomnia were observed in subjects receiving PTC299. Sporadic episodes of diarrhea, constipation, and headache were observed in subjects receiving placebo. The incidence and severity of the treatment-emergent adverse events were not dose dependent. No deaths or serious adverse events occurred during the study. No subject prematurely terminated the study for safety reasons.

In both stages, there were no safety concerns based on subjects' physical examinations, vital sign measurements, or ECGs. No clinically significant changes in hematology, coagulation, or chemistry parameters were observed. Similarly, no clinically meaningful urinalysis abnormalities were seen.

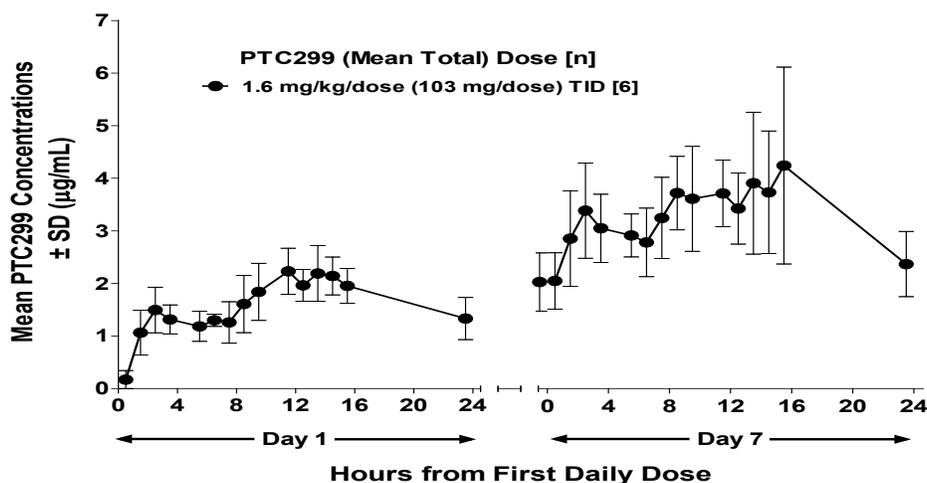
Mean plasma concentration-time profiles for PTC299 are shown in Figure 3 and Figure 4 below. PTC299 appeared in plasma after a ~30-minute lag time. On Day 1, mean  $C_{max}$  values after the second dose were almost double those of the first dose, while by Day 7, the mean  $C_{max}$  values of the first and second daily doses appeared similar; this pattern suggests accumulation of drug concentrations over time rather than diurnal variation in drug exposures. At all doses, the mean trough plasma concentrations in humans exceeded the target value of 0.10 to 0.15  $\mu\text{g}/\text{mL}$  established in preclinical tumor models.

**Figure 3. Plasma Concentrations of PTC299 by Dose During BID Dosing of PTC299 for 7 Days in Healthy Volunteers**



Abbreviations: BID = 2 times per day, SD = standard deviation

**Figure 4. Plasma Concentrations of PTC299 During TID Dosing of PTC299 for 7 Days in Healthy Volunteers**



Abbreviations: TID = 3 times per day, SD = standard deviation

PK parameters for PTC299 are shown in Table 2. The mean  $T_{max}$  was in the range of ~3 hours. During Stage 1 and Stage 2, increases in mean values for  $C_{max}$  and  $AUC_{0-24}$  were generally dose proportional. When comparing Day 1 to Day 7, there was an increase in the mean  $C_{max}$  and  $AUC_{0-24}$  over time at all doses, indicating some accumulation when PTC299 is dosed continuously. A 2-compartment model could be readily fit to all of the individual subject data throughout the 7-day course of treatment.

**Table 2. Mean (SD) Noncompartmental PK Parameters for Plasma PTC299 by Stage and Dose after BID or TID Dosing of PTC299 in Healthy Volunteers**

Parameter, units	PTC299 (Mean Total) Dose, mg/kg/dose (mg/dose) [n]							
	Stage 1						Stage 2	
	0.3 (21) BID [6]		0.6 (44) BID [6]		1.2 (78) BID [6]		1.6 (103) TID [6]	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
$T_{max}$ (after PM dose), hours	3.17 (0.41)	3.33 (0.52)	3.17 (0.41)	3.33 (0.52)	3.00 (0.00)	3.33 (0.52)	2.50 (1.05)	2.33 (1.37)
$C_{max}$ (after PM dose), µg/mL	0.48 (0.15)	0.59 (0.17)	0.97 (0.24)	1.16 (0.26)	1.97 (0.30)	2.47 (0.56)	2.36 (0.46)	4.65 (1.86)
$C_{24h}$ , µg/mL	0.094 (0.036)	0.21 (0.09)	0.26 (0.095)	0.54 (0.21)	0.41 (0.17)	0.85 (0.32)	1.33 (0.40)	2.37 (0.62)
$AUC_{0-24}$ , µg•hr/mL	4.31 (1.20)	8.44 (2.84)	10.1 (2.61)	18.6 (4.92)	18.0 (3.90)	32.9 (9.43)	37.2 (5.90)	78.6 (19.4)
Dose-normalized $C_{max}$ , µg/mL/mg/kg	0.79 (0.24)	0.99 (0.29)	0.81 (0.20)	0.97 (0.22)	0.82 (0.12)	1.03 (0.24)	0.51 (0.10)	0.98 (0.38)
Dose-normalized $AUC_{0-24}$ , µg•hr/mL/mg/kg	7.2 (2.0)	14.1 (4.7)	8.4 (2.2)	15.5 (4.1)	7.5 (1.6)	13.7 (3.9)	7.7 (1.2)	16.4 (4.0)

Abbreviations: AUC = area under the concentration-time curve,  $C_{max}$  = maximum concentration,  $T_{max}$  = time of maximum concentration; BID = 2 times per day, TID = 3 times per day

Gender-related differences were analyzed by ANOVA. This analysis did not confirm the suggestion from the Phase 1 single-dose study of somewhat higher exposures in females. In this study, no significant differences in  $C_{max}$  or  $AUC_{0-24}$  values were observed between males and females.

Plasma and serum VEGF-A concentrations were assayed in all subjects. When considering both stages of the study, no clear dose-dependent effects of PTC299 on physiological concentrations of circulating VEGF-A were noted.

#### 1.2.6.3 Ongoing Studies in Patients with Neoplastic Conditions

In addition to the data available from the experience with PTC299 in healthy volunteers, additional safety data have been obtained from ongoing studies of PTC299 in patients with cancer, including this Phase 1/2 study in patients with HIV-associated Kaposi's sarcoma, a Phase 1b study at MSKCC, a multicenter Phase 1b trial in women with metastatic breast cancer, and a Phase 2 study in patients with neurofibromatosis type 2 being conducted at Massachusetts General Hospital of Harvard Medical School. To date, doses of 0.3 mg/kg/dose (~20 mg/dose) (n=6), 0.6 mg/kg/dose (~40 mg/dose) (n=9), 1.2 mg/kg/dose (~80 mg/dose) (n=12) BID have been tested for at least 4 weeks. In addition, 100 mg/dose BID (n=52), 100 mg/dose TID (n=6), 120 mg/dose TID (n=6), and 160 mg/dose TID (n=3) have been tested for at least 6 weeks of continuous treatment. Across this entire experience, PTC299 has been generally well tolerated; adverse events have been infrequent, usually Grade 1 or 2 in severity, and not usually considered to be PTC299-related. Three patients have died during PTC299 studies: one was a 44-year-old female with breast cancer receiving PTC299 1.2 mg/kg (~80 mg/dose) BID who had rapidly progressive metastatic disease; one was a 50-year-old patient with KS and a history of depression receiving PTC299 100 mg BID on this study who died due to uncertain causes (coroner's report indicated use of multiple illicit drugs and chronic hypertensive cardiovascular disease); and one was a 68-year-old female with melanoma receiving PTC299 120 mg/dose TID who also had rapidly progressive metastatic disease. One 25-year-old female patient with neurofibromatosis type 2 receiving 100 mg BID of PTC299 for 12 weeks, developed Grade 4 serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin elevations and required hepatic transplantation. Complicating factors in determining a definitive etiology include an antecedent history of hepatitis in association with lapatinib, with subsequent normalization of liver function tests, and concurrent use of acetazolamide, which has been associated with rare cases of fulminant hepatic necrosis. At the time of hepatic transplantation, the patient's native liver demonstrated hepatitis with necrosis involving 80 to 90% of the parenchyma, and the pathologic differential diagnosis included acute viral hepatitis versus drug-induced hepatitis. Viral serologies, including hepatitis A, B, C; cytomegalovirus; and Epstein-Barr virus demonstrated no evidence of acute or chronic infection. Serologies for autoimmune hepatitis were negative, and there was no evidence of copper

or iron accumulation in the liver. No MTD has been established. Evidence of tumor stabilization, tumor response, and/or pharmacodynamic activity has been observed in patients with breast cancer, thyroid cancer, melanoma, neurofibromatosis type 2, chondrosarcoma, head & neck cancer, and KS.

## **2.0 OBJECTIVES**

### **2.1 Primary Objectives**

- 2.1.1 To define the safety and toxicity of PTC299 in patients with HIV-associated KS.
- 2.1.2 To establish the maximum safely tolerated dose of PTC299 in patients with HIV-associated KS.
- 2.1.3 To estimate the response rate of HIV-associated KS to treatment with PTC299.

### **2.2 Secondary Objectives**

- 2.2.1 To describe the pharmacokinetics of PTC299 in patients with HIV-associated KS.
- 2.2.2 To describe the effects of PTC299 on serum and plasma VEGF, VEGFR and cytokine profiles in patients with HIV-associated KS.
- 2.2.3 To describe the effects of PTC299 on HIV and KSHV viral loads.
- 2.2.4 To describe the effects of PTC299 on T-lymphocyte subsets (CD4 and CD8).
- 2.2.5 To describe the effects of PTC299 on KS tumor biopsies with respect to expression of VEGF, the VEGFR-2 and -3, phospho-Akt, p53, HIF-1 $\alpha$  and proliferation, measured by Ki-67 staining.
- 2.2.6 To describe the effects of PTC299 on viral gene expression and cellular gene transcription in KS tumor biopsies using real-time QPCR-based profiling.

### **3.0 PATIENT SELECTION**

#### **3.1 Inclusion Criteria**

Subjects must meet all of the following conditions to be eligible for enrollment into the study:

- 3.1.1 Biopsy-proven KS involving the skin, with or without lymph node, oral cavity, gastrointestinal (GI) tract and/or lung involvement. GI and pulmonary involvement must be asymptomatic or minimally symptomatic and not require systemic cytotoxic chemotherapy. At least five measurable, previously non-radiated, cutaneous lesions must be present, which can be used as indicator lesions. Additionally, patients will need a sufficient number of non-indicator cutaneous lesions measuring  $\geq 4 \times 4$  mm to obtain a total of four (4) 3-mm punch biopsies, two at baseline and two during the course of treatment.
- 3.1.2 Serologic documentation of HIV infection at any time prior to study entry, as evidenced by positive ELISA, positive Western Blot, or other federally approved licensed HIV test, or a detectable blood level of HIV RNA.
- 3.1.3 Karnofsky performance status  $\geq 60$ .
- 3.1.4 The following laboratory parameters within 21 days prior to enrollment:
  - 3.1.4.1 Hemoglobin  $\geq 8$  g/dL.
  - 3.1.4.2 Absolute neutrophil count  $\geq 1,000$  cells/mm<sup>3</sup>.
  - 3.1.4.3 Platelet count  $\geq 75,000$ /mm<sup>3</sup>.
  - 3.1.4.4 Creatinine  $\leq 2.0$  mg/dL.
  - 3.1.4.5 Bilirubin values:
    - For patients who are not receiving indinavir or atazanavir, total serum bilirubin should be Grade 0 ( $\leq$  upper limit of normal [ULN]).
    - For patients who are receiving indinavir or atazanavir, there is no specific limit on total serum bilirubin, but direct serum bilirubin should be  $\leq 30\%$  of the total bilirubin.
  - 3.1.4.6 AST (SGOT) and ALT (SGPT)  $\leq$  Grade 1 (2.5 x ULN).
  - 3.1.4.7 INR and aPTT  $\leq$ ULN.
  - 3.1.4.8  $< 2+$  proteinuria.
- 3.1.5 Life expectancy  $\geq 3$  months.

- 3.1.6 Age  $\geq$  18 years.
- 3.1.7 Ability and willingness to give written informed consent.
- 3.1.8 Women of child-bearing potential must have a negative pregnancy test within 72 hours before initiation of study drug dosing. Post menopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential.
- 3.1.9 Male and female patients of reproductive potential must agree to employ an effective barrier method of birth control throughout the study and for up to 3 months following discontinuation of study drug. (Note: A woman of childbearing potential is one who is biologically capable of becoming pregnant. This includes women who are using contraceptives or whose sexual partners are either sterile or using contraceptives.)
- 3.1.10 Patients must, in the opinion of the investigator, be capable of complying with the protocol.
- 3.1.11 Antiretroviral therapy for HIV infection is not required, but is recommended. Patients receiving antiretroviral therapy must be on a stable regimen for at least 12 weeks prior to study entry. Patients may receive any FDA approved antiretroviral therapy or agents available through a treatment IND. For patients receiving antiretroviral therapy, there should be no evidence for improvement in KS in the 3 months prior to study entry, unless there is evidence for progression of KS in the 4 weeks immediately prior to study entry.

## **3.2 Exclusion Criteria**

The presence of any of the following conditions will exclude a subject from study enrollment:

- 3.2.1 Concurrent, acute, active opportunistic infection other than oral thrush or genital herpes within 14 days of enrollment.
- 3.2.2 Symptomatic visceral KS requiring cytotoxic therapy.
- 3.2.3 Concurrent neoplasia requiring cytotoxic therapy.
- 3.2.4 Acute treatment for an infection (other than oral thrush or genital herpes) or other serious medical illness within 14 days prior to enrollment.
- 3.2.5 Anti-neoplastic treatment for KS (including chemotherapy, radiation therapy, local therapy, biological therapy, or investigational therapy) within 4 weeks of study entry.
- 3.2.6 Previous local therapy of any KS-indicator lesion within 60 days prior to enrollment unless the lesion has clearly progressed since treatment. Any prior local treatment to indicator lesions regardless of the elapsed time should not be allowed unless there is evidence of clear-cut progression of said lesion.

- 3.2.7 Use of any investigational drug or treatment (excluding antiretroviral therapy or agents available on a treatment IND) within 28 days prior to enrollment.
- 3.2.8 A history of any of the following: myocardial infarction, severe/unstable angina, coronary/peripheral artery bypass graft, symptomatic congestive heart failure, cerebral vascular accident, or transient ischemic attack, pulmonary embolism, deep vein thrombosis, or other significant thromboembolic event.
- 3.2.9 Known coagulopathy or bleeding diathesis.
- 3.2.10 Known history of drug-induced liver injury.
- 3.2.11 Any history of the following: central nervous system, pulmonary, gastrointestinal, or urinary bleeding.
- 3.2.12 Resting systolic blood pressure >160 mmHg or diastolic blood pressure >100 mmHg.
- 3.2.13 Female patients who are pregnant or breast-feeding.
- 3.2.14 Prior or ongoing clinically significant illness, medical condition, surgical history, physical finding, ECG finding, or laboratory abnormality that, in the investigator's opinion, could affect the safety of the subject; alter the absorption of the study drug; or impair the assessment of study results.
- 3.2.15 Major surgery within 30 days prior to enrollment, the presence of unhealed surgical wounds at the time of enrollment or anticipated need for surgical procedures during the period of study participation.
- 3.2.16 Ongoing systemic corticosteroid treatment, other than replacement doses.
- 3.2.17 Ongoing use of anticoagulant therapy, including coumadin, heparin or antiplatelet drugs. Note: Use of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) at doses not to exceed the maximum recommended dose is permitted.

### **3.3 Number of Subjects to be Enrolled**

- 3.3.1 Three to 38 evaluable subjects will be enrolled. In Stage 1, three successive cohorts of three subjects have been enrolled at each of the following dose levels: 40 mg/dose BID, 80 mg/dose BID, and 100 mg/dose BID. An additional eight subjects have been enrolled in Stage 2 at the highest safely tolerated dose (e.g., 100 mg/dose BID) established in Stage 1 to better define the safety, antitumor activity, and biological activities of PTC299 at this dose level. Dose escalation will continue in Stage 3 for two additional successive cohorts of 3 to 6 subjects at the following dose levels: 160 mg/dose TID and 200 mg/dose TID. Once the safety profile at each level has been established based on first cycle dose-limiting toxicities (DLTs), an additional 9 to 12 subjects will be enrolled at the highest safely tolerated dose (e.g., 200 mg/dose TID) for a total number of 15 subjects at this dose level. If the dose level of 200 mg/dose TID is not well tolerated (i.e.,  $\geq 2/6$  subjects experience a first-cycle DLT), an additional 9 to 12 subjects will be enrolled at the dose level of 160 mg/dose TID for a

total number of 15 subjects at the MTD. If the dose level of 160 mg/dose TID is not well tolerated in the initial cohort (e.g.,  $\geq 2/3$  or  $\geq 2/6$  subjects experience a first-cycle DLT), 3 to 15 subjects will be enrolled at the dose level of 120 mg/dose TID. If the dose level of 120 mg/dose TID is not well tolerated (e.g.,  $\geq 2/3$  or  $\geq 2/6$  subjects experience a first-cycle DLT), a total of 9 to 15 subjects will be enrolled at the 100 mg/dose BID dose level in Stage 3. Collectively, up to 21 patients will be enrolled in Stage 3.

- 3.3.2 Estimated accrual rate: four subjects per month.
- 3.3.3 Replacement of subjects who cannot be evaluated: A subject who does not complete cycle 1 can be replaced if, based on a review of the available safety for that dose level, the principal investigator agrees that the events leading to subject withdrawal are unlikely to constitute a safety risk for further subject enrollment and dose escalation. This means that as long as subject safety is not compromised, a subject who withdraws from the study for administrative reasons prior to experiencing DLT during cycle 1 of therapy can be replaced by another subject to complete the number of subjects evaluable per dose level required by the protocol. Similarly, as long as subject safety is not compromised, if an eligibility criterion or protocol violation occurs that substantially impairs evaluation of DLT during cycle 1, another subject can enter the cohort to complete the number of subjects evaluable per dose level required by the protocol. Any replacement subject will be enrolled into the same dosing cohort as that for the subject who withdrew. Patients withdrawn because of PTC299-related DLT may not be replaced.

### **3.4 Subject Enrollment Procedures**

This trial is open to all AMC sites for subject accrual. Sites must have this protocol approved by their Institutional Review Boards (IRB) and be registered with the AMC Operations Center before they may enroll subjects.

After the subject has signed the informed consent and it has been determined that the subject is eligible, the subject must be registered on-line via the AMC AdvantageEDC<sup>SM</sup> Internet Data Entry System. Enrollment and data collection will occur via the AMC Internet Data Entry System.

The participating site will ensure the subject meets all eligibility criteria prior to completing the protocol-specific eligibility checklist. Subjects will be enrolled on-line via the AMC Internet Data Entry System no more than 1 week prior to the initiation of treatment (enrollment 1 day prior to or on the day of treatment is strongly encouraged). Once the eligibility checklist is submitted a system generated confirmation email will be sent to the enroller upon successful completion of the subject enrollment. If the on-line system is inaccessible, the site should notify the AMC Operations Center (via email at [amcpm@emmes.com](mailto:amcpm@emmes.com) or via phone at 301-251-1161) for further instructions.

## 4.0 CLINICAL AND LABORATORY EVALUATIONS

All signs, symptoms, HIV-related and AIDS-defining events (refer to Appendix XII), death and toxicities must be documented. All signs, symptoms and laboratory results  $\geq$  Grade 2 that are felt to be clinically significant or drug-related and all HIV-related and AIDS-defining events and deaths must be recorded on the case report forms (CRFs).

All prescription medications taken within 14 days prior to study enrollment and during the interval between each visit must be recorded on CRFs. The duration of all anti-HIV medications and all opportunistic infection (OI) treatment and/or prophylaxis medications at the time of enrollment must be recorded in the CRF. All non-prescription medications must be recorded in the clinic record.

### 4.1 Screening/Baseline Evaluations

4.1.1 Biopsy diagnostic of KS at any time prior to study entry.

4.1.2 Documentation of HIV infection at any time prior to study entry.

4.1.3 Chest X-ray to rule out pulmonary KS (must be done within 28 days prior to study enrollment). Pulmonary involvement must be asymptomatic or minimally symptomatic and not require systemic cytotoxic therapy in the judgment of the Investigator. Subjects with a positive chest X-ray or symptoms suggestive of pulmonary disease will have a chest CT performed at entry. Findings suggestive of pulmonary KS should be followed up with bronchoscopy to evaluate the presence and extent of pulmonary KS and evaluate for the presence of other pulmonary diseases.

4.1.4 Electrocardiogram within 21 days of enrollment.

4.1.5 A medical history within 21 days of starting study drug to include the following information:

- Previous HIV-related and non-HIV related diagnoses.
- Complete prior anti-HIV therapy, immune based therapy and prior anti-tumor therapy, including start dates of current anti-HIV therapy.
- All prescription medications taken within the preceding 2 weeks.
- An assessment of signs and symptoms occurring within the 2 weeks prior to beginning study drug, including history of weight change.
- Complete physical exam including the following: vital signs (temperature, pulse, blood pressure, respiratory rate), height, weight, and Karnofsky performance status (Appendix II).

4.1.6 Laboratory studies must be obtained within 21 days (unless noted otherwise) prior to beginning study drug and will include the following:

- Complete blood count with differential and platelets.
- INR and aPTT.
- Serum chemistries: liver enzymes (AST, ALT, alkaline phosphatase), BUN, creatinine, electrolytes (sodium, potassium, chloride, bicarbonate), calcium,

phosphorus, total protein, glucose, albumin, bilirubin (direct and indirect), triglycerides and total cholesterol.

- Blood for ACTH and cortisol.
- Urinalysis.
- For women of child bearing potential, a serum beta human chorionic gonadotropin ( $\beta$ -HCG) pregnancy test within 72 hours of initiating treatment.
- CD4 count.
- Plasma HIV-1 RNA.

4.1.7 KS tumor assessments may be performed on Day 1 prior to receiving study medication but may be performed no earlier than 14 days before initiating treatment. Tumor measurements should include the following:

- Identify marker lesions: Select five bi-dimensionally measurable marker lesions for assessing changes in lesion dimension. Select the largest lesions with clearly defined margins. Marker lesions will be photographed to document lesion appearance and affected areas at baseline, in accordance with the guidelines for photography in Appendix XIV. Additionally, patients will need a sufficient number of non-indicator cutaneous lesions measuring  $\geq 4 \times 4$  mm to obtain a total of four (4) 3-mm punch biopsies, two at baseline and two during the course of treatment.
- For subjects with  $< 50$  total skin and oral lesions, all lesions must be evaluated for changes in number and characteristics. For subjects with  $\geq 50$  total skin and oral lesions, choose up to three representative areas for evaluating change in lesion numbers and characteristics (preferably an area with  $\geq 5$  lesions), with a total of at least 20 lesions.

NOTE: A representative area is a single extremity, the back, chest, or face that has lesions similar in characteristics, i.e., nodularity, size, color, and number, to those found on other parts of the body. A representative area does not need to be the area with the largest number of lesions but should contain lesions that are truly representative of those throughout the remainder of the body.

4.1.8 Staging Criteria (to be done within 28 days prior to study enrollment). KS staging will be based on the modified AIDS Clinical Trials Group (ACTG) Oncology Committee Staging Criteria (see Appendix III).

4.1.9 If specific consent obtained (see Appendix VIII), specimens for the AIDS and Cancer Specimen Resource (ACSR) will be collected within 28 days prior to enrollment (see Appendix VII for specimen handling).

4.1.10 KSHV viral load (see Appendix VI).

## 4.2 Evaluations During Treatment

Evaluations may occur up to 3 days before or after the end of the study visit indicated, unless otherwise noted. Each cycle will be 28 days. Evaluations will continue as outlined below.

- 4.2.1 Clinical assessment at Days 15 ( $\pm$  2) of cycle 1, and then Day 1 on cycle 2 and every cycle thereafter to include an assessment of the following at every visit unless otherwise specified:
- 4.2.1.1 KS tumor assessment Day 1 of cycle 2 and every cycle thereafter. Response of KS to treatment will be assessed as described in Section 8.0. Photographic documentation of lesions will be completed at each visit when the KS response category changes. For example, if a participant's KS response category changes from no response (stable disease) to partial response, the site will take photos of this to document the category change. If there was no change in the KS response category, no photos are required. Refer to Appendix XIV for lesion photography guidelines.
  - 4.2.1.2 A complete physical exam including: vital signs, weight, Karnofsky performance status (Appendix II) and toxicity evaluation.
  - 4.2.1.3 Signs and symptoms review.
  - 4.2.1.4 Complete blood count with differential and platelets.
  - 4.2.1.5 INR and aPTT.
  - 4.2.1.6 Serum chemistries: liver enzymes (AST, ALT, alkaline phosphatase), BUN, creatinine, electrolytes (sodium, potassium, chloride, bicarbonate), calcium, phosphorus, glucose, total protein, albumin, bilirubin (direct and indirect), triglycerides and total cholesterol. If the triglycerides and/or total cholesterol increases to  $\geq$  grade 3, then these values should be rechecked in a fasting state and the cholesterol fractionated.
  - 4.2.1.7 Urinalysis
  - 4.2.1.8 For women of childbearing potential: serum  $\beta$ -HCG at any time pregnancy is suspected.
  - 4.2.1.9 All HIV-related and AIDS-defining events (Appendix XII) and concomitant prescription and over the counter medications, vitamins and supplements taken since the last visit.
  - 4.2.1.10 CD4 count on Day 29 (Day 1 of cycle 2) and every three cycles (12 weeks) thereafter.
  - 4.2.1.11 Plasma HIV-1 RNA on Day 29 (Day 1 of cycle 2) and every three cycles (12 weeks) thereafter.
  - 4.2.1.12 KSHV viral load on Day 29 (Day 1 of cycle 2) and every three cycles (12 weeks) thereafter.

4.2.1.13 Blood for ACTH and cortisol on Day 29 (Day 1 of cycle 2) and every cycle thereafter.

#### 4.2.2 Biologic Endpoints

4.2.2.1 A 3-mm punch biopsy of a non-indicator KS lesion will be performed at baseline and between Days 22 and 28 of cycle 1. Biopsies will be fixed in formalin and studied by immunohistochemistry (IHC) for expression of the following: VEGF, VEGFR-2, VEGFR-3, phospho-Akt, KSHV LANA, orf59, p53 and HIF-1 $\alpha$ . Tumor cell proliferation will be assessed by Ki-67 staining. Change in the intensity or distribution of a particular marker will be evaluated. Ki 67 staining will be quantified as a percentage of tumor cells. All other immunohistochemical staining will be described with a score (0, 1+, 2+) and a description of the distribution of the signal (endothelial cells, spindle cells, infiltrating lymphocytes, etc.). Depending on the results of these assays, other potential immunohistochemical markers characteristic for KS may also be measured. A detailed protocol is provided in Appendix XIII.

Evaluation by IHC of the effects of PTC299 on KSHV LANA and orf 59 is based on the hypothesis that either VEGF depletion directly, or VEGF inhibition-induced hypoxia indirectly, may induce KSHV lytic gene expression. Because reduction of VEGF production is expected to reduce capillary-dependent oxygen flow to the tumor, this will, in turn, induce hypoxia. Hypoxia causes p53-dependent apoptosis but also, via HIF-1 $\alpha$ , induces VEGF. HIF-1 $\alpha$  and the cellular response to hypoxia are modulated by KSHV. Hence, p53, HIF-1 $\alpha$ , and some of their representative targets will be characterized by IHC as secondary biological endpoints.

4.2.2.2 A separate 3-mm biopsy of a non-indicator KS lesion will be performed at baseline and again between Days 22 and 28 of cycle 1 to assess viral gene expression within tumor at the messenger RNA level. Real-time QPCR based profiling of KSHV transcription will be used to test the hypothesis that depletion of VEGF directly or indirectly through the induction of hypoxia may lead to changes in KSHV transcription. This has previously been observed in laboratory studies.[134-137] A detailed protocol is provided in Appendix V.

4.2.2.3 Using the same biopsies as described in Section 4.2.2.2, Real-time QPCR based profiling will be used to test the hypothesis that depletion of VEGF directly, or indirectly through the induction of hypoxia, may lead to changes in cellular gene transcription.

4.2.2.4 In addition, as a control, a 3-mm skin biopsy from morphologically normal skin in the same anatomic location as the tumor specimen will be obtained at baseline only. A detailed protocol is provided in Appendix V.

#### 4.2.3 Pharmacokinetics of PTC299

Blood for PK assessments should be collected immediately pre-dose; and at 1, 2, 3, 4, 5, 6, and 8 hours after the AM dose on Day 1 and again at 1, 2, 3, 4, 5, 6, and 8 hours after the AM dose on Day 28 of cycle 1. (**Note:** the Day 28/cycle 1 PK samples can be collected up to 3 days prior to Day 28). A PK sample will also be collected prior to the AM dose on Day 15 ( $\pm$  1 day) during cycle 1 and prior to the AM dose on Day 28 of cycle 2 (or Day 1 of cycle 3 if the subject's last dose of study treatment was the evening prior). Blood samples for PTC299 PK assessments will not be collected after cycle 2. The actual sample collection times should be recorded. Please see Appendix IX for detailed collection, processing, storage and shipping instructions for PK samples.

4.2.4 Assaying secreted cytokines characteristic for KS: To measure PTC299's effect on KS we will evaluate serum and plasma for levels of the cytokines (e.g., VEGF-A, VEGF-C, VEGF-D, VEGF-R1, VEGF-R2, VEGF165b, PlGF, IL-6, and IL-8) at baseline (pre-dose of study treatment on Day 1) and mid-cycle (Day 15) of cycle 1, on Day 1 of all subsequent cycles, and at the time of treatment discontinuation and 30 days post discontinuation of study therapy. Please see Appendix IV for detailed collection, processing, storage and shipping instructions for these samples. Depending on the results of these assays, other potential serum markers characteristic for KS may also be measured.

### 4.3 Evaluations at the Time of Treatment Discontinuation

At the time of treatment discontinuation all evaluations should be completed as soon as possible (see Appendix I, Schedule of Evaluations). The following should be performed upon discontinuation of study drug:

4.3.1 Complete blood count with differential and platelets.

4.3.2 Serum chemistries: liver enzymes (AST, ALT, alkaline phosphatase), BUN, creatinine, electrolytes (sodium, potassium, chloride, bicarbonate), calcium, phosphorus, glucose, total protein, albumin, bilirubin (direct and indirect), triglycerides and total cholesterol.

4.3.3 CD4 count and HIV plasma RNA viral load, and KSHV viral load.

4.3.4 Complete physical examination including: vitals signs, weight, Karnofsky performance status (Appendix II) and toxicity evaluation.

4.3.5 Blood for ACTH and cortisol.

4.3.6 KS tumor assessment. Response of KS to treatment will be assessed as described in Section 8.0. Marker lesions and other areas will be photographed (Appendix XIV).

4.3.7 Cytokine assay (Appendix IV).

#### **4.4 Post-treatment Evaluation and Evaluation at Early Discontinuation of Therapy**

Thirty days after discontinuation of therapy, a follow-up visit will include the following procedures: complete physical examination (including vitals signs, weight, and tumor assessment, and lesion photography if the response category changed), Karnofsky performance status (Appendix II), toxicity evaluation and cytokine assay.

At a subject's final study visit, the Off-Study Form will be completed and permanent discontinuation of drug will be documented.

Subjects who withdraw for toxicity reasons should be followed until the toxicity resolves/returns to baseline, or for at least 1 month, whichever is later. In addition, subjects who go off study for reasons other than toxicity should be followed for at least 1 month after discontinuing drug.

#### **4.5 Final Evaluations/Off Drug, Off Study**

Adverse experiences must be reported if the event began any time within 30 days of receiving the study treatment. Additionally, if a site learns of any incidence of death, cancer or fetal anomaly, which is possibly, probably or definitely related to the drug, at any time after the study is closed, the event should be reported to the NCI through AdEERS (see Section 6.2) within 10 calendar days of when the Investigator learns of the event. This information may provide additional insight into the safety of PTC299.

## 5.0 TREATMENT PLAN

### 5.1 Study Drug

- 5.1.1 Study Medication Description: PTC299 is manufactured following cGMP. Each capsule contains 20 mg of the active drug substance provided as size 00 hard gelatin capsules with an orange color. Excipients present in the formulation include Solutol<sup>®</sup> HS15, Gelucire<sup>®</sup> 44/14, and butylated hydroxytoluene (BHT). The drug product in the capsules appears as an opaque, off-white soft wax at room temperature.
- 5.1.2 Study Medication Packaging: PTC299 capsules will be packaged and supplied in bulk for the study. PTC299 capsules will be provided in the bulk supply bottles. Labels for bulk supply bottles will be printed in English and will contain the following information: name and potency, bottle fill count, lot number, space to enter the subject number, dosing instructions with a space to enter the number of capsules to be taken per dose, storage conditions, sponsor address, and other information as per regulatory requirements for the United States.
- 5.1.3 Storage and Stability: Bottles containing capsules of PTC299 will be shipped and stored at room temperature (~15 to 30°C). While stability of capsules stored at up to 40°C for 6 months has been confirmed, excursions to temperatures ≥38°C are not recommended because the hard gelatin capsule shell may be damaged or the waxy material in the capsules may soften. Refrigerating or freezing must be avoided since the drug may crystallize out of the solid wax matrix in the capsules. Stability data at the start of study will support the use of the drug product for ≥6 months. The AMC will be updated by PTC Therapeutics as more stability data become available.

### 5.2 Drug Orders, Transfers, Returns, and Accountability

- 5.2.1 Drug Orders: PTC 299 will be supplied free of charge by PTC Therapeutics. AMC sites will be considered qualified to receive study drug (from the local depot of PTC) as soon as the site becomes eligible for subject enrollment (i.e., all required regulatory documents have been received by the AMC Operations Center). Upon receiving an order request from an eligible site, PTC's Clinical Supplies Manager will send an e-mail notification to the site's pharmacist, Study Site Coordinator, and the study site monitor regarding the pending study drug shipment. Drug supplies will be shipped Mondays to Thursdays only. Sites should allow at least 5 to 10 working days from the day that the order is received at PTC and the day drug is to be received at the site. To order additional study drug, please contact the Study Sponsor Drug Supply Coordinator:

Leslie Callahan  
PTC Therapeutics, Inc.  
100 Corporate Court  
South Plainfield, NJ 07080  
Telephone: (908) 912-9422  
Facsimile: (908) 222-1669  
Email: lcallahan@ptcbio.com

Additional instructions regarding drug ordering, including a form for ordering study drug will be provided on the AMC website ([www.amcoperations.com](http://www.amcoperations.com)).

- 5.2.2 Drug Dispensing: The clinic pharmacist or an alternative qualified person at each participating AMC site will be responsible for dispensing study medication. The total amount of drug necessary for the 28-day treatment cycle will be dispensed to the subject at the beginning of the cycle, with written instructions on the number of capsules to be taken at each dose. A modest overage will be supplied such that subjects have sufficient drug in case of loss, spillage, or necessary deviations in scheduling clinic returns (e.g., due to inclement weather, etc).
- 5.2.3 Returns: Subjects should return all unused study medication to the study site at the end of each cycle. Appropriate source documentation confirming the amount of study drug supplied to the subjects and documenting the return of any unused drug must be maintained by the site for compliance assessments. Clinic staff will record the study medication administration information, including the exact clock time of each dose, for doses of study drug administered in the clinic or hospital.
- 5.2.4 Drug Accountability: Study personnel must ensure that all study medication supplies are kept in a secure locked area with access limited to authorized personnel. This study product must not be used outside the context of this protocol. Under no circumstances should the investigator or site personnel supply study product to other investigators or clinics, or allow the supplies to be used other than as directed by this protocol.

The investigator and/or the responsible site personnel must maintain accurate records of the receipt of all study medication shipped by PTC Therapeutics or its designee, including, but not limited to, the date received, lot number, amount received, and the disposition of all study medication. Current dispensing records must also be maintained that include the date and amount of medication dispensed, relevant batch or bottle code numbers, and study number assigned to the subject.

All used containers of study medication should be discarded according to standard institutional policy only after the study monitor has examined them. Depending upon the decision of PTC Therapeutics, unused clinical supplies must be returned to PTC Therapeutics or its designee after the study is completed. Records documenting the date of study medication destruction or shipping, relevant batch or bottle code numbers, and amount destroyed or shipped should be kept.

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all drugs received using the NCI Drug Accountability Record Form (DARF) (available on the CTEP home page (<http://ctep.cancer.gov>) or by calling the Pharmaceutical Management Branch at 301-496-5725) or a site-specific form that captures the same elements as the NCI DARF.

## 5.3 Dosing

### 5.3.1 PTC299 Treatment

Each cycle with the study drug includes administration of 56 to 84 doses of PTC299 during a 4-week (28-day) period. The maximum duration of treatment is 12, 4-week cycles. PTC299 will be administered on a BID or TID schedule at approximately the same times each day. At each dose administration, the capsule number corresponding to the appropriate dose level of PTC299 is to be swallowed whole with a glass of tap water (150 to 200 mL). Subjects should be instructed not to bite or chew on the capsules. In case of breakage of the capsules in the oral cavity, an additional glass of water should be taken immediately.

On Day 1 and on Day 28 of cycle 1, the drug will be administered in the clinic with dosing appropriately timed relative to blood sampling for PTC299 PK. Thereafter, subjects will be given an adequate supply of capsules to take at home for the duration of each single cycle (28 days) of treatment.

Ideally, when given BID, doses should be taken at ~12-hour intervals (e.g., at ~7:00 AM and at ~7:00 PM) and, when given TID, PTC299 doses should be taken at ~8-hour intervals (eg, at ~7:00 AM, at ~3:00 PM, and at ~11:00 PM). If convenient for the subject, the drug may be taken during or within ~30 minutes after a meal; however, administration with food is not required. Yogurt or pudding may be taken together with the capsules in case of difficulty in swallowing. While it is realized that variations in dosing schedule may occur in the outpatient setting, the prescribed regimen (dosing intervals) should be followed as closely as possible, especially in the clinic. In particular, on study days of PK profiling, patients administered TID dosing should take the first dose immediately after the pre-dose blood draw and not dose again until all profile blood draws are completed.

### 5.3.2 Dose Escalation Rules and Definition of MTD

In Stage 1, each subject will be assigned to Dose Level 1 (40 mg/dose BID), Dose Level 2 (80 mg/dose BID), or Dose Level 3 (100 mg/dose BID) sequentially according to the dose level being explored at the time of subject enrollment. A Dose Level -1 (20 mg/dose BID) is provided in case a subject assigned to Dose Level 1 (40 mg/dose BID) requires dose reduction. In Stage 2, 8 subjects were enrolled at the Stage 1 MTD, Dose Level 2 (100 mg/dose BID). In Stage 3, each subject will be assigned to Dose Level 6 (160 mg/dose TID), or Dose Level 7 (200 mg/dose TID) sequentially according to the dose level being explored at the time of subject enrollment. Dose Level 5 (120 mg/dose TID) is provided in case a subject assigned to Dose Level 6 (160 mg/dose TID) requires dose reduction. Dose Level 4 (100 mg/dose BID) is provided in case a subject assigned to dose Level 5 (120 mg/dose TID) requires dose reduction.

During the study, dose escalation will depend on evaluations of safety in cycle 1 of therapy. The following dose escalation rules will be employed:

- Three subjects will be studied at the first dose level. If none of these three subjects experience DLT during cycle 1, then the dose will be escalated to the next higher level in the three subsequent subjects.
- If one of three subjects experiences DLT at the current dose during cycle 1, then three more subjects will be accrued at the same dose. If none of these three additional subjects experience DLT during cycle 1, then the dose will be escalated in subsequent subjects. If one or more of the three additional subjects experiences DLT in the first cycle, the MTD has been exceeded and three more subjects will be treated at the next lower dose (if only three subjects were previously treated at that prior dose).
- If two or more subjects experience DLT in the first cycle, then the MTD has been exceeded and three more subjects will be treated at the next lower dose level (if only three subjects were previously treated at that prior dose).
- No intrasubject dose escalations in subsequent cycles of therapy will be permitted.

At the first and at all subsequent dose levels, each group of three subjects within a cohort must be observed for a minimum period of at least 28 days without DLT before subsequent subjects are enrolled at the next dose level. Escalation to the next dose level can occur with the concurrence of the Protocol Chair, AMC Group Statistician, and the AMC Medical Monitor upon review of the safety data from the ongoing group of three subjects. The PTC medical monitor should be notified promptly of the dose escalation decision.

The establishment of an MTD will be based on a review of the overall first-cycle safety data by the Protocol Chair, AMC Group Statistician, and the AMC Medical Monitor. The MTD is the starting dose level at which 0/6 or  $\leq 1/6$  subjects experience DLT with the next higher dose having at least 2/3 or 2/6 subjects encountering DLT during the first treatment cycle. Effectively, the Stage 1 MTD is that dose associated with first-cycle DLT in  $< 33.3\%$  of subjects. If none of the dose levels tested is associated with 2/3 or 2/6 subjects experiencing DLT during the first treatment cycle, subjects in the Stage 2 (Section 5.3.5) of the study will be treated at a dose of 100 mg/dose BID. Similarly, in Stage 3, if neither of the 2 dose levels (160 mg/dose TID nor 200 mg/dose TID) tested is associated with at least 2/3 or 2/6 subjects experiencing DLT during the first treatment cycle, subjects in the expansion cohort will be treated at a dose of 200 mg/dose TID.

### 5.3.3 Definitions of Dose-Limiting Toxicity

The occurrence of DLTs during cycle 1 will be used to define the MTD. In cycle 1 and in subsequent cycles the occurrence of DLTs will determine the need for dose interruptions or reductions.

DLT will be defined as the occurrence of any of the following:

- Grade  $\geq 2$  PTC299-related vomiting despite maximal oral antiemetic therapy, or a requirement for intravenous (IV) antiemetics to control PTC299-related nausea and vomiting
- Grade  $\geq 2$  proteinuria

- In patients not receiving atazanavir or indinavir, change in total serum bilirubin from baseline value of Grade 0 to on-treatment value of Grade  $\geq 3$  ( $>3.0 \times \text{ULN}$ ), whether or not serum ALT or AST are elevated
- In patients receiving atazanavir or indinavir, on-treatment serum direct bilirubin  $>40\%$  of total bilirubin, whether or not serum ALT or AST are elevated
- Change in serum ALT or AST from baseline value of Grade 0 to on-treatment value of Grade  $\geq 2$  ( $>2.5 \times \text{ULN}$ )
- Change in serum ALT or AST from baseline value of Grade 1 to on-treatment value of Grade  $\geq 3$  ( $>5 \times \text{ULN}$ )
- Grade  $\geq 3$  PTC299-related toxicities (for purposes of defining the DLT, only toxicities considered by the investigator to be possibly, probably or definitely related to PTC299 will be considered).
- Failure to complete  $\geq 80\%$  of the planned 56 doses (i.e.,  $\geq 45$  doses) of a PTC299 treatment course due to PTC299-related toxicities.

Toxicities will be graded according to the CTCAE, Version 3.0. If multiple toxicities are seen, the presence of DLT will be based on the most severe toxicity experienced. At the end of each cycle, each subject must be assessed by the investigator as to whether or not the subject experienced DLT, and this information must be recorded on the Adverse Event CRF.

NOTE: If DLT is based on PTC 299-related increases in serum triglycerides or cholesterol, the increase must be confirmed in the fasting state and the grade of toxicity must be at least one grade higher than the baseline value.

#### 5.3.4 Study Drug Dose Interruptions and Dose Modification for Toxicity

Subjects on a BID PTC299 treatment schedule who inadvertently have a delay in administration of a dose of the study drug of  $< 6$  hours should take the planned dose as soon as possible after the intended time of administration. For subjects who inadvertently have a delay in administration of study drug of  $\geq 6$  hours, the dose should not be taken. Study drug administration may continue but the missed dose should not be made up and the planned timing of subsequent study drug dosing should not be altered.

For subjects on a TID PTC299 treatment schedule who inadvertently have a delay in administration of a dose of the study drug of  $\leq 1$  hour, the planned dose should be taken with no changes to the subsequent dose schedule. For subjects who have a delay of  $>1$  hour but  $\leq 4$  hours, the planned dose should be taken; however, all future doses for that day should be shifted later by a corresponding amount. It is recommended that subjects take the last dose of study medication no later than 12:00 midnight on any study treatment day. For example if the 07:00 AM dose is taken at 10:00 AM, the next dose should be taken at 5:00 PM, and the last dose should be taken at 12:00 midnight. For subjects who have a delay in administration of study drug of  $>4$  hours, the dose should not be taken. Study drug administration may continue but the missed dose should not be made up and the planned timing of subsequent study drug dosing should not be altered.

Vomiting upon dose administration is considered as a missed dose; the dose should not be made up and the planned timing of subsequent study drug dosing should not be altered.

Because of a case of liver injury in a patient with a history of prior drug induced liver injury, laboratory parameters reflective of liver injury should be monitored closely. If criteria for DLT are observed based on monitoring ALT, AST, or bilirubin, PTC299 treatment should be held and the parameters (plus alkaline phosphatase) should be retested within 72 hours. If retesting does not confirm DLT-severity abnormalities, PTC299 treatment may be resumed. If the retesting confirms DLT-severity abnormalities, the AMC and PTC Therapeutics medical monitors should be notified. Efforts should be made to investigate the possibility of new or progressive hepatic tumor. Follow-up evaluations of liver-related laboratory parameters (ALT, AST, bilirubin, alkaline phosphatase) should be performed at least 2 times per week, until they are stable or resolved. Further workup may be considered in consultation with the AMC and PTC Therapeutics medical monitors. Further workup may include: obtaining a history of recent symptoms/illnesses and of relevant past history (e.g., history of hepatitis or of hepatitis A or hepatitis B vaccination); obtaining information regarding concomitant drug use (prescription and nonprescription medications, dietary supplements, alcohol consumption, illicit drug use, special diets); questioning the patient regarding potential exposure to environmental toxins; ruling out viral hepatitis A, B, C, D (if hepatitis B is positive), and E, autoimmune hepatitis, alcoholic hepatitis, nonalcoholic steatohepatitis, hypoxic/ischemic hepatopathy, and biliary tract disease; obtaining additional tests to evaluate liver function (e.g., PT, aPTT, INR, albumin); and considering gastroenterology or hepatology consultation [138].

If on-treatment serum ALT, AST, or bilirubin level doubles from baseline, in the absence of a similar change alkaline phosphatase, but has not reached the DLT levels described above, tests for ALT, AST, bilirubin, and alkaline phosphatase should be monitored closely (e.g., at least once per week). PTC299 treatment may continue while the laboratory tests are being performed.

If a subject experiences a DLT during the 28-day course of PTC299 therapy, then study drug administration can be omitted, as necessary, until the adverse event resolves or stabilizes to an acceptable degree (i.e., to < Grade 2). Thereafter, the dose of PTC299 for the remainder of the treatment course during that cycle should be reduced by one dose level; reduction to Dose Level -1 (20 mg/dose BID) may be made for subjects experiencing PTC299-related DLT at Dose Level 1 (40 mg/dose BID); and reduction to Dose Level 4 (100 mg/dose BID) may be made for subjects experiencing PTC299-related DLT at Dose Level 5 (120 mg/dose TID) and reduction to Dose Level 6 (160 mg/dose TID) may be made for subjects experiencing PTC299-related DLT at Dose Level 7 (200 mg/dose TID). Doses during missed days of treatment should not be made up (e.g., if a subject experiences an AE on Day 7 of the treatment course and the event lasts for 3 days, the reduced dose should be administered only for a further 18 days so that the total treatment course duration remains 28 days).

A new cycle of PTC299 treatment may begin when AEs or laboratory abnormalities have returned to baseline levels. If AEs or laboratory abnormalities are not resolved to baseline, week-by-week delays in initiating the new cycle of treatment should be instituted. If it takes longer than 4 weeks for the AE to resolve to baseline, protocol treatment will be permanently discontinued. Once all toxicities have returned to baseline, the next cycle of therapy can be initiated.

Subjects experiencing any of the DLTs described in Section 5.3.3 in the current cycle of therapy should have the dose in the next cycle of therapy reduced by 1 dose level; reduction to Dose Level -1 (20 mg/dose BID) may be made for subjects experiencing PTC299-related DLT at Dose Level 1; reduction to Dose Level 4 (100 mg/dose BID) may be made for subjects experiencing PTC299-related DLT at Dose Level 5 (120 mg/dose TID) and reduction to Dose Level 6 (120 mg/dose TID) may be made for subjects experiencing PTC-related DLT at Dose Level 7 (160 mg/dose TID).

In general, after a dose is reduced for PTC299-related toxicity it should not be re-escalated, even if there is minimal or no toxicity with the reduced dose. However, if further evaluation reveals that the adverse event that led to the dose reduction was not study drug-related, the dose may be re-escalated to the original dose level.

When there is a dose modification, the subject should be notified of the change in dose and the appropriate clinic staff should instruct the subject about the revised number of study medication capsules to be used per dose according to the new dose level.

Clinical use of other anti-VEGF compounds (e.g., bevacizumab) can be associated with delayed wound healing. For this reason, subjects who require surgery or a deep invasive procedure should have PTC299 held for  $\geq 2$  weeks before the procedure, if possible, and PTC299 should be held for  $\geq 2$  weeks after the procedure, or until complete wound healing, to avoid the theoretical risk of impaired wound closure.

#### 5.3.5 Treatment in Phase 2

Once the safety profile at each dose level has been established based on first cycle DLTs in Phase 1, up to 21 additional subjects whose response to treatment can be evaluated will be enrolled in the Phase 2 portion of the study at the highest safely tolerated dose established in the Phase 1 portion to better define the safety, antitumor activity and biological activities of PTC299 at this dose level. In the Phase 2 portion of the study, Simon's 2-stage design will be used, as described in Section 11.1. Treatment will consist of 4 week cycles of therapy (56 or 84 doses) as described in Section 5.3.2. The maximum duration of treatment is 12, 4-week cycles. Definitions of DLT will be as described in Section 5.3.3. Study drug interruptions and dosage reductions will be managed as described in Sections 5.3.1 and 5.3.4.

#### 5.3.6 Study Medication Dose Calculation

The number of capsules of study drug to be administered will be the same for all the subjects enrolled to the same dosing cohort. Two 20-mg capsules per dose will be administered to subjects at Dose Level 1, four 20-mg capsules per dose will be

administered to subjects at Dose Level 2, five 20-mg capsules per dose will be administered to subjects at Dose Level 3 and 4, six 20-mg capsules per dose will be administered to subjects at Dose Level 5, eight 20-mg capsules per dose will be administered to subjects at Dose Level 6, and ten 20-mg capsules per dose will be administered to subjects at Dose Level 7.

Analyses of PK data do not reveal an advantage for body-weight-based dosing in minimizing variations of PTC299 plasma exposure. Using a fixed dosing regimen offers convenience for pharmacies in dispensing the drug and helps to ensure subject compliance in drug administration. To date, six cancer patients have received 0.3 mg/kg/dose of PTC299 BID and six cancer patients have received 0.6 mg/kg/dose of PTC299 BID for at least 4 weeks. PTC299 has been generally well tolerated. No PTC299-related AEs or dose-limiting toxicities have been reported. A preliminary PK analysis suggests that the target plasma concentration of ~0.15 µg/mL is readily achievable with the planned dose levels.

#### 5.4 Concomitant Medication

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the subject are allowed, provided their use is documented in the subject records and on the appropriate CRF. Specific exceptions include warfarin, heparin and antiplatelet agents. Use of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) at doses not to exceed the maximum recommended dose is permitted.

The administration of any other therapies intended to treat KS including chemotherapy and biologic agents is NOT permitted. The use of other concurrent investigational drugs is not allowed.

##### Antiemetics

Prophylactic treatment with antiemetics is not allowed on the first day of treatment in cycle 1, but can be administered on subsequent treatment days and in subsequent cycles, based on the judgment of the treating physician. At the occurrence of nausea or vomiting of severity grade  $\geq 1$ , it is suggested that the subject receive a 5HT3 blocker such as:

- Ondansetron hydrochloride (Zofran®) 8 mg orally up to 1 hour before PTC299 dosing and up to 2 additional times daily, or
- Granisetron hydrochloride (Kytril®) 1 mg orally up to 1 hour before PTC299 dosing, and 1 mg 12 hours later as needed on the day of dosing.

In the presence of recurring unacceptable nausea or vomiting, the investigator may consider additional medications. Possible agents include:

- Lorazepam (Ativan®) 1 to 2 mg orally every 4 hours.

Nausea and vomiting requiring IV antiemetics should be considered a DLT (see Section 5.3.3).

### Other Concomitant Medications

To the extent possible, administration of any prescription or over-the-counter drug products other than study medication should be minimized during the study period. It is expected, however, that subjects will be receiving antiretroviral therapy for their HIV infection, and may be receiving other drugs to prevent OIs; these are specifically allowed, as discussed in Section 5.4.1. Subjects should be discouraged from use of street drugs, health foods, herbal remedies, self-prescribed drugs, tobacco products, or excessive alcohol at any time during clinical studies of PTC299.

If considered necessary for the subject's well being, drugs for concomitant medical conditions or for symptom management may be given at the discretion of the investigator. The decision to authorize the use of any drug other than study medication should take into account subject safety, the medical need, the potential for drug interactions, the possibility for masking symptoms of a more significant underlying event, and whether use of the drug will compromise the outcome or integrity of the study.

Because it is possible that PTC299 may slow the clearance of drugs that are primarily metabolized by CYP2D6, investigators should pay specific attention to use of drugs that are known substrates of this enzyme, particularly when such drugs may have a low therapeutic index. Concomitantly administered substrates for CYP2D6 that may require special attention include certain chemotherapeutic drugs (anthracyclines and vinca alkaloids) most antidepressants (tricyclic antidepressants, selective serotonin reuptake inhibitors, and others), many antipsychotics, many beta-adrenergic receptor blockers, and certain anti-arrhythmics. In addition, several antiretroviral agents, including ritonavir, lopinavir/ritonavir, and delavirdine, rely to some extent on CYP2D6 for their clearance.

Subjects should be instructed about the importance of the need to inform the clinic staff of the use of any drugs or remedies (whether prescribed, over-the-counter, or illicit) before and during the course of the study. Any concomitant drugs taken by a subject during the course of the study and the reason for use will be recorded on the CRFs.

#### 5.4.1 Permitted Medications

- Antiretroviral therapy for HIV infection
- Treatment, maintenance or chemoprophylaxis with approved agents for opportunistic infections as clinically indicated
- Topical and/or oral antifungal agents
- Antibiotics as clinically indicated
- Erythropoietin is permitted at the discretion of the Investigator
- Granulocyte colony stimulating factors as clinically indicated for > grade 3 neutropenia at the discretion of the Investigator.
- Regularly prescribed medications such as antipyretics, analgesics, allergy medications, antidepressants, sleep medications, oral contraceptives, megestrol acetate, testosterone or any other medications are permitted except for prohibited medications (see Section 5.4.2).

- Alternative therapies such as vitamins not exceeding the recommended daily allowance, acupuncture and visualization techniques will be permitted. Subjects should report the use of these therapies and they will be recorded.
- Use of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) at doses not to exceed the maximum recommended dose is permitted.

#### 5.4.2 Prohibited Medications

- Investigational drugs with the exception of antiretroviral agents that are being obtained through an expanded access protocol.
- Warfarin at any dose.
- Heparins, including low molecular weight heparins.
- Antiplatelet agents (e.g., clopidogrel bisulfate)
- Systemic cytotoxic chemotherapy or any other treatment specifically prescribed to treat KS (e.g., radiation, etc.).
- Routine use of systemic corticosteroid therapy other than replacement doses is not permitted.

#### 5.4.3 Diet

- There are no specific dietary restrictions in the study. PTC299 may be taken with or without food.

### 5.5 Treatment Compliance

Records of study medication used, dosages administered, and intervals between visits will be kept during the study. Drug accountability will be noted at the completion of the trial. Subjects will be asked to return all unused medication at the monthly visits.

Subjects will also be given a diary to carry home and will be instructed to record each time study drug is administered. The subject will be asked to bring the diary to each clinic visit (see Appendix XI).

## 6.0 REPORTING OF ADVERSE EVENTS

This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 for reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP home page (<http://ctep.info.nih.gov>). All appropriate treatment areas should have access to a copy of the CTCAE version 3.0. The document “CTEP, NCI Guidelines: Adverse Event Reporting Requirements” clearly outlines reporting criteria and can be downloaded from the CTEP web site (<http://ctep.cancer.gov/reporting/adeers.html>).

This study will be monitored by the Clinical Data System (CDS). Cumulative CDS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31.

### 6.1 Classification of AEs by Severity and Relationship to Study Drug Administration

#### Adverse Event

Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite).

#### Life-Threatening Adverse Event

Any AE that places the subject or subject in view of the Investigator, at immediate risk of death from the reaction.

#### Serious Adverse Event (SAE)

Any AE occurring at any dose that results in any of the following outcomes: Death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

Please note for hospitalization – All hospitalizations (or prolongation of existing hospitalization) for medical events equivalent to CTCAE Grade 3, 4, 5 must be reported regardless of the requirements for the phase of study, expected or unexpected, and attribution. For example, do not report an admission for pharmacokinetic sampling, but do report an admission for a myocardial infarction.

#### Toxicity

Toxicity is a term NOT clearly defined by regulatory organizations. Toxicity has been described as an AE that has an attribution of possibly, probably or definitely related to investigational treatment. To minimize confusion the NCI would recommend that the term toxicity NOT be utilized for AE reporting purposes. The CTCAE continues to use the term ‘toxicity’ because of familiarity.

### Unexpected Adverse Event

Any AE that is not listed in available sources including the package insert, the Investigator's Brochure, or the protocol.

### Adverse Event Expedited Reporting System (AdEERS)

An electronic system for expedited submission of AE reports.

### Attribution

The determination of whether an AE is related to a medical treatment or procedure. Attribution categories:

**Table 3. Relationship of Study Drug To Adverse Event**

<b>Relationship</b>	<b>Description</b>
Definite	A clinical event in which a relationship to the use of the study drug seems definite because of such factors as consistency with known effects of the drug; a clear temporal association with the use of the drug; lack of alternative explanations for the event; improvement upon withdrawal of the drug (de-challenge); and recurrence upon resumption of the drug (rechallenge). Thus, the AE is clearly related to the study drug.
Probable	A clinical event in which a relationship to the study drug seems probable because of such factors as consistency with known effects of the drug; a reasonable temporal association with the use of the drug; lack of alternative explanations for the event; and improvement upon withdrawal of the drug (de-challenge). Thus, the AE is likely related to the study drug.
Possible	A clinical event occurring coincident with administration of the study drug and which is not likely to be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking. Thus, the AE may be related to the study drug.
Unlikely	A clinical event with a temporal relationship to study drug administration that makes a causal relationship improbable and in which other factors suggesting an alternative etiology exist. Such factors include a known relationship of the adverse event to concomitant drug, the subject's disease state, or environmental factors including common infectious diseases. Thus, the AE is doubtfully related to the study drug.
Unrelated	A clinical event, including a laboratory test abnormality, in which a relationship to the study drug seems improbable because of factors such as inconsistency with known effects of the study drug; lack of a temporal association with study drug administration; lack of association of the event with study drug withdrawal or rechallenge; and/or presence of alternative explanations for the event. Alternative explanations might include a known relationship of the adverse event to a concomitant drug, past medical history of a similar event, the subject's disease state, or environmental factors. Thus, the AE is clearly NOT related to the study drug.

An AE is considered to be related to study drug treatment if the causality assessment is ranked as possible, probable, or definite.

## 6.2 Expedited AE Reporting Procedures

Expedited AE reporting for this study must use AdEERS, accessed via the CTEP home page (<http://ctep.cancer.gov>). The reporting procedures to be followed are presented in the CTEP, NCI Guidelines: Adverse Event Reporting Requirements which can be downloaded from the CTEP home page (<http://ctep.cancer.gov>). These requirements are briefly outlined in the table below.

In the rare occurrence when internet connectivity is lost, an AE report may be submitted using CTEP's Adverse Event Expedited Report-Single Agent or Multiple Agent paper template (available at <http://ctep.cancer.gov>) and faxed to the AMC Operations Center at 240-238-2842. A 24-hour notification is to be made to AMC by telephone at 301-251-1161, only when internet connectivity is disrupted. Once internet connectivity is restored, an AE report submitted on a paper template, or a 24-hour notification phoned in, must be entered electronically into AdEERS by the original submitter at the site.

AdEERS is programmed for automatic electronic distribution of reports to the following individuals: Protocol Chair, Principal Investigator at the local AMC treating institution, the PTC Therapeutics Medical Monitor and AMC Operations Center. AdEERS provides a copy feature for other e-mail recipients.

### 6.2.1 Expedited Reporting Requirements for AEs that occur within 30 Days of Last Protocol Treatment (Investigational Agent)

**Table 4. Expedited Reporting Timelines for Investigational Agents**

Grade	1	2	2	3		3		4 & 5 <sub>1</sub>	4 & 5 <sub>2</sub>
	Unexpected and Expected	Unexpected	Expected	Unexpected		Expected		Unexpected	Expected
				with Hospitalization	without Hospitalization	with Hospitalization	without Hospitalization		
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

<sup>1</sup> Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment require reporting as follows:

- 24-hour notification followed by complete report within 5 calendar days for:
  - Grade 4 and Grade 5 unexpected events
- Complete SAE report within 10 calendar days:
  - Grade 3 unexpected events with hospitalization or prolongation of hospitalization
  - Grade 5 expected events

<sup>2</sup> Although 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

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6.2.1.1 Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

6.2.1.2 Expedited AE reporting timelines defined:

“24 hours; 5 calendar days”– The Investigator must initially report the AE via AdEERS, according to the procedures outlined in section 6.2, within 24 hours of learning of the event, and followed by a complete AE report submitted via AdEERS within 5 calendar days of the initial 24-hour report. Use the NCI protocol number and protocol-specific subject ID assigned during trial registration on all reports.

“10 calendar days”– A complete AdEERS report on the AE must be submitted within 10 calendar days of the Investigator learning of the event. Use the NCI protocol number and protocol-specific subject ID assigned during trial registration on all reports.

Any medical event equivalent to CTCAE Grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited AE reporting exclusions.

Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS according to the guidelines as described above.

All expedited reports should be submitted to the local IRBs according to local IRB policies and procedures.

### **6.3 Routine AE Reporting**

AEs reported through AdEERS must also be reported in routine study data submissions. Routine reporting of all AEs, regardless of grade, should be reported on the Adverse Event Form CRF.

### **6.4 PTC Therapeutics Adverse Event Reporting Requirements**

Each SAE reported through AdEERS received from AMC sites must be evaluated by the Investigator, the AMC medical monitor, and the PTC Therapeutics medical monitor. The medical monitors are required to review all unanticipated problems involving risk to subjects or others, serious adverse events, and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitors should comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitors should also indicate whether they concur with the details of the report provided by the AMC clinic site investigator.

For regulatory reporting purposes, the event is classified as related if the AMC clinic site investigator, the AMC medical monitor, or the PTC Therapeutic medical monitor determines that the event is definitely, possibly or probably related to the study drug. An AE will be considered expected if the protocol or Investigator Brochure indicates that such an event of similar or greater severity has already been observed in association with use of the study medication.

The PTC Therapeutics Awareness Date (PTCAD) is used in determining AE regulatory reporting timelines. The PTCAD is defined as the earliest date PTC Therapeutics or an agent becomes aware of an AE. This is the date the regulatory reporting clock begins.

PTC Therapeutics SAE regulatory reporting requirements are described in the table below.

**Table 5. PTC Therapeutics Serious Adverse Event Regulatory Reporting Requirements**

Type of Event			Type of Report	Timeframe for Reporting To Health Authorities
Fatal or Life-Threatening	Unexpected	Related		
Yes	Yes	Yes	Telephone/Fax notification or report	Within 7 calendar days of PTCAD or according to local regulations
			FDA Form 3500A or CIOMS I form	Within 15 calendar days of PTCAD or according to local regulations
No	Yes	Yes	FDA Form 3500A or CIOMS I form	Within 15 calendar days of PTCAD or according to local regulations
Yes	No	Yes	Annual report	Annually
No	No	Yes		
Yes	Yes	No		
No	Yes	No		
Yes	No	No		
No	No	No		

a. Includes possibly or probably related events

Abbreviations: CIOMS I = Council for International Organizations of Medical Sciences, FDA = Food and Drug Administration, PTCAD = PTC Therapeutics Awareness Date

If notification of an adverse event requiring expedited reporting is received, PTC Therapeutics will contact each investigator using PTC299 by e-mail or regular mail within 15 working days from the PTCAD such that the Investigator can promptly notify the site IRB. A complete written report will follow the initial notification. PTC will receive all SAE notifications via AdeERS. Although AdeERS reports are automatically forwarded to the FDA, PTC will maintain responsibility for ensuring all events are reported to the FDA according to federal requirements according to the Regulatory Reporting Requirements listed in Table 5 above. Routine reporting of all AEs will be recorded by the sites on the Adverse Event Form CRF.

## **7.0 CRITERIA FOR DISCONTINUATION**

- Study medication will be interrupted or permanently discontinued for treatment related toxicities as outlined in Section 5.3.3.
- Disease progression.
- Subjects who show no more than stable disease after six 4-week cycles of treatment will be permanently removed from treatment.
- Subjects who show a partial response will be treated for up to twelve 4-week cycles.
- Subjects who show a complete response will be treated for two 4-week cycles beyond confirmation of complete response.
- Subjects who become pregnant or breast-feed or who require systemic chemotherapy for the treatment of a malignancy other than KS.
- Subjects who require treatment with medications which are disallowed as outlined in Section 5.4.2.
- Subjects who are noncompliant with respect to taking drugs, keeping appointments or having tests required for the evaluation of drug safety and efficacy.
- Subjects have the right to withdraw from the study at any time for any reason.
- The Investigator has the right to remove subjects from the study for clinical reasons which he/she believes are life threatening to the subject even if such reasons do not fall into the toxicity classifications discussed above.

## 8.0 EVALUATION OF RESPONSE

All subjects will be evaluated for response by physical examination and by relevant imaging studies at Day 29 and every 28 days thereafter ( $\pm 7$  days).

### 8.1 Definition of Response

8.1.1 Complete response (CR) is defined as the absence of any detectable residual disease, including tumor-associated edema, persisting for at least 4 weeks. In subjects in whose pigmented (brown or tan) macular skin lesions persist after apparent CR, biopsy of at least one representative lesion is required in order to document the absence of malignant cells. In subjects known to have had visceral disease, an assessment at restaging with appropriate endoscopic or radiographic procedures should be made.

8.1.2 Partial response (PR) is defined as no new lesions (skin or oral), or new visceral sites of involvement (or the appearance or worsening of tumor-associated edema or effusions); AND

- A 50% or greater decrease in the number of all previously existing lesions lasting for at least 4 weeks; OR
- Complete flattening of at least 50% of all previously raised lesions (i.e., 50% of all previously nodular or plaque-like lesion become macules); OR
- A 50% decrease in the sum of the products of the largest perpendicular diameters of the marker lesions

Note: Subjects with residual tumor-associated edema or effusion who otherwise meet the criteria for CR will be classified as having a PR.

8.1.3 Stable disease is defined as any response not meeting the criteria for CR, PR, or progressive disease.

8.1.4 Progressive disease (PD) is defined as follows:

For subjects with <50 cutaneous lesions:

- $\geq 25\%$  increase in the sum of perpendicular diameters of the indicator lesions; OR
- $\geq 25\%$  increase in the total lesion count, or a minimum of 5 new lesions, whichever is greater; OR
- $\geq 25\%$  increase in the number of raised lesions (minimum of 5 new raised lesions if there are very few raised lesions, for example  $\leq 8$ ), whichever is greater.

Note: There are body sites where disease is particularly difficult to evaluate, and a few new lesions may be counted in spite of the fact that a subject is not actually progressing. For example, lesions of the foot, particularly those which are flat, are difficult to evaluate because their intensity may be variable based on how much edema is present, how much the person walked the day before, how long their feet have been in a dependent position prior to the physical exam, etc.

For subjects with >50 cutaneous lesions:

- $\geq 25\%$  increase in the sum of the perpendicular diameters of the indicator lesions; OR

- $\geq 25\%$  increase in the total number of lesions in the prospectively defined anatomic sites containing representative numbers of lesions; OR
- A total of 5 new lesions in anatomic sites which were previously documented as having no evidence of cutaneous disease on the whole body diagram; OR
- $\geq 25\%$  increase in the number of raised lesions (minimum of 5 raised lesions if there are very few raised lesions, for example  $<8$ ) whichever is greater. Photographic documentation of “gross” or significant progression, particularly in areas that were not being followed, will be of particular value.

In order to classify a response as PR, the subject must have at least a PR in either the cutaneous or noncutaneous sites of disease and no evidence of progression as defined in the above criteria. In order to classify a response as a CR, the subject must have a CR in both the cutaneous (if applicable) and noncutaneous (if applicable) sites of disease and no evidence of progression as defined by the above criteria.

#### Noncutaneous Progression

PD includes new visceral sites of involvement or progression of visceral disease or the development of new or increasing tumor-associated edema or effusion lasting at least 1 week, which interferes with the subject’s normal activities. Progressive visceral disease, for measurable and evaluable disease, should be analogous to non-KS response criteria.

- 8.1.5 Recurrent disease is defined as the appearance of tumor following documentation of a complete remission.
- 8.1.6 Time to response is defined as time from the first dose of chemotherapy until documentation of first response.
- 8.1.7 Time to progression is defined as time from initiation of chemotherapy to documentation of first progression.
- 8.1.8 Response duration is defined as the time from first documentation of response to documentation of first progression.

## **9.0 RECORDS TO BE KEPT**

CRFs will be provided for each subject via the AMC AdvantageEDC<sup>SM</sup> Internet Data Entry System upon enrollment. Subjects must not be identified by name on any study documents. Data will be recorded on the CRFs using the unique subject identification number assigned at registration. Sample CRFs will be available on the AMC Operations Center website.

## **10.0 ROLE OF DATA MANAGEMENT**

### **10.1 CRF Instructions**

Instructions concerning the recording of study data on CRFs will be provided by the AMC Operations Center.

### **10.2 Data Quality**

It is the responsibility of the AMC Operations Center to assure the quality of data for the study. This role extends from protocol development to generation of the final study database.

## 11.0 STATISTICAL CONSIDERATIONS

### 11.1 Design and Sample Size

In Stage 1, three to six subjects were to be treated at each proposed dose levels to evaluate the MTD. In Stage 3, three to six initial subjects are to be treated at each proposed dose level to evaluate the potential MTD. Using the proposed dose escalation scheme, the probability of escalating to the next dose level, based on the true rate of DLT at the current dose, is given below:

**Table 6. Probability of Dose Escalation within Stage (Stage 1 & Stage 3)**

True Incidence of DLT	Probability of Escalating
10%	0.91
20%	0.71
30%	0.49
40%	0.31
50%	0.17
60%	0.08

Abbreviations: DLT = dose-limiting toxicity

Thus, if the true underlying proportion of DLTs is 20% at the current dose level, there is a high probability (0.71) of dose escalation to the next dose level. Conversely, if the true underlying proportion of DLTs is 50% at the current dose level, there is a low probability (0.17) of escalation to the next dose level.

In the Phase 2 portion of the study, Simon's 2-stage design is used to test the null hypothesis that the response rate (CR + PR) is  $\leq 10\%$  against the alternative that it is  $35\%$  at the one-sided 0.05 significance level with power of 0.90. Eleven subjects will be enrolled at the first dose level administered in Phase 2 (eg, 100 mg/dose BID) considering subjects from both the Phase 1 and Phase 2 portions of the study. If  $\leq$  one out of 11 subjects respond, the study will be stopped. If  $\geq$  two out of 11 subjects respond, the study will enroll 18-21 additional subjects in Stage 3. Assuming that the true response rate is  $\leq 10\%$ , the probability of stopping the trial at the end of Stage 2 is 0.697. However, if the true RR is  $\geq 35\%$ , then there is only a probability of 0.059 that the trial will be stopped erroneously in Stage 2.

Using the proposed dose escalation scheme from 100 mg/dose BID to 160 mg/dose TID, the probability of dose escalation based on the true rate of DLT at the lower dose, is given in Table 7 below:

**Table 7. Probability of Dose Escalation  
(100 mg/dose BID to 160 mg/dose TID)**

True Incidence of DLT	Probability of Escalating
10%	0.96
20%	0.82
30%	0.59
40%	0.35
50%	0.18
60%	0.08

Abbreviations: DLT = dose-limiting toxicity

Thus, if the true underlying proportion of DLTs is 20% at the 100 mg/dose BID dose level, there is a high probability (0.82) of dose escalation to the next dose level. Conversely, if the true underlying proportion of DLTs is 50% at the 100 mg/dose BID dose level, there is a low probability (0.18) of escalation to 160 mg/dose TID.

At the end of Stage 3, if  $\geq 6$  subjects respond, then the null hypothesis that the true response probability is  $\leq 10\%$  will be rejected, and further investigation of PTC299 in this patient population is warranted.

In Stage 3, a minimum of 12 patients will be treated at the maximally treated dose. If the underlying response rate is no greater than 10%, then the probability of observing 4 or more responses among 12 patients will be  $< 5\%$ . Thus, if there are 4 or more responses among 12 patients, it can be inferred that the response rate is  $> 10\%$ .

The minimum number of subjects to be entered in the study is **three** (i.e., if  $\geq$  two subjects experience DLT at the lowest dose level in Stage 1).

The maximum number of evaluable subjects to be enrolled in the study is **38** (i.e., 9 subjects have been enrolled into Stage 1, 8 subjects have been enrolled into Stage 2, and up to 21 subjects will be enrolled to Stage 3). Assuming that  $\sim 15\%$  of subjects will not be evaluable for response, we estimate that the maximum number of subjects in the study will be **44**.

## 11.2 Statistical Analysis Plan

Descriptive statistics will be used to summarize the demographic and baseline characteristics of the study population. Subjects who do not complete the required observations will be described and evaluated separately, as necessary. Reasons for study discontinuation and date of withdrawal from study will be described

The binomial proportion and its 95% confidence interval will be used to estimate the objective (complete + partial) response rate as defined in Section 8.0. With 12 patients at the maximally treated dose, the response rate can be estimated with a 95% confidence interval with width no greater than  $\pm 28.3\%$ .

The effects of PTC299 on serum and plasma VEGF, VEGFR and cytokine profiles will be evaluated by comparing the Day 28 value with the pre-treatment value, and the Day 28 value with the lowest value obtained subsequent to Day 28. These changes will be evaluated using the paired t-test, or if the data are not normally distributed, the Wilcoxon signed rank test. Comparisons between dose levels with respect to these changes will be evaluated using ANOVA or the Kruskal-Wallis test if the data are not normally distributed.

Comparisons of on-treatment values with the pre-treatment baseline value, and with the corresponding baseline value within each cycle will be performed using paired t-tests. If the distribution of the data deviates substantially from normality, the analysis will be performed on log-transformed data. Differences in change from baseline among dose levels will be analyzed by means of ANOVA (including a test for linear dose response); if a significant effect is observed, individual comparisons among dose levels will be performed with t-tests. The pattern of responses across doses will be considered in interpreting the logical validity of the results. Results will be presented in tabular and graphic form, as appropriate.

Immunohistochemistry (IHC) will be used to determine expression of the following: VEGF, VEGFR-2, VEGFR-3, phospho-Akt, KSHV LANA, orf59, p53 and HIF-1 $\alpha$ . We will count the number of positive cells per HPF for a total of 10 fields per section and derive an index value. We will consider a 50% change from baseline in the index value for the aforementioned markers a biological response. For each measure (VEGF, VEGFR-2, VEGFR-3, phospho-Akt, KSHV LANA, orf59, p53 and HIF-1 $\alpha$ ), we will estimate the proportion of patients who demonstrate a 50% change between baseline and on treatment (between day 22 and day 28) value. The exact binomial 95% confidence interval will be derived for each measure.

To evaluate the effects of PTC299 on tumor cell proliferation, the change in immunohistochemistry of Ki67 from the pre-treatment to the post-treatment biopsy will be tested using the Wilcoxon sign test to determine if the change is less than zero, suggesting a decrease. To evaluate the drug's effects on circulating concentrations of angiogenic factors and cytokines, HIV and KSHV loads, and T-lymphocyte subsets, changes in these levels from pre-treatment to indicated sampling times will be tested by repeated-measures ANOVA, single and multivariate analysis.

For each subject, study drug administration will be described in terms of the actual doses administered and the proportion of drug actually delivered relative to the amount that should have been delivered. The total number of cycles administered; the median (range) of cycles administered; dose modifications, dose delays, and dose omissions; and reasons for deviations from planned therapy will be described by starting dose level. The type and timing of use of concomitant drugs will also be described.

AEs and laboratory abnormalities will be summarized by type and severity grade. First-cycle DLTs, adverse events leading to death or to discontinuation from treatment, and serious adverse events will be considered with special attention.

Pharmacokinetic parameters will be calculated using the actual sample collection times. Pharmacokinetic variables will be calculated from the plasma concentration data using standard compartmental and noncompartmental methods, as appropriate for the data. Variables of interest may include  $T_{max}$ ,  $C_{max}$ ,  $C_{24h}$ ,  $AUC_{0-24}$ , and  $t_{1/2}$ . Plasma concentrations and PK parameter results will be presented in tabular and graphic form by dose level and

time period (Day 1 and Day 28). Modeling of the PK data may be performed using noncompartmental and compartmental models, as appropriate.

## **12.0 ETHICAL AND REGULATORY CONSIDERATIONS**

(See Model Informed Consent)

### **12.1 Informed Consent**

The principles of informed consent described in Food and Drug Administration (FDA) regulations (21 CFR Part 50) must be followed. IRB approval of the protocol and the informed consent form must be given in writing.

The AMC Operations Center must receive a copy of the letter of approval from the IRB, which specifically approves the protocol and informed consent, before subject enrollment. The IRB must also approve any significant changes to the protocol and documentation of this approval must be sent to the AMC Operations Center. Records of all study review and approval documents must be kept on file by the Investigator and are subject to FDA inspection during or after completion of the study. AEs must be reported to the IRB. The IRB should receive notification of completion of the study within 3 months of study completion and termination. The Investigator will maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

### **12.2 Changes to the Protocol**

Any change or addition to this protocol requires a written protocol amendment that must be approved by CTEP and the site's local IRB before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study, require additional approval by the IRB/IEC/REB.

### **12.3 Women and Minorities**

This study is being conducted by the NCI-sponsored AIDS Malignancy Clinical Trials Consortium (AMC). As part of their contractual obligations, each participating site within the AMC and the AMC as a whole is required to assure that the participation of women and minority subjects reflects the percentage representation of these populations in their geographic region and, for the AMC, the United States as a whole. As such, it is expected that the representation of subjects on this trial will reflect the constitution of the respective populations.

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### APPENDIX I: SCHEDULE OF EVALUATIONS

	Eligibility	Screening/ Baseline	Day 1/ (Cycle 1)	Day 15 (Cycle 1)	Day 28 (Cycle 1)	Day 29 (Day 1 of Cycle 2)	Day 57 (Day 1 of Cycle 3)	Day 85 (Day 1 of Cycle 4)	Day 113 (Day 1 of Cycle 5)	Every 28 Days Thereafter	Treatment Discont.	Post- Treatment/ Early Discont. of Therapy
Informed Consent	X											
HIV Documentation	X <sup>1</sup>											
KS Biopsy (Diagnostic)	X <sup>1</sup>											
KS Biopsy (non-indicator KS lesion; IHC)		X			X <sup>9</sup>							
KS Biopsy (non-indicator KS lesion; viral gene expression & cellular gene transcription)		X			X <sup>9</sup>							
Skin Biopsy (morphologically normal skin)		X										
Complete Medical History		X <sup>4</sup>										
Complete Review of Signs and Symptoms		X <sup>4</sup>		X <sup>7</sup>		X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>		
Complete Physical Exam (vital signs [temperature, pulse, BP, respiratory rate] height, weight)		X <sup>4</sup>		X <sup>7</sup>		X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X	X <sup>12</sup>
KS Tumor Assessment	X <sup>6</sup>					X	X	X	X	X	X	X <sup>12</sup>
Karnofsky Performance Status	X <sup>4</sup>			X <sup>7</sup>		X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X	X <sup>12</sup>
Concomitant Medication Review		X <sup>4</sup>		X <sup>7</sup>		X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>		
Review of current and prior anti-HIV therapy, prior immune based therapy, and prior anti-tumor therapy		X <sup>4</sup>		X <sup>7</sup>		X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>		
KS Staging		X										
Toxicity Evaluation				X <sup>7</sup>		X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X	X <sup>12</sup>
CBC with Differential and Platelets		X <sup>4</sup>		X <sup>7</sup>		X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X	
INR and aPTT	X <sup>4</sup>	X <sup>4</sup>		X <sup>7</sup>		X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>		

**APPENDIX I: SCHEDULE OF EVALUATIONS – cont'd**

	Eligibility	Screening/ Baseline	Day 1/ (Cycle 1)	Day 15 (Cycle 1)	Day 28 (Cycle 1)	Day 29 (Day 1 of Cycle 2)	Day 57 (Day 1 of Cycle 3)	Day 85 (Day 1 of Cycle 4)	Day 113 (Day 1 of Cycle 5)	Every 28 Days Thereafter	Treatment Discont.	Post- Treatment/ Early Discont. of Therapy
Serum chemistries: liver enzymes (AST, ALT, alkaline phosphatase), BUN, creatinine, electrolytes (sodium, potassium, chloride, bicarbonate), calcium, phosphorus, total protein, glucose, albumin, bilirubin (direct and indirect), fasting triglycerides and total cholesterol	X <sup>4</sup>	X <sup>4</sup>		X <sup>7</sup>		X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X	
Urinalysis	X <sup>4</sup>	X <sup>4</sup>		X <sup>7</sup>		X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>		
Serum HCG Pregnancy Test	X <sup>4</sup>	X <sup>5</sup>										
CD4 count		X <sup>4</sup>				X <sup>8</sup>			X <sup>8</sup>		X	
HIV-1 plasma RNA		X <sup>4</sup>				X <sup>8</sup>			X <sup>8</sup>		X	
KSHV viral load		X				X <sup>8</sup>			X <sup>8</sup>		X	
ACTH and cortisol		X				X	X	X	X	X	X	
PK Sampling			X <sup>10</sup>	X <sup>11</sup>	X <sup>10</sup>		X <sup>11</sup>					
Cytokine Assays			X <sup>13</sup>	X <sup>7</sup>		X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X <sup>7</sup>	X	X <sup>12</sup>
ACSR Donation (Baseline or Day 1 – optional, if patient consents)		X <sup>2</sup>										
Chest X-ray		X <sup>2</sup>										
CT Scan		X <sup>3</sup>										
Electrocardiogram		X <sup>4</sup>										

<sup>1</sup> To be done at any time prior to study entry.

<sup>2</sup> To be done within 28 days prior to study entry.

<sup>3</sup> Subjects with a positive chest X-ray or symptoms suggestive of pulmonary disease will have a chest CT performed at study entry.

<sup>4</sup> To be done within 21 days of study entry.

<sup>5</sup> For women of child bearing potential, serum beta human chorionic gonadotropin ( $\beta$ -HCG) pregnancy test to be completed within 72 hours of initiating study treatment and at any time pregnancy is suspected.

## APPENDIX I: SCHEDULE OF EVALUATIONS – *cont'd*

- <sup>6</sup> KS tumor assessments may be performed on Day 1 prior to receiving study treatment but may be performed no earlier than 14 days before initiating study treatment. Marker lesions and body areas must be photographed at baseline, then at each assessment when the response category changes.
- <sup>7</sup> To occur at Day 15 ( $\pm 2$  days) of Cycle 1, on Day 29 (Day 1 of Cycle 2) and every cycle (28 days) thereafter.
- <sup>8</sup> To occur at Day 29 (Day 1 of Cycle 2) and every three cycles thereafter.
- <sup>9</sup> To occur between Day 22 and 28 of Cycle 1. (Biopsy can occur as late as Day 29, but will need to be performed PRIOR to the first dose of study treatment in Cycle 2.)
- <sup>10</sup> To be collected immediately pre-dose; and at 1, 2, 3, 4, 5, 6 and 8 hours after the AM dose of study treatment on Day 1 of Cycle 1 and again at 1, 2, 3, 4, 5, 6 and 8 hours after the AM dose of study treatment on Day 28 of Cycle 1. **Note:** Day 28/Cycle 1 PK sampling may occur up to 3 days prior to Day 28 for scheduling purposes.
- <sup>11</sup> To be collected prior to the AM dose of study treatment on Day 15 ( $\pm 1$  day) of Cycle 1 and prior to the AM dose on Day 28 of Cycle 2 (or Day 1 of Cycle 3 if the subject's last dose of study treatment was the evening prior).
- <sup>12</sup> To be completed 30 days after discontinuation of study therapy.
- <sup>13</sup> To be collected pre-dose of study treatment on Day 1 of Cycle 1

**APPENDIX II: KARNOFSKY PERFORMANCE SCALE**

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

### APPENDIX III: KS STAGING CRITERIA

	<b>GOOD RISK (0) (All of the following)</b>	<b>POOR RISK (1) (Any of the following)</b>
Tumor (T)	- Confined to skin and/or lymph nodes and/or minimal oral disease <sup>1</sup>	- Tumor-associated edema or ulceration - Extensive oral KS - Gastrointestinal KS - KS in other nonnodal viscera
Immune system (I)	- CD4 cells $\geq$ 200/ $\mu$ L	- CD4 cells $<$ 200/ $\mu$ L
Systemic illness (S)	- No history of OI or thrush - No "B" symptoms <sup>2</sup> - Performance status $\geq$ 70 (Karnofsky)	- History of OI and/or thrush - "B" symptoms present - Performance status $<$ 70 - Other HIV-related illness (e.g., neurological disease, lymphoma)

T<sub>0</sub> =tumor confined to skin, lymph nodes and/or minimal oral disease.

T<sub>1</sub> =any tumor falling under the "Poor Risk" criteria.

S<sub>0</sub> =no history of OI or thrush, no "B" symptoms, and Karnofsky Performance status  $\geq$  70.

S<sub>1</sub> =any "Poor Risk" systemic illness signs and symptoms.

Note: Staging criteria taken from: Krown SE, Metroka C, Wernz JC. Kaposi's sarcoma in the acquired immunodeficiency syndrome: A proposal for uniform evaluation, response, and staging criteria. *J Clin Oncol* 1989;7:1201-1207. These criteria were adopted by the Oncology Committee.

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<sup>1</sup> Minimal oral disease is nonnodular KS confined to the palate.

<sup>2</sup> "B" symptoms are unexplained fever, night sweats,  $>$  10% involuntary weight loss, or diarrhea persisting more than 2 weeks.

## **APPENDIX IV: SAMPLE COLLECTION AND SHIPPING INSTRUCTIONS FOR AMC #059 STUDY (CIRCULATING ANGIOGENIC FACTORS/CYTOKINES)**

### **Collection Materials**

Upon site registration, PTC Therapeutics will ship sample collection materials to site personnel based on the contact information that the site provides in the site registration packet.

During the study, additional materials may be requested from Leslie Callahan at PTC Therapeutics.

Telephone: (908) 912-9422

Email: [lcallahan@ptcbio.com](mailto:lcallahan@ptcbio.com))

### **Collection Requirements for Serum**

#### Materials (Per Patient)

- 1 x 6.0 mL serum separator tube SST; (Red-Gray/Tiger top) blood collection tube (minimum blood volume 4 mL)
- 2 x 1.5 mL polypropylene cryovials
- 2 x transfer pipettes, non-sterile

#### Procedure: Serum

1. Use one 6.0 mL SST tube and allow samples to clot for 30 minutes at room temperature.
2. Centrifuge for 15 minutes at approximately 1000 x g.
3. Remove serum fraction using a transfer pipette and aliquot at least 1.5 mL of the serum fraction into 1 cryovial for the first shipment and store samples at -70°C for up to 3 months.
4. Ship cryovial overnight, on dry ice, to Quest Diagnostics. Additional frozen sample(s) should be held frozen at the site until notification that the samples are to be sent to Quest.

Note: at least 1.5 mL of the serum fraction should be placed in 1 cryovial; all other serum fractions should be placed in additional cryovials. Additional cryovial sample(s) should be held at site until notification to ship in the event that the assay needs to be repeated.

### **Collection Requirements for EDTA Plasma**

#### Materials (Per Patient)

- 1 x 6.0 mL EDTA (Lavender top) blood collection tube (minimum blood volume 6 mL)
- 3 x 1.5 mL polypropylene cryovials
- 2 x transfer pipettes, non-sterile

#### Procedure: EDTA Plasma

1. Collect plasma using 6 mL EDTA as an anticoagulant.
2. Centrifuge at 1000 x g for 15 minutes within 30 minutes of collection.
3. Remove plasma using a transfer pipette and aliquot at least 1.5 mL of the plasma fraction into 1 cryovial for the first shipment and store samples at -70°C for up to 3 months.

4. Ship cryovial via overnight courier, on dry ice, to PTC Therapeutics. Additional frozen sample(s) should be **held frozen at the site** until notification that the samples are to be sent to PTC Therapeutics.

Note: at least 1.5 mL of the plasma fraction should be placed in 1 cryovial all other plasma fractions should be placed in additional cryovials. Additional cryovial sample(s), should be held at site until notification to ship in the event that the assay needs to be repeated.

### **Shipping Procedure**

Prior to shipping, the samples will be packed in thermal insulated containers with sufficient dry ice to ensure they remain frozen and are protected from breakage during shipment. Samples will be shipped via priority courier to PTC Therapeutics Inc. Containers are only to be shipped on Monday, Tuesday, or Wednesday to avoid weekend delays in receipt that may subject the samples to thawing. The site will be notified when to send the second shipment.

The shipping address is:

**PTC Therapeutics Inc**  
**Attn: Liangxian Cao**  
**100 Corporate Court**  
**South Plainfield, NJ 07080**  
**Telephone: (908)222-7000**  
**Fax: (908)222-0567**  
**Email: lcao@ptcbio.com**

### **Courier Information**

Sites may use either World Courier or Fed Ex for sample shipping. To request shipment supplies or a sample pick-up: sites need to call their preferred shipper (World Courier or Fed Ex) provide the PTC Therapeutics account number (Fed-Ex 2275-2524-0, World Courier 10663) and study number (PTC299-ONC-005-KS). World Courier and Fed Ex will supply site(s) with shipping air bills, containers, and dry ice (if needed).

Sites should notify PTC Therapeutics via e-mail of the date of shipment and the courier tracking number. E-mail notification of the shipment is to be sent to: [jpichardo@ptcbio.com](mailto:jpichardo@ptcbio.com) and [lcallahan@ptcbio.com](mailto:lcallahan@ptcbio.com).

### **Record of Specimens**

This study will track specimens via GlobalTrace<sup>SM</sup>, a component of the AMC AdvantageEDC<sup>SM</sup> system. The GlobalTrace<sup>SM</sup> shipment manifest must accompany all specimen shipments.

## APPENDIX V: DETECTION OF VIRAL AND HOST RNA LEVELS IN KS TISSUES

### Description of the Assay

Changes in gene expression are a fundamental hallmark of cancer progression and invaluable tool of cancer staging. In case of KS, KSHV/HHV-8 has been identified as the etiological agent and this assay is designed to identify KSHV genes that might change in response to therapy. We will use reverse-transcription (RT) coupled to amplification using polymerase chain reaction (PCR) to measure the RNA levels of all KSHV/HHV-8 mRNAs in tumor biopsies. Secondly, we will use a custom-made array for cellular targets, which we presume to be regulated by VEGF, hypoxia or both. This assay is a research test only and will not be used to make clinical decisions.

We will use a targeted array of 96 mRNAs that were previously shown to be involved in angiogenesis, endothelial cell remodeling or hypoxic responses. This array is based upon PAHS-024 (SuperArray, Inc.). It may be modified based upon literature review but most likely will include the following growth factors and receptors: ANGPT1, ANGPT2, ANPEP, ECGF1, EREG, FGF1, FGF2, FIGF, FLT1, JAG1, KDR, LAMA5, NRP1, NRP2, PGF, PLXDC1, STAB1, VEGFA, VEGFC. Adhesion molecules: ANGPTL3, BAI1, COL4A3, IL8, LAMA5, NRP1, NRP2, STAB1. Proteases, inhibitors and other matrix proteins: ANGPTL4, PECAM1, PF4, PROK2, SERPINF1, TNFAIP2. Transcription factors and others: HAND2, SPHK1. Cytokines and chemokines: CCL11, CCL2, CXCL1, CXCL10, CXCL3, CXCL5, CXCL6, CXCL9, IFNA1, IFNB1, IFNG, IL1B, IL6, MDK, TNF. Other growth factors and receptors: EDG1, EFNA1, EFNA3, EFNB2, EGF, EPHB4, FGFR3, HGF, IGF1, ITGB3, PDGFA, TEK, TGFA, TGFB1, TGFB2, TGFBRI. Adhesion molecules: CCL11, CCL2, CDH5, COL18A1, EDG1, ENG, ITGAV, ITGB3, THBS1, THBS2. Proteases, inhibitors and other matrix proteins: LECT1, LEP, MMP2, MMP9, PLAU, PLG, TIMP1, TIMP2, TIMP3. Transcription factors and others: AKT1, HIF1A, HPSE, ID1, ID3, NOTCH4, PTGS1.

Reverse-transcription (RT) coupled to amplification using polymerase chain reaction (PCR) is widely recognized as the most sensitive method to detect the presence of specific RNAs. We will use real-time quantitative RT-PCR. This assay measures the amount of PCR product based on hybridization to a sequence-specific dual-labeled fluorogenic oligonucleotide (Taqman™) or intercalation of a fluorescent dye. Fluorescence is recorded at each cycle. So-called Ct-values indicate the cycle at which the fluorescence crosses a particular threshold (3 times standard deviation (SD) of the non-template control (NTC)). Hence, Ct-values indicate the abundance of a given mRNA on a log scale. A low Ct value represents a highly abundant target mRNA (Heid, Genome Res. 6:968-94,1996).

Specifically, we will use biopsies of Kaposi's sarcoma preserved in RNAlater (Ambion) at baseline and at indicated intervals after treatment. Total RNA will be isolated using RNeasy (Qiagen, Crawfordsville, IN) according to the supplier's protocol and reverse-transcribed using Mo-MuLV reverse transcriptase and 120 pmol random hexanucleotide primers (Taqman™MRT, Applied Biosystems Inc., Foster City, CA). After incubation at 42°C for 35 min, the reaction is stopped by heating to 95°C for 5 min, the cDNA pool is diluted, and the resulting sample analyzed by real-time quantitative PCR using a dedicated Roche LC480 machine and universal cycle conditions. We will use commercial SYBR-green-based PCR (Roche Inc., Indianapolis, IN) as a uniform detection method.

Biopsies should be obtained by standard punch technique, using a THREE (3) mm punch. Tumor biopsies should be completely within the margins of the lesions. A second biopsy should be obtained at baseline only from adjacent normal skin.

Each specimen should then be immersed in 1.5 ml RNAlater (Ambion Inc. cat#AM7022) and stored at FOUR (4) °C until shipment and labeled as follows:

Samples must be labeled with the bar-coded labels provided. Each sample should be labeled with the following information:

- **Protocol #:** “AMC-059”
- **Nine (9) Digit Subject ID:** “XXX-XXX-XXX”
- **Date Sample Collection**
- **Specimen Type** “KS tumor” or “normal skin”
- **Specimen Purpose:** “RNA levels”

**Supplies:** AMC sites will be provided five pre-filled vials of RNAlater and supplies will be replenished upon request to the Dittmer Laboratory.

The samples should be shipped by overnight courier on wet ice within 2 weeks to:

**Dirk P. Dittmer, Ph.D.**

Department of Microbiology and Immunology  
University of North Carolina at Chapel Hill  
CB# 7290, 804 Mary Ellen Jones Bldg  
Chapel Hill, NC 27599-7290

**Phone: (919) 966-7960**

**Fax: (919) 962-8103**

**Email: [ddittmer@med.unc.edu](mailto:ddittmer@med.unc.edu)**

**NO SAMPLES WILL BE RECEIVED ON SATURDAYS.**

**Record of Specimens**

This study will track specimens via GlobalTrace<sup>SM</sup>, a component of the AMC AdvantageEDC<sup>SM</sup> system. The GlobalTrace<sup>SM</sup> shipment manifest must accompany all specimen shipments.

## APPENDIX VI: COLLECTION, PROCESSING, AND SHIPPING INSTRUCTIONS FOR KSHV VIRAL LOAD SPECIMENS

### A. DESCRIPTION OF ASSAY

This assay is used to quantify the burden of KSHV (Human Herpes virus Type 8, or Kaposi's sarcoma associated virus) present in circulating peripheral blood mononuclear cells (PBMC). Cells are separated from whole blood by Ficoll centrifugation, DNA extracted from purified PBMC by column extraction, and the number of KSHV copies determined using a quantitative competitive DNA PCR assay. Results are expressed in numbers of copies per million PBMC. The limit of detection for this assay depends upon the DNA available, but generally  $\leq 50$  copies/ $10^6$  PBMC. Changes  $\geq 1.7$ -fold are unlikely to be due to assay variation, but KSHV burden does not appear to be as constant as HIV viral load. While some preliminary data indicates that untreated individuals with an average KSHV burden of  $\geq 100$  copies/ $10^6$  cells may be at greater risk of progression, this assay is a research test only, and should not be used to make clinical decisions. The purpose of including this assay as part of this trial is to determine its usefulness as a prognostic marker.

### B. GENERAL

To ship KSHV specimens, use a diagnostic shipper approved for a volume of at least 30 cc. AMC recommends the use of the SAF-T-PAK STP 210 diagnostic cardboard shipper. These shippers may be ordered at the SAF-T-PAK website [www.saftpak.com](http://www.saftpak.com). The following instructions below are for use with the recommended STP-210 shipper. If using another federally approved diagnostic shipper, please follow instructions provided for that specific shipper.

Note: Specimens **MUST BE SHIPPED Mondays through Thursdays as PRIORITY OVERNIGHT**. Specimens are **NOT ACCEPTED ON SATURDAYS** in the **Ambinder Lab**.

### C. SPECIMEN PREPARATION

- a. Draw three 10 cc (mL) green top (heparin) tubes from study patient.
- b. With a black, water resistant, sharpie pen, label each specimen label with the following information:
  - Protocol #: AMC-059
  - 9 digit Patient Study ID #
  - Patient initials
  - Date and time of collection
  - Specimen type- "Whole Blood"
  - Specimen purpose: "KSHV"
- c. Seal the tops of the three 10 cc heparin green tops with parafilm

### D. PACKAGING and FED-EX FORMS

- a. Place the three sealed tubes into bubble wrap (provided in STP-210 kit).
- b. Tape around the bubble wrap so that the roll stays together and the tubes cannot fall out or break.
- c. Place absorbent material sheet around the bubble wrapped tubes and slip into a biohazard poly-bag and "self-seal".

- d. Place poly-bag containing tubes into the white TYVEK bag and seal.
- e. Place the TYVEK bag into the STP-210 diagnostic cardboard shipper. Seal the cardboard shipper with clear packing/shipping tape.
- f. Affix the FED-EX airbill on blank side of the shipper making sure that it is marked “FED-EX PRIORITY OVERNIGHT”.
- g. Mark “OTHER” in the airbill under “Packaging”.
- h. Under airbill section “Special Handling” indicate “YES-SHIPPERS DECLARATION NOT REQUIRED”.
- i. Enter FED-EX account # [REDACTED].
- j. Place “From/To” information onto areas provided on the shipper.

Shipping Address is:

**Ambinder Lab**  
**Johns Hopkins Oncology**  
**1650 Orleans Street, CRB1-384**  
**Baltimore, MD 21287**  
**TEL: (410) 955-8721**  
**FAX: (443) 287-3217**

Make certain that shipper is already either pre-labeled with ‘UN#3373’ stamp, or make a paper label with ‘UN#3373’ and affix it to the shipper. Make certain that the net volume of the specimen being shipped is written in the space provided on the shipper or make a separate label with the volume in ml (so three 10 cc tubes is 30 mL) and affix to the shipper. Affix airbill to shipper so that the ‘UN’ and ‘VOLUME’ labels are visible. **RETAIN THE TOP COPY OF THE AIRWAY BILL FOR YOUR RECORDS.** Place the box in the FedEx pickup area at your site or call to request a package pickup. **Please Note:** The shippers will be mailed back to each AMC site.

#### **E. RECORD OF SPECIMENS**

This study will track specimens via GlobalTrace<sup>SM</sup>, a component of the AMC AdvantageEDC<sup>SM</sup> system. The GlobalTrace<sup>SM</sup> shipment manifest must accompany all specimen shipments.

## **APPENDIX VII: AIDS AND CANCER SPECIMEN RESOURCE (ACSR) – SPECIMEN PREPARATION AND SHIPPING INSTRUCTIONS**

### **A. BLOOD COLLECTION**

Consent subject for ACSR donation. Collect 20 cc of whole blood in ACD tubes.

### **B. SHIPPING**

To ship bloods, place the tubes into a canister of a STP-100 SAF-T-PAK shipper wrapping each tube in bubble wrap and using the absorbent paper at the bottom of the canister. Each sample tube should be labeled using a sharpie pen (permanent marker) with the following information:

- AMC Protocol #059
- AMC Subject ID#
- Date and time of collection
- Specimen type, i.e., WB=Whole Blood, P=Plasma, S=Serum
- Specimen purpose: Repository

Place the lid on the canister and place it inside of the ambient SAF-T-PAK shipper. Fold and pack ACSR form inside shipping box. Seal the ambient shipper with cellophane shipping tape. Label the ambient shipper with the "UN 3373" diamond shaped label. On one side, in black marker write, your name or name of responsible person, date of collection and phone number of the person responsible for the package.

#### **Specimen Shipment**

Specimens are accepted MONDAY through WEDNESDAY. Specimens are not accepted on Friday. Please use the AMC Operations FedEx account number to ship samples: # [REDACTED].

**Blood specimens** should be shipped by overnight express at room temperature to:

Ambinder Lab  
Attention: ACSR Blood Receiving Lab  
Johns Hopkins Oncology  
1650 Orleans Street, CRB1-384  
Baltimore, MD 21287  
Phone: (410) 955-8721  
Fax: (443) 287-3217

### **C. TISSUE/BIOPSY COLLECTION**

Tissue specimens are to be snap frozen DO NOT COAT IN OCT. Tissue specimens for donation may be batched for shipping after storage in -80°C freezer.

With a black, water resistant, sharpie pen, label each specimen with the following information:

- AMC Protocol #059
- AMC Subject ID#
- Date and time of collection
- Specimen type: Tissue
- Specimen purpose: Repository

### **Specimen Shipment**

Allowable shipment days are Monday through Wednesday. Shipping frozen tissue requires the use of packaging acceptable for dry ice and Class 9 label with weight of dry ice written on package. Please use the AMC Operations FedEx account number to ship samples: # [REDACTED]. Tissue specimens should be shipped by overnight express to:

Dr. Sylvia Silver/George Washington University Medical Center  
 2300 I Street, NW, Room 507  
 Washington, DC 20037  
 Phone: (202) 994-1444  
 Fax: (202) 994-5056

## **D. INSTRUCTIONS FOR SPECIMENS COLLECTED ON FRIDAY**

### **Preparation of plasma and mononuclear cells**

It is preferable that separation occurs as soon as possible. If necessary, whole blood in acid citrate dextrose (yellow top tube) can be held at room temperature for no more than 24 hours.

#### Materials:

- **Lymphocyte Separation Medium (LSM Solution, Ficoll-Hypaque - sterile)**
- 15 ml conical centrifuge tubes (sterile)
- PBS (sterile)
- 1, 5 mL and 10 ml serologic pipettes (sterile)
- NUNC tubes
- Alcohol-saturated, control rate freezer container
- DMSO freezing media:
- **50% Cryoprotective Medium, Cambrex (catalog no.:12-132A)**
- 50% Heat Inactivated Fetal Bovine Serum

#### 1. Preparation of Plasma Samples

- a. The 7 mL tube of whole blood in acid citrate dextrose should be rotated gently 2 or 3 times before being centrifuged. Do not transfer before centrifugation.
- b. The cells are separated by centrifugation at 500 g for 10 minutes.
- c. 0.5 mL aliquots of plasma are removed and put into 1.5 mL screw top tubes and transferred to liquid nitrogen storage.

#### 2. Peripheral Blood Mononuclear Cell Separation and Freezing

- a. The cells and plasma remaining from the previous step are transferred into a 15 mL conical tube, capped and re-suspended by gently tapping the bottom of the tube.
- b. Sterile PBS should be added to the suspended cells until the final volume is 8 mL; invert to mix.

- c. The 8 mL whole blood-PBS mixture should be carefully overlaid onto 4 mL of room temperature LSM or Ficoll-Hypaque solution in a sterile 15 mL conical tube. A sharp interface should exist between the LSM and the whole blood mixture. (If the layer of LSM gets mixed with the blood-PBS, the tube should be gently rotated to mix the blood, PBS, and LSM, and transfer to a 50 mL sterile conical tube. An equal volume of PBS is added, and the cells are separated at 600 g for 15 minutes. After removal of LSM-PBS supernatant, return to Step b).
- d. The 15 mL conical tube for 30 minutes at 900 g at room temperature. The mononuclear leukocytes (principally lymphocytes and monocytes) will band at plasma/LSM interface.
- e. The fluffy white layer just below the plasma layer should be aspirated off, along with approximately half of the LSM layer under it, and transferred to an appropriately labeled 15 mL sterile conical centrifuge tube. Mix by gentle rotation.
- f. Washed twice in sterile PBS - centrifuge at 500 g for 10 minutes.
- g. Cell pellet should be mixed well with a gentle finger-tapping action.
- h. Using a 1 mL pipette, the \*DMSO freezing mixture should be added drop wise to the cell pellet suspension. Gently finger-tap between drops. If the cell pellet is small, only 1 0.5 mL of freezing media is added (and only one aliquot of cells is frozen). If the cell pellet is large, up to 4 2 mL of freezing media can be added in a drop wise fashion. (Cell densities of 1 - 10 million PBMC/mL are best for cryopreservation. If a hemocytometer is available, the optimal concentration is 5 million PBMC/mL).

**IMPORTANT-Do not put the DMSO containing media on the cell button all at once.**

3. Freeze the cell suspension in 0.5 mL aliquots in sterile NUNC vials by placing the NUNC tubes in a room temperature, alcohol saturated, control rate freezer container and store in the -80°C freezer overnight. Transfer the cell suspension into the liquid nitrogen temperature freezer for long-term storage the next working day.

**\*\*PLEASE DOUBLE CHECK PACKAGING OF SHIPPER AND DO NOT DEVIATE FROM REQUESTED LABELING\*\*** Shipping frozen aliquots requires the use of packaging acceptable for dry ice and Class 9 label with weight of dry ice written on package.

**Please Note:** The shipper will be mailed back to the AMC site.

The STP-100 SAF-T-PAK shipper is a complete kit with all trappings, bubble wrap, absorbent paper, labels, everything (but to reuse the shipper, you will need new labels, wrap, etc).

## **E. Record of Specimens**

This study will track specimens via GlobalTrace<sup>SM</sup>, a component of the AMC AdvantageEDC<sup>SM</sup> system. The GlobalTrace<sup>SM</sup> shipment manifest must accompany all specimen shipments.

**APPENDIX VIII: INFORMED CONSENT FORM**  
**RESEARCH STUDY AIDS AND CANCER SPECIMEN RESOURCE (ACSR)**

**A. INTRODUCTION**

You are being asked to donate tissue for research. Before you decide to be a part of this research study, you need to understand the risks and benefits so that you can make an informed decision. This is known as informed consent.

This consent form provides information about the research study, which has been explained, to you. Once you understand the study and the tests it requires, you will be asked to sign this form if you want to take part in the study. Your decision to take part in the study is voluntary. This means that you are free to choose if you will take part in the study.

**B. PURPOSE**

The National Cancer Institute has set up a Bank for tissues and biological fluids from HIV-positive and HIV-negative individuals in order to have specimens available for scientists studying malignancies associated with HIV disease. Individuals who have had biopsies to determine a malignancy are being asked for permission to store some of the tissue in the Bank. Only tissue in excess of that required for decision making will be given to the Bank. If it turns out that your physician needs more of your tissue for additional studies, the Bank will release all of your tissue back to your doctor. No additional tissues will be taken from your body for the Bank.

In addition, you are requested to donate some of your blood to the Bank so that scientists will also be able to look for any deviation in these body fluids that may explain the malignancy.

**C. PROCEDURES**

You are being asked for consent to place some of the biopsy material in the ACSR. If you agree to allow the ACSR to have some of your tissue, we would also like to:

1. Confidentially obtain some clinical information from your medical records that could be useful to research investigators. The report of the information retrieved from your medical record that is given to research investigators will not have your name, or include any information which could personally identify you.
2. Obtain some blood for the Bank. Up to twenty (20) milliliters of blood will be obtained at your next visit to your physician.

If during the course of treatment by your physician, it is necessary to perform any of the following procedures for diagnostic reasons, you will be asked, at that time, to consent to having a portion of that specimen sent to the Bank. These requests will not require you to make any additional visits to your doctor or have any additional specimens taken just for the Bank. The Bank will only receive part of your specimen, and only what is in excess. No additional materials will be removed for the purposes of the Bank alone. Samples of interest would include, (but are not limited to):

1. Spinal fluid.
2. Airway washes.
3. Fluid around lungs and intestines.

4. Additional biopsy material.

You will not be asked to fill out any forms for any of these specimens.

#### **D. POSSIBLE RISKS**

There is a possibility of a bruise and slight pain at the time the blood samples are taken. There is also the possibility of fainting and infection at the site of the blood draw.

#### **E. POSSIBLE BENEFITS**

It may be that there will be no direct benefit to you by consenting to allow the Bank to have portions of your biopsies and biological fluids. However, there may be possible benefits to medical knowledge and HIV-infected individuals in the future.

#### **F. COSTS**

There will not be any additional costs to you for consenting to participate in the AIDS and Cancer Specimen Resource.

#### **G. PAYMENT FOR INJURY OR HARM**

As the lists of risks shows, taking part in this research study may result in injury or harm to you. In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge. No funds have been set aside to compensate you in the event of injury. You or your insurance company will be charged for continuing medical care and/or hospitalization. (**Institution**) will not pay for the care. Likewise, (**Institution**) will not pay you for pain, worry, lost income, or non-medical care costs that might occur from taking part in this research study.

#### **H. PRIVACY**

My hospital medical records will be confidentially reviewed to obtain clinical information that could be useful to research investigators. However, the report of this information will not have my name or social security number anywhere on the report, so I will not be easily identified. The results of this research study will be given to the sponsor, the National Cancer Institute (NCI), AIDS Malignancy Consortium, and may be asked for by the Department of Health and Human Services. In addition, the Institutional Review Board may see my records. Except for these people, records from this study will be kept private unless I authorize their release or release is required by law (i.e. court subpoena). Any publications of this study will not use my name, identify me personally, or include any information that could personally identify me.

#### **I. QUESTIONS**

If you have any questions about this research study, you should contact Dr. (\_\_\_\_\_) at (**Phone Number**) (day) or (**Phone Number**) (night), or the person in charge of the study, (\_\_\_\_\_), the study coordinator, at (**Phone Number**). If you have any questions about your rights as a research subject, you should call (**IRB Representative**), in (**Institution**) Office of Human

Research at (\_\_\_\_\_). (**IRB Representative**) is your representative and is not employed by the individuals conducting the study.

**J. SIGNATURES**

➤ **Statement of professional obtaining consent**

I have fully explained this research study to the subject or guardian of subject. In my judgment and the subject's or guardian's, there was sufficient access to information, including risks and benefits to make an informed decision.

Date: \_\_\_\_\_ Physician's Signature: \_\_\_\_\_

Physician's Name: \_\_\_\_\_  
(Print)

➤ **Patient's/subject (or guardian's) statement**

I have read the description of the clinical research study or have had it translated into a language I understand. I have also talked it over with the doctor to my satisfaction. I understand that my/the subject's participation is voluntary. I know enough about the purpose, methods, risks, and benefits of the research study to judge that I want (the patient/subject) to take part in it.

Date: \_\_\_\_\_ Patient/Subject Signature: \_\_\_\_\_

Patient's/Subject's Name: \_\_\_\_\_  
(Print)

\_\_\_\_\_  
**Patient**

\_\_\_\_\_  
**Date**

\_\_\_\_\_  
Person obtaining consent

\_\_\_\_\_  
Date

\_\_\_\_\_  
Witness

\_\_\_\_\_  
Date

## APPENDIX IX: PHARMACOKINETIC (PK) SAMPLING PROCEDURES

**Collection Kits:** Upon site registration, PTC Therapeutics will ship sample collection kits to site personnel based on the contact information that the site provides in the site registration packet. During the study, additional supplies may be requested from Leslie Callahan at PTC Therapeutics (Telephone: (908) 912-9422; Email: [lcallahan@ptcbio.com](mailto:lcallahan@ptcbio.com)).

Blood for PK assessments should be collected immediately pre-dose; and at 1, 2, 3, 4, 5, 6, and 8 hours after the AM dose on Day 1 and again at 1, 2, 3, 4, 5, 6, and 8 hours after the AM dose on Day 28 of cycle 1. A blood sample will also be collected prior to the AM dose on Day 15 ( $\pm 1$  day) during cycle 1 and prior to the AM dose on Day 28 of cycle 2 (or Day 1 of cycle 3 if the subject's last dose of study treatment was the evening prior). Blood samples for PTC299 PK assessments will not be collected after cycle 2. The actual sample collection times should be recorded.

**Schedule of PK Sample Collection**

Cycle	Day	Time Relative to AM Dose							
		Pre-dose	Post-dose (hours)						
			1	2	3	4	5	6	8
1	1	X	X	X	X	X	X	X	X
	15	X							
	28	X	X	X	X	X	X	X	X
2	28	X <sup>a</sup>							

<sup>a</sup> (or Day 1 of cycle 3 if the subject's last dose of study treatment was the evening prior)

### **Collection, Processing and Storage**

- 1) Each sample will comprise  $\leq 2$  mL of venous blood drawn into a 5-mL Vacutainer<sup>®</sup> tube with K<sub>2</sub>-EDTA as the anticoagulant. Immediately after collection, the tube should be gently inverted 8 to 10 times to mix the anticoagulant with the blood sample. The tube should be stored upright on ice until centrifugation; centrifugation and sample processing should be performed within 1 hour of sample collection.
- 2) The plasma fraction should be separated by placing the collection tube into a refrigerated centrifuge (4 to 8°C) in a horizontal rotor (with a swing-out head) for a minimum of 15 minutes at 1500 to 1800 relative centrifugal force (RCF).
- 3) The plasma fraction will be withdrawn by pipette and divided into 2 polypropylene freezing tubes (with each tube receiving approximately equal aliquots).
- 4) All sample collection and freezing tubes will be clearly labeled in a fashion that identifies the subject, the study period, and the collection date and time. Labels will be fixed to freezing tubes in a manner that will prevent the label from becoming detached after freezing.
- 5) After processing, samples should be placed into a freezer at approximately  $-70^{\circ}\text{C}$ .

With a black, water resistant, sharpie pen, label each specimen label with the following information:

- Protocol #: AMC 059
- 9 digit Study ID #

- Study Visit
- Specimen type: "Plasma"
- Specimen purpose: Enter the time point of the blood draw\*
- Date collected:
- Time collected:

The nominal time point of the blood draw should be entered in the specimen purpose field (e.g. pre-dose, 1 hour, 2 hour, etc.)

### **Record of Specimens**

This study will track specimens via GlobalTrace<sup>SM</sup>, a component of the AMC AdvantageEDC<sup>SM</sup> system. The GlobalTrace<sup>SM</sup> shipment manifest must accompany all specimen shipments.

## **Guidelines for Shipping Samples to Covance – Madison**

### **Regulatory Information**

Specific Federal and International Regulations define classes of “Hazardous Materials”<sup>1</sup> and “Dangerous Goods”<sup>2</sup>. Specimens transported to the Covance Madison - Bioanalytical Chemistry Department should be evaluated for their Hazardous Material Class and categorized, packaged, labeled, documented, and transported in accordance with the applicable regulations. Facilities shipping Hazardous Materials are required to maintain designated personnel trained in accordance with part 493 CFR-Subpart H within the last 36 months and International Air Transportation Association (IATA) regulations (if shipping by air) within the last 24 months. The IATA regulation manual also lists additional regulations imposed individually by a variety of Commercial Carriers and Airlines.

The information provided here are Covance Labs guidelines to assist in the proper and safe transport of samples for assay in this facility. They are not to be construed as a replacement or complete summary of applicable DOT (CFR) or IATA regulations.

### **Guidelines for Packaging Samples**

Please organize samples by subject where possible (i.e. box all of subject 001 together, with dividers all of subject 002 together with dividers, etc.)

1. Sample container caps should be securely fastened.
  - a. Samples should not be transported in glass vials. Samples should be transferred to plastic tubes for transport.  
(If glass must be used, containers must be immobilized. Note: the use of glass greatly increases the risk of breakage and sample loss).
  - b. Use labels that will not smear or fall off under cold or moist conditions.
2. Samples should be placed in a primary receptacle (insulated cooler), then into a secondary receptacle (sturdy cardboard box). These primary and secondary containers are available commercially as combination packaging. The package contents are placed in this order:
  - a. Wrap the samples in enough absorbent material to absorb at least three times the contents should leakage occur.
  - b. Place the wrapped samples in a plastic bag and seal (heat seal or zip lock)
  - c. Place the sealed bag in the bottom of the primary receptacle.
  - d. Add styrofoam peanuts or equivalent (barrier to dry ice and shock stabilizer)
  - e. Add a sheet of cardboard onto the top of the primary receptacle.

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1 Term used by Department of Transportation (DOT) in the Code of Federal Regulations (CFR)

2 Similar term used by the International Air Transportation Association (IATA), will use the term Hazardous Material in this document

3 Part 49 is “Hazardous Materials in Commerce” in the CFR

- f. If samples are to be frozen, adequate dry ice should be included in the container to last the duration of the journey. (48-72 hrs, at least 7 Kg or 15 pounds, approximately 4 pounds per day of transit).
  - g. If there is room remaining, add filler material to avoid content movement during transport.
  - h. The primary Styrofoam container should be taped shut and placed in the secondary cardboard container.
3. Include a copy of the GlobalTrace<sup>SM</sup> shipping manifest with the shipment. If possible please send this electronically and include all information that would be required in final data tables. **Please indicate sample storage conditions on the shipping manifest.**
  4. Seal the sample inventory form(s) as well as a copy of the GlobalTrace<sup>SM</sup> shipping manifest into a protected plastic bag. Place the plastic bag containing the sample inventory form(s) and the GlobalTrace<sup>SM</sup> shipping manifest on top of the secured styrofoam primary container lid so that it is immediately accessible upon opening the box.
  5. Tape the shipping box securely closed. Use tape that is resistant to moisture and cold.
  6. Place Biohazard warnings on outside of box (if applicable).
  7. Label the box exterior in accordance with the applicable DOT CFR / IATA Regulations.
  8. Complete an address label and attach it to the outside of the box.

\*\*\*\*\*

### Guidelines for Shipping Samples

Send samples to:

For Shipments:

**Covance Bioanalytical Laboratory Services Inc.  
3301 Kinsman Boulevard  
Madison, WI 53704-2523  
Attn: Sample Management – Bioanalytical (Rm131D 1S)**

1. Samples should be shipped at **least 2 days prior to a USA National Holiday.**
2. Frozen samples should not be shipped after Wednesday (**samples should arrive at Covance by Friday, not on Saturday or Sunday**)
3. Call the Covance Bioanalytical Services Sample Management department **on the day prior to shipment (1-888-541-7377 Ext 2540, Ext 2187, or Ext 2327)**, as notification of the intended shipment, **OR** e-mail [Madison.SA@Covance.com](mailto:Madison.SA@Covance.com) with shipment information (tracking numbers and number of boxes sent).
4. For samples shipped within the U.S.A, use World Courier account number **10663**. To request shipment supplies, sites are to call World Courier and provide the study number. World Courier will supply site(s) with shipping air bills, containers, and dry ice (if required).
5. Any questions regarding shipping instructions may be directed to the Sample Management Group at 1-888-541-7377 Ext 2540, Ext 2187, or Ext 2327, or via Fax 608-245-7082. International phone code is 001.

## APPENDIX X: AMC DATA AND SAFETY MONITORING PLAN

Version 4.0 • September 13, 2010

### Monitoring the Progress of Trials and the Safety of Participants

All AMC protocols that collect safety data follow the *National Cancer Institute (NCI), Cancer Therapy Evaluation Program (CTEP) Guidelines: Adverse Event Reporting Requirements* (<http://ctep.cancer.gov/guidelines/index.html>). All adverse events that meet the NCI's expedited reporting requirements are reported to the Investigational Drug Branch (IDB) of the NCI via the Adverse Event Expedited Reporting System (AdEERS) web application. All expedited adverse event reports are also required to be submitted to the local Institutional Review Board (IRB) of the reporting institution. If NCI holds the IND or no IND is required for a study, the AMC site reports serious adverse events directly to the AMC Operations and Data Management Center (ODMC) via AdEERS. In some instances, the AMC sites may report serious adverse events directly to a commercial sponsor holding the IND, who will then report the event to the AMC ODMC. Most AMC protocols require sites to report all serious adverse events via AdEERS and the AMC ODMC to forward a copy of the report to the sponsor. The AMC ODMC also distributes all IND safety reports to all investigators upon receipt, and makes these reports available on the password-protected section of the AMC Operations web site. Unless an AMC protocol specifies an alternate plan for the review and submission of serious adverse events, all serious adverse events received by the AMC ODMC will be reviewed by the AMC Medical Monitor at the AMC ODMC prior to submission to NCI and the sponsor. For protocols for which the IDB does not have an assigned drug monitor to review serious adverse event reports, in the event of disagreement between the reporting physician and the AMC Medical Monitor regarding the attribution of the event to the investigational agent(s) (i.e., determination of whether the relationship is unrelated, unlikely, possible, probable, or definite), the AMC Medical Monitor will provide the final determination of the relationship.

The AMC ODMC provides listings of all reported adverse events and serious adverse events to the Protocol Chair and Co-chair(s) for review on a regular basis. The AMC ODMC compiles these events in a tabular format and posts them on the password-protected section of the AMC web site where these reports are updated nightly. The AMC web site is accessible to all AMC investigators, co-investigators, and their staff. Email notification that this information is available on the web site will be sent to all site PIs. It is the responsibility of each site to provide this information to their respective IRBs, if required by their IRB. For blinded studies, the serious adverse events are reviewed and tabulated without treatment assignment. The AMC Medical Monitor will review listings of all reported adverse events on a quarterly basis for safety concerns.

Accrual summaries for each AMC trial are updated nightly on the password-protected section of the AMC web site. The progress of each AMC trial is reviewed regularly by the Protocol Chair and also by the appropriate disease-oriented Working Group during scheduled conference calls. For phase I dose escalation trials, dose escalation (or dose de-escalation) is based on the rules in the protocol and the Protocol Chair, AMC Medical Monitor, and Group Statistician determine whether these criteria have been met. For phase II trials, stopping the trial for toxicity or efficacy, or suspending enrollment pending observation of responses in a multi-stage phase II trial, is based on meeting criteria stated in the protocol, and the Protocol Chair, AMC Medical Monitor, and Group Statistician determine whether these criteria have been met.

For phase III trials, the AMC has formed an independent Data Safety and Monitoring Board (DSMB). Voting members of the DSMB are physicians, a statistician, and a patient advocate. All voting members are from outside the AMC. Nonvoting members are the AMC Group Statistician, the Statistician listed on the protocol, an AMC Operations Center staff member, two representatives (normally a clinician or

statistician) from the Office of HIV AIDS Malignancy (OHAM) or from the Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, of the National Cancer Institute (NCI). The AMC Data Safety and Monitoring Board reviews AMC phase III studies in accordance with the National Cancer Institute's Policy for Data Safety and Monitoring. Confidential reports of all phase III trials are prepared by the AMC Group Statistician with support from the AMC ODMC. A written report containing the current status of each trial monitored, and when appropriate, any toxicity and outcome data, are sent to DSMB members by the AMC ODMC within the timelines specified by the AMC DSMB Charter. This report addresses specific toxicity concerns as well as concerns about the conduct of the trial. The report may contain recommendations for consideration by the DSMB concerning whether to close the trial, report the results, or continue accrual or follow-up.

The results of each DSMB meeting are summarized in a formal report sent by the DSMB Chair to the Group Chair and AMC ODMC. The DSMB report contains recommendations on whether to close each study reviewed, whether to report the results, and whether to continue accrual or follow-up. A primary recommendation (e.g., continue with no change; recommended or required modification; stop) must be included in the document. The Group Chair is then responsible for notifying the Protocol Chair and relevant Disease-oriented Working Group Chair before the recommendations of the DSMB are carried out. In the unlikely event that the Protocol Chair does not concur with the DSMB, then the NCI Division Director or designee must be informed of the reason for the disagreement. The Study Chair, relevant Disease-oriented Working Group Chair, Group Chair, DSMB Chair, and NCI Division Director or designee will be responsible for reaching a mutually acceptable decision about the study. CTEP approval of a formal amendment will be required prior to any implementation of a change to the study.

Following a DSMB meeting, a summary of the serious adverse events reported to the DSMB is posted to the AMC web site. It is each site's responsibility for conveying this information to its IRB.

#### **Plans for Assuring Compliance with Requirements Regarding the Reporting of Adverse Events (AE)**

For trials monitored by the NCI's Clinical Data Update System (CDUS), adverse event information is transmitted electronically to NCI on a quarterly basis. For trials monitored by NCI's Clinical Trials Monitoring Service (CTMS), adverse event information is transmitted electronically to NCI every two weeks.

The Protocol Chair, AMC Group Chair, and the AMC ODMC share responsibility in assuring that participating investigators comply with the protocol requirements for adverse event reporting. All AMC investigators certify compliance with NCI and FDA requirements for adverse event reporting by signing the AMC Adherence Statement for site membership, the protocol signature page for each protocol active at the site, and Form FDA-1572 for CTEP investigator registration and IND studies sponsored by AMC investigators. Investigators are responsible for identifying and reporting all adverse events to the AMC ODMC, AdEERS, and/or sponsors according to the protocol requirements, and assuring compliance with reporting to the local IRB. Protocol compliance with adverse event reporting requirements is assessed by the AMC ODMC during routine site monitoring visits by reviewing the site's source documentation.

The data entry system used for AMC studies, AdvantageEDC<sup>SM</sup> (a web-based data entry and enrollment system), is programmed to notify the site investigator, protocol chair, and AMC ODMC via email in the event that a site reports an adverse event that meets expedited reporting criteria to NCI and/or FDA. If the site does not follow with an AdEERS report, the AMC ODMC contacts sites to request an expedited report. Additionally, the protocol chair, AMC ODMC, and the AMC Medical Monitor review reported adverse events on a routine basis to identify adverse events reported by sites that require expedited reporting via AdEERS. The Protocol Chair, AMC Group Chair, and IND sponsors have general

oversight for assuring that routine and expedited adverse reporting requirements are met by the responsible parties.

### **Plans for Assuring that any Action Resulting in a Temporary or Permanent Suspension of an NCI-Funded Clinical Trial is Reported to the NCI Grant Program Director Responsible for the Grant**

In the event that termination of the trial or major modification to the protocol is under consideration, the Protocol Chair will convene the AMC Data Coordinator and Disease-oriented Working Group Chair by conference call to discuss the options. For phase I and II trials, the Protocol Chair also has the option of asking the AMC DSMB to review the study. The AMC ODMC will inform the CTEP Protocol Information Office (PIO) when studies are temporarily or permanently closed. The Cancer Treatment and Evaluation Program (CTEP) of the National Cancer Institute (NCI) must approve all protocol amendments prior to distributing to the AMC sites.

### **Plans for Assuring Data Accuracy and Protocol Compliance**

All study data for AMC clinical trials are entered directly by AMC site staff into AdvantageEDC<sup>SM</sup>. During data entry, the system performs validation checks on many fields and performs consistency checks between select fields. Range checks are placed on each field to eliminate entry of out-of-range values. Edit check programs are run on the database on a set schedule to identify and resolve inconsistencies between forms or data collected at different points in time. AMC ODMC staff routinely interacts with site staff to resolve any data problems.

In accordance with NCI guidelines, the AMC ODMC conducts monitoring visits at the AMC sites to evaluate compliance with regulatory issues, and to review data for specific cases by checking source documents. These reports are sent to the site Principal Investigator and to the NCI. In the event that major violations are identified, sites are asked to provide a plan to correct deficiencies within 30 days. If needed, a repeat site visit is conducted. In the event that a site does not correct deficiencies in a pre-determined time frame, the AMC Executive Committee has the option of taking action against the site. Possible actions include, but are not limited to, suspending enrollment of new patients to AMC trials until deficiencies are corrected; recommending a decrease in funding to the site; and requiring specific training for site investigators or staff members.

**APPENDIX XI: PATIENT STUDY DRUG COMPLIANCE LOG**

<b>PROTOCOL NUMBER: AMC-059</b>	<b>Patient Study Drug Compliance Log Cycle __</b>
Site ID _____ Subject ID: <u>059</u> -____-____	Subject's Initials _____

<b>Planned Dose (number of pills to be taken per dose)</b>		
<b>Morning Dose</b>	<b>Afternoon Dose</b>	<b>Evening Dose</b>

**Patient Instructions:** Please fill in the calendar dates and enter the number of PTC299 pills actually taken at each dosing time on each day. If a particular dose is missed, please write '0' in the column of that dose. When writing the date, please record in the dd/mmm/yyyy format (e.g., 15/Nov/2007).

Day	Date (dd/mmm/yyyy)	Morning Dose	Afternoon Dose	Evening Dose
1	___/___/20__			
2	___/___/20__			
3	___/___/20__			
4	___/___/20__			
5	___/___/20__			
6	___/___/20__			
7	___/___/20__			
8	___/___/20__			

<b>PROTOCOL NUMBER: AMC-059</b>	<b>Patient Study Drug Compliance Log Cycle __</b>
Site ID _____ Subject ID: <u>059</u> -____-____	Subject's Initials _____

Day	Date (dd/mmm/yyyy)	Morning Dose	Afternoon Dose	Evening Dose
9	___ / ___ / 20__			
10	___ / ___ / 20__			
11	___ / ___ / 20__			
12	___ / ___ / 20__			
13	___ / ___ / 20__			
14	___ / ___ / 20__			
15	___ / ___ / 20__			
16	___ / ___ / 20__			
17	___ / ___ / 20__			
18	___ / ___ / 20__			
19	___ / ___ / 20__			
20	___ / ___ / 20__			

<b>PROTOCOL NUMBER: AMC-059</b>	<b>Patient Study Drug Compliance Log Cycle __</b>
Site ID _____ Subject ID: <u>059</u> -____-____	Subject's Initials _____

Day	Date (dd/mmm/yyyy)	Morning Dose	Afternoon Dose	Evening Dose
21	___ / ___ / 20__			
22	___ / ___ / 20__			
23	___ / ___ / 20__			
24	___ / ___ / 20__			
25	___ / ___ / 20__			
26	___ / ___ / 20__			
27	___ / ___ / 20__			
28	___ / ___ / 20__			

## APPENDIX XII: DEFINITION OF AIDS INDICATOR CONDITIONS

- Aspergillosis, invasive \*
- *Bartonella henselae* infection, disseminated (bacillary angiomatosis, peliosis hepatis)\*
- Candidiasis of bronchi, trachea, or lungs \*
- Candidiasis, esophageal \*
- Cervical cancer, invasive \*
- Coccidioidomycosis, disseminated or extrapulmonary \*
- Cryptococcosis, extrapulmonary \*
- Cryptosporidiosis, chronic intestinal (> 1 month's duration) \*
- Cytomegalovirus disease, invasive \*
- Cytomegalovirus retinitis \*
- Encephalopathy, HIV-related \*
- Herpes simplex: chronic ulcer(s) (> 1 month's duration), bronchitis, pneumonitis, or esophagitis \*
- Histoplasmosis, disseminated or extrapulmonary \*
- Isosporiasis, chronic intestinal (> 1 month's duration) \*
- Kaposi's sarcoma (progression to visceral disease)
- Lymphoma, Burkitt's (or equivalent term) \*
- Lymphoma, immunoblastic (or equivalent term) \*
- Lymphoma, primary, of brain \*
- Microsporidiosis, diarrhea > 1 month or disseminated \*
- *Mycobacterium avium* complex or *M. kansasii*, disseminated or extrapulmonary \*
- *Mycobacterium tuberculosis*, any site (pulmonary<sup>1</sup> or extrapulmonary) \*
- *Mycobacterium*, other species or unidentified species, disseminated or extrapulmonary \*
- Nocardiosis, pulmonary, brain or disseminated \*
- *Pneumocystis carinii* pneumonia (new or recurrent diagnosis)
- Progressive multifocal leukoencephalopathy \*
- *Salmonella* septicemia, recurrent \*
- Toxoplasmosis of brain \*
- Wasting syndrome due to HIV \*
- New Diagnosis \*

## APPENDIX XIII: DETECTION OF VIRAL AND HOST PROTEIN EXPRESSION IN KS TISSUES

### Description of the Assay

Changes in protein expression are a fundamental hallmark of cancer progression and an invaluable tool of cancer staging. In case of KS, KSHV/HHV-8 has been identified as the etiological agent and this assay is designed to identify KSHV proteins and cellular proteins that might change in response to therapy. The previous assay used PCR to look at changes in mRNA level. This assay uses histochemistry to look at changes in protein expression.

Biopsies will be fixed in formalin and studied by immunohistochemistry (IHC) for expression of the following: VEGF, VEGFR-2, VEGFR-3, phospho- Akt, KSHV LANA, orf59, p53 and HIF-1a. Tumor cell proliferation will be assessed by Ki-67 staining. Change in the intensity or distribution of a particular marker will be evaluated.

This assay is a research test only and will not be used to make clinical decisions.

Biopsies should be obtained by standard punch technique, using a THREE (3) mm punch from non-indicator KS lesions. Tumor biopsies should be completely within the margins of the lesions.

The first biopsy should be obtained at baseline (see section 4.2.2.1)

A second biopsy should be obtained between days 22 and 28 of cycle 1.

Each specimen should then be immersed in 1.5 ml formalin and stored at room temperature until shipment and labeled as follows:

Samples must be labeled with the bar-coded labels provided. Each sample should be labeled with the following information:

- **Protocol #:** “AMC-059”
- **Nine (9) Digit Subject ID:** “XXX-XXX-XXX”
- **Date of Sample Collection**
- **Specimen Type** “KS tumor” or “normal skin”
- **Specimen Purpose:** “IHC”

The samples should be shipped by overnight courier on wet ice within two (2) days to:

#### **Dirk P. Dittmer, Ph.D.**

Department of Microbiology and Immunology

University of North Carolina at Chapel Hill

CB# 7290, 804 Mary Ellen Jones Bldg

Chapel Hill, NC 27599-7290

Phone: (919) 966-7962

Fax: (919) 962-8103

Email: [ddittmer@med.unc.edu](mailto:ddittmer@med.unc.edu)

**NO SAMPLES WILL BE RECEIVED ON SATURDAYS.**

## **Record of Specimens**

This study will track specimens via GlobalTrace<sup>SM</sup>, a component of the AMC AdvantageEDC<sup>SM</sup> system. The GlobalTrace<sup>SM</sup> shipment manifest must accompany all specimen shipments.

## **APPENDIX XIV: PHOTOGRAPHIC DOCUMENTATION OF KS LESIONS**

In Phase II of this protocol, the collection of photographs of KS marker lesions will be required according to the guidelines described below.

### Photographic Guidelines

Photographs will be taken to assist in documentation of the diagnosis of KS and for clinical monitoring purposes. The difficulty in standardizing these photographs is acknowledged.

In all participants, photographs will be needed of the five marker lesions, defined at study enrollment and used for clinical assessment of response. The five marker lesions must be labeled in the photographs A – E, to correspond with the designations in the Physical Evaluation form in AdvantageEDC<sup>SM</sup>. The same lesions must be consistently labeled throughout the trial. For identification purposes, sites may include a tag or card in the photo with the subject ID number, visit number on which the photos are taken, and the marker lesion designation.

For each lesion, two photos will be taken. The first photo will be a close-up of the lesion. A millimeter ruler should be included in the photograph to demonstrate the size of the lesion. The second photo will be a larger view photo that will show the lesion's location on the body.

All participants will also need photos of larger views of the back, chest, arms (front and back), legs (front and back), feet (including soles), whether involved with KS or not. In addition, photos should be taken of any other area with significant involvement at baseline. For subject privacy and informed consent considerations, at no time should the subject's entire face be photographed, and any distinguishing characteristics (e.g., tattoos, birthmarks, or other features) should be avoided or removed from photographs.

In participants with >50 cutaneous lesions, photographs will be taken of the three representative areas (each with  $\geq 5$  lesions), defined at study enrollment and used for clinical assessment of response. Photographic documentation will be completed at baseline and then at each visit when the KS response category changes. For example, if a participant's KS response category changes from no response (stable disease) to partial response, the site will take photos of this to document the category change. If there was no change in the KS response category, no photos are required.

### Informed Consent for Photograph Collection

The collection of photographs is addressed in the model informed consent form for this study, as follows, and this text should be present in your site's IRB-approved informed consent form.

The study doctor will examine your KS lesions, will take measurements of some of the lesions and will count them, and may take photographs of the lesions to document their appearance. Additional photographs will be taken during the course of the study to document any changes in appearance. At no time will your entire face appear, and any distinguishing features will be removed from the photo so that you cannot be identified. Only your initials and subject number will be used to identify the photo.

### Photograph Electronic Labeling and Storage

Photographs will be electronically stored at sites in a file with back-up storage, with access limited to active study personnel (e.g., password protected). Photos should be saved in a common file format such as JPEG, TIFF, or GIF files. Electronic files should be labeled in a manner that includes the subject ID number, the visit number, and the marker lesion designation.

Instructions on the secure transmission of photographs to the AMC Operations and Data Management Center, Study Sponsor, and Study Chair will be available on the AMC Operations web site. When uploading photos to these parties named above, subject initials should not be included in file names or anywhere in the photographs.

Please direct any questions regarding lesion photography to the AMC Operations and Data Management Center via e-mail at [amcpm@emmes.com](mailto:amcpm@emmes.com).