

SUMMARY OF CHANGES
A Phase II Study of sEphB4-HSA in Kaposi Sarcoma
Version 6.0

NCI Protocol #: AMC-096
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I. Scientific and Substantive Changes

#	Section	Comments
1.	5.4	Dose Modifications for Hypertension grade 3 section was updated to match Hypertension Management Schema.

II. Administrative and Editorial Changes

#	Section	Comments
2.	Global	The protocol version number and version date have been updated from version 5.0 dated 29JUL2020 to version 6.0 dated 31AUG2023.
3.	Global	Grammatical corrections were applied throughout the document.
4.	Global	Figures and tables were renumbered.
5.	Global	The AMC Biorepository Director, Syliva Silver, was replaced with the AMC Network Resources Laboratory Director, Jeffrey Bethony.
6.	Global	The shipping site for peripheral blood samples was updated to replace Bui Lab (Dr. Bui) with George Washington University Medical Center (Dr. Jeffrey Bethony) throughout the document.
7.	Protocol Roster	The Protocol Statistician was updated to replace Shelly Lensing with Deukwoo Kwon. The AMC Data Management and Operations email address was updated to amc-096@emmes.com.
8.	AMC Protocol Signature Page	The AMC Protocol signature page was moved before table of contents to be consistent with AMC protocol template.

#	Section	Comments
9.	List of Abbreviations	List of Abbreviations table was added.
10.	7.2.1	Vasgene's drug disposal and return policy was added.
11.	8.2 Appendix I	The evaluations during treatment were updated to clarify that the CBC with differential on Days 1 and 15 must be completed within 7 days prior to day 1 and day 15 of each cycle.



AIDS MALIGNANCY CONSORTIUM

AMC PROTOCOL # 096:

A Phase II Study of sEphB4-HSA in Kaposi Sarcoma A Trial of the AIDS Malignancy Consortium (AMC)

Funded by:	National Cancer Institute Office of HIV and AIDS Malignancy (OHAM)
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Investigational Agent:	sEphB4-HSA (NSC 782348)
IND #:	112629
IND Sponsor:	VasGene Therapeutics, Inc.
Protocol Chair:	Ida Wong-Sefidan, MD
Protocol Co-Chair:	Erin Reid, MD

*Version 6.0, 31AUG2023
NCI Version Date: 31AUG2023*

AMC PROTOCOL SIGNATURE PAGE

I, _____, Principal Investigator at site _____, agree to conduct and follow this protocol: **AMC Protocol # 096 - A Phase II Study of sEphB4-HSA in Kaposi Sarcoma (Version 6.0, 17MAR2023)**, as written according to AMC, NCI, and FDA guidelines. I understand that no deviations from the protocol eligibility criteria or waivers for protocol deviations will be permitted.

Signature

Date (mm/dd/yyyy)

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PROTOCOL ROSTER
AMC Protocol # 096
A Phase II Study of sEphB4-HSA in Kaposi Sarcoma

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PROTOCOL SYNOPSIS

Title:	A Phase II Study of sEphB4-HSA in Kaposi Sarcoma
Phase of Study:	Phase II
Participating Institutions:	This protocol will be open to all AMC domestic member sites
Accrual Target:	20 participants
Population:	Participants with Kaposi sarcoma (KS), with or without Human Immunodeficiency Virus (HIV) seropositivity
Regimen:	sEphB4-human serum albumin (HSA) will be administered via intravenous infusion. Each cycle of sEphB4-HSA will be 28 days (4 weeks). Each cycle of the study drug includes administration of 2 doses of sEphB4-HSA at 10 mg/kg I.V. administered on Days 1 and 15.
Duration:	<p>Participants may continue on study protocol as long as their Kaposi Sarcoma (KS) is continuing to respond or is clinically stable on study medication for up to 12 treatment cycles. If complete response (CR) is achieved, the participant will receive one cycle beyond CR. Treatment may be resumed if KS progresses off treatment after a CR. The participant will resume the previous dose that he/she was receiving when CR was achieved. Participants who achieve partial response (PR) or stable disease (SD) may continue the treatment at the dose he/she was receiving when PR or SD was achieved, for a maximum of 12 cycles, if the participant and treating physician feel that it is beneficial to continue treatment. Treatment will be discontinued if the participant experiences unacceptable toxicity or develops one of the protocol-defined reasons for treatment discontinuation. Participants who have partial response or better will be followed up every three months for up to one year, or until an earlier time of disease progression requiring additional treatment.</p> <p>All participants will be followed for 4 weeks after completion of study, removal from study treatment, or until death, whichever occurs first. Participants removed from study treatment for unacceptable adverse event(s) (AE) will be followed until resolution or stabilization of the AE. Participants who withdraw for toxicity reasons should be followed until the toxicity resolves/returns to baseline, or for 4 weeks, whichever is later. In addition, participants who go off study for reasons other than toxicity should be followed for at least 4 weeks after discontinuing drug.</p>

- Primary Objective:** Evaluate the clinical response and toxicity of sEphB4-HSA (at initial dosing of 10 mg/kg every 2 weeks) in participants with KS.
- Secondary Objectives:**
1. Assess the safety of sEphB4-HSA in participants with KS.
 2. Determine trough level exposure of sEphB4-HSA and correlate with tumor response.
 3. Characterize the pharmacodynamics of sEphB4-HSA and correlate these effects with clinical response:
 - i. Effects on viral replication and gene expression of HHV-8.
 - ii. Changes in VEGF-Notch-EphrinB2 angiogenic pathway.
 - iii. Effects on immune response and modulation.
 - iv. Effects on tumor cell apoptosis and proliferation.
 - v. Effect of sEphB4-HSA on HIV plasma viral loads in participants with HIV.
 4. Archive peripheral blood mononuclear cells (PBMCs) and tissue samples to be used in conjunction with samples collected in subsequent trials of sEphB4-HSA for future studies including identification of biomarkers predictive of response.
- Exploratory Objective:** Describe baseline quality of life (QOL) scores, using the functional assessment of HIV Infection (FAHI) + Kaposi sarcoma (KS) questionnaire, in participants with KS, and explore changes in QOL of participants on treatment with sEphB4-HSA.

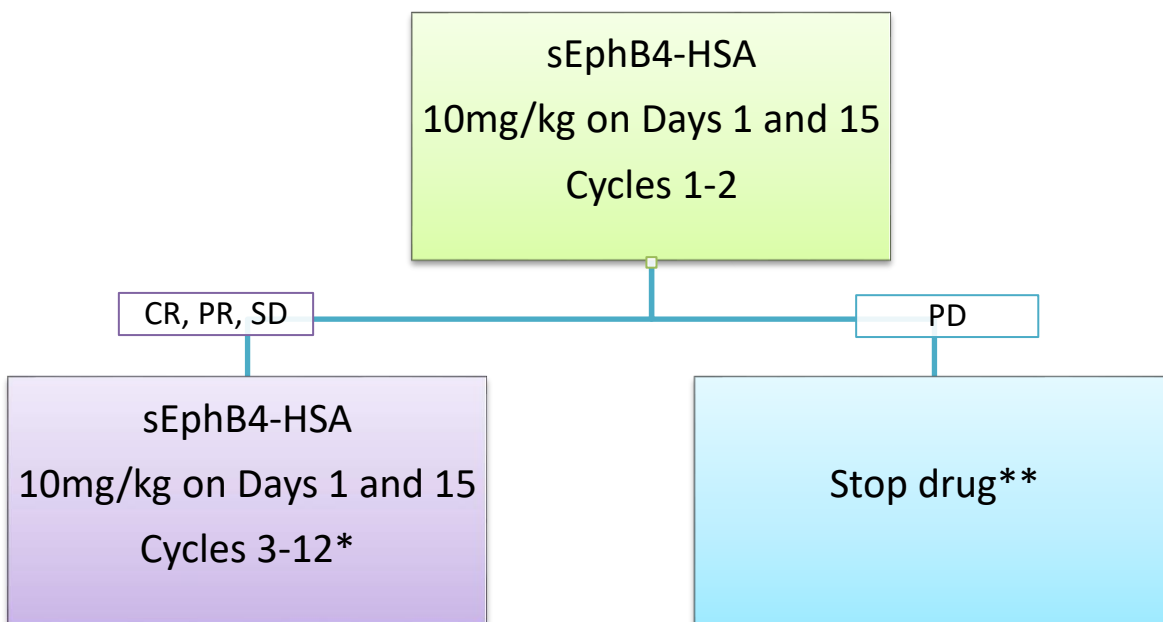
LIST OF ABBREVIATIONS

Ab	Antibody
AB	Associate Basic
ACD	Acid Citrate Dextrose
ACSR	AIDS And Cancer Specimen Resource
ACTG	Aids Clinical Trials Group
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
AMC	Aids Malignancy Consortium
AML	Acute Myelocytic Leukemia
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
AUC	Area Under Curve
BP	Blood Pressure
CBC	Complete Blood Count
CDUS	Clinical Data Update System
CHO	Chinese Hamster Ovary
CI	Clinical Investigator
COPD	Chronic Obstructive Pulmonary Disease
CR	Complete Response
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTMB	Clinical Trials Monitoring Branch
CTSU	Cancer Trials Support Unit
DARF	Drug Accountability Record Form
DBP	Diastolic Blood Pressure
DHHS	Department Of Health and Human Services
DLT	Dose limiting toxicity
DMU	Data Mapping Utility

DSMB	Data And Safety Monitoring Board
DSMP	Data And Safety Monitoring Plan
DTL	Delegation of Tasks Log
ECOG	Eastern Cooperative Oncology Group
EKG	Electrocardiogram
ELISA	Enzyme-Linked Immunosorbent Assay
ELISPOT	Enzyme-Linked Immunosorbent Spot
FAHI	Functional Assessment of HIV
FCBP	Females of Childbearing Potential
FDA	Food and Drug Administration
FAHI	Functional Assessment of Human Immunodeficiency Virus Infection
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HSA	Human Serum Albumin
HUVEC	Human Umbilical Vein Endothelial Cells
IAM	Identity and Access Management
IDB	Investigational Drug Branch
IL	Interleukin
IME	Important Medical Events
IRB	Institutional Review Board
I.V.	Intravenous
IVR	Investigator
KPS	Karnofsky Performance Score
KS	Kaposi Sarcoma
KSHV	Kaposi Sarcoma Associated Herpes Virus
LANA	Latency-Associated Nuclear Antigen
MDS	Myelodysplastic Syndrome
MSC	Mesenchymal Stem Cells
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NRTI	Nucleoside (and Nucleotide) Reverse Transcriptase Inhibitors

NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NPIVR	Non-Physician Investigator
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
NYHA	New York Heart Association
ODMC	Operations and Data Management Center
OHAM	Office of HIV and AIDS Malignancy
PBMC	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PI	Principal Investigator
PIO	Protocol Information Office
PMB	Pharmaceutical Management Branch
PR	Partial Response
QOL	Quality of life
RCR	Registration and Credential Repository
RNA	Ribonucleic Acid
RTK	Receptor Tyrosine Kinase
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Stable Disease
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOC	System Organ Class
SOP	Standard Operating Procedure
SPEER	Specific Protocol Exceptions to Expedited Reporting
SUSAR	Serious and Unexpected Suspected Adverse Drug Reaction
SWG	Scientific Working Group
TORO	Transfer of Regulatory Obligations
ULN	Upper Limit of Normal
VEGF	Vascular Endothelial Growth Factor
VEGFR	VEGF Receptors
WBC	White Blood Cell

PROTOCOL SCHEMA



*If complete response (CR) is achieved, the participant will receive one cycle beyond CR. Treatment may be resumed if KS progresses off treatment after a CR. The participant will resume the previous dose that he/she was receiving when CR was achieved. Participants who achieve PR or stable disease (SD) may continue the treatment at the dose he/she was receiving when PR or SD was achieved, for a maximum of 12 cycles, if the participant and treating physician feel that it is beneficial to continue treatment.

**Assuming at least 4 doses of sEphB4-HSA administered.

1.0 OBJECTIVES

1.1 Primary Objective

To evaluate the clinical response and toxicity of sEphB4-human serum albumin (HSA) (at initial dosing of 10 mg/kg every 2 weeks) in participants with Kaposi sarcoma.

1.2 Secondary Objectives

1.2.1 To assess the safety of sEphB4-HSA in participants with KS.

1.2.2 To determine trough level exposure of sEphB4-HSA and correlate with tumor response.

1.2.3 To characterize the pharmacodynamics of sEphB4-HSA and correlate these effects with clinical response.

1.2.3.1 Effects on viral replication and gene expression of HHV-8.

1.2.3.2 Changes in VEGF-Notch-EphrinB2 angiogenic pathway.

1.2.3.3 Effects on immune response and modulation.

1.2.3.4 Effects on tumor cell apoptosis and proliferation.

1.2.3.5 Effects on sEphB4-HSA on Human Immunodeficiency Virus (HIV) plasma viral loads in participants with HIV.

1.2.4 To archive peripheral blood mononuclear cells (PBMCs) and tissue samples to be used in conjunction with samples collected in subsequent trials of sEphB4-HSA for future studies including identification of biomarkers predictive of response.

1.3 Exploratory Objective

Describe baseline quality of life (QOL) scores, using the functional assessment of HIV Infection (FAHI) + Kaposi sarcoma (KS) questionnaire, in participants with KS, and explore changes in QOL of participants on treatment with sEphB4-HSA.

2.0 BACKGROUND

2.1 Kaposi Sarcoma

Kaposi sarcoma (KS) is a multifocal angioproliferative disorder of vascular endothelium, most associated with infection with the Kaposi sarcoma associated herpes virus (KSHV), also known as human herpes virus-8 (HHV-8). KS is associated with a number of epidemiologic and pathophysiologic factors. KS is classified into four distinct clinical types: classic Mediterranean KS, African-endemic KS, immunosuppressive drug-related KS, and HIV-related KS. A rare disease before the era of HIV and AIDS, HIV-related KS is the most frequent malignancy in HIV-infected patients.

The pathogenesis of all four types of KS is alike. Initiation of KS starts with HHV-8 infection and transformation of human endothelial cells, eventually forming the neoplastic spindle-cell component of the KS.¹ Viral gene products of HHV-8 affect both cell cycle regulation and the control of apoptosis, and segments of the HHV-8 genome contain viral oncogenes that are important in the pathogenesis of tumor formation.² Like all herpesviruses, HHV-8 alternates between two phases of its life cycle—the latent and lytic phases. Latent and lytic phases of the HHV-8 life cycle are both involved in KS development. During latency, the virus is maintained as episomes attached to the host chromosome, and the virus is replicated with the host chromosome, and subsequently passed to descendant cells.³ Though gene expression is limited during the latent phase, latently expressed viral proteins have been shown to promote tumorigenesis by dysregulating the cellular mechanisms which would normally protect cells from atypical proliferation.⁴ Eventually, this leads to transformation of endothelial-derived spindle cells with hyperproliferation and reactivation of the latent HHV-8 with lytic replication of viral proteins that can promote angiogenesis and activate further inflammation.⁵ Characteristic of the lytic phase is active viral replication and active expression of a wide range of HHV-8-gene products.⁶ Vascular cells that are infected with HHV-8 can lead to transformation of endothelial-derived spindle cells with hyperproliferation and development of characteristic KS lesions.⁵ The microscopic features of all four different types of KS do not differ. All forms show evidence of angiogenesis, inflammation, and spindle cell proliferation.

KS can affect many organs. KS manifests most frequently as a disease of the skin. In many advanced cases, KS involves organs such as the lungs, liver, or gastrointestinal tract.⁷ At this time, KS is incurable. Available therapies are for palliation. Systemic chemotherapy is generally used for patients with more advanced disease or evidence of rapid progression of disease. The major goals of treatment are symptom palliation, prevention of disease progression, and reduction of tumor burden to alleviate lymphedema, organ compromise, and psychological stress. The standard therapies for visceral or advanced cutaneous KS include cytotoxic chemotherapy such as liposomal anthracycline and paclitaxel.⁸ Liposomal doxorubicin has superior efficacy and favorable tolerability and toxicity compared to the combination of non-liposomal doxorubicin, vincristine, and bleomycin with overall response rates of 59% in HIV patients.⁹ In classical KS, response rates to liposomal doxorubicin can be higher.¹⁰ However, CR rates are uncommon and there is no cure. At this point in time, no targeted therapy has been fully developed for KS. Given the role of angiogenesis in KS tumorigenicity, antiangiogenic approaches represent potential therapeutic targets. A novel, effective drug would be welcomed for patients with KS. Given

KS skin lesions are visibly apparent; the disease can often result in significant psychological problems and social stigma for patients with KS. Symptoms of systemic KS include pain, diarrhea, weight loss, edema, and oral sores. In many cases, KS can severely compromise the QOL by causing physical suffering, scarring, and deformity. Lymphedema, particularly in the face, genitalia, and lower extremities are not uncommon and impair functional ability.¹¹ Survival can often be marked by poor QOL, making it difficult for patients to deal with their disease. While the introduction and use of highly active antiretroviral therapy (HAART) therapy has resulted in a steep decline in the incidence of HIV-related KS with improvements in survival, CRs are rare and the disease remains incurable.^{12,13} Therefore, QOL for patients with KS still remains a major issue. Since QOL has been, and continues to be, such a vital issue in KS, QOL measures have been used as endpoints in AIDS-related KS.¹⁴⁻¹⁶ In a phase II trial with low-dose etoposide, responders had a statistically improvement in pain, physical and bodily pain distress compared to non-responders.¹⁵ In an AIDS Malignancy Consortium (AMC) trial of liposomal doxorubicin versus paclitaxel, two common drugs used in advanced KS, the KS Functional Assessment of HIV Infection (FAHI) QOL instrument with three supplemental questions on pain, swelling and physical appearance was used to collect QOL data.^{17,18} This study was able to show that with treatment, there were significant improvements reported between baseline and treatment values of pain and swelling; these values are of clinical value as this was not captured by the given response values (i.e., complete response, partial response). Clinicians currently take QOL improvement into account when treating KS patients with chemotherapy drugs like liposomal doxorubicin and paclitaxel, which have clear side effects. Therefore, it is also important to consider QOL changes when moving a therapy forward into the clinical arena.

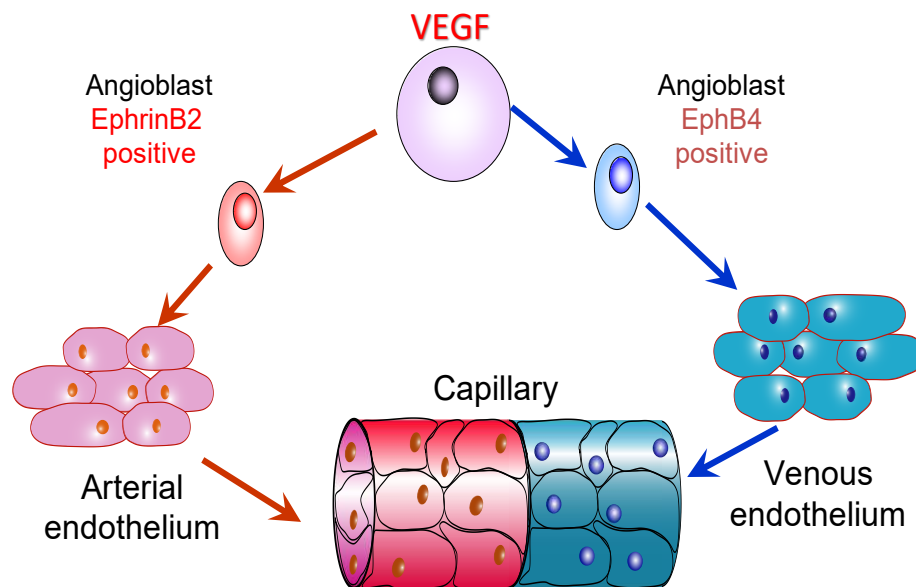
2.2 Rationale

Angiogenesis is one of the most important features of KS. Therefore, there is rationale to target the angiogenic pathway. Vascular endothelial growth factor (VEGF) is a major regulator of both physiological and pathological neovascularization and angiogenesis. VEGF functions as an autocrine growth factor, as well as stimulates tumor vessels in a paracrine manner.¹⁹ VEGF is crucial in the development and pathogenesis of KS.²⁰ HHV-8 induces VEGF in KS, and KS cells have been shown to contain and secrete VEGF.^{21,22}

KS tumor cells have high expression of VEGF receptors (VEGFR). VEGF activates KS tumor cells via the VEGF-Notch-Ephrin pathway. In this pathway, VEGF induces the Notch ligand Delta-like 4 (Dll4). Activation of Notch induces high expression of EphrinB2.²³⁻²⁵ In important feedback loops, VEGF signaling requires EphrinB2 activation, and VEGFRs physically bind EphrinB2.

EphrinB2 is a transmembrane ligand; one of its receptors EphB4, a tyrosine kinase receptor, is also a transmembrane protein. Typical endothelial progenitors and mature cells of venous lineage express EphB4, while arterial lineage cells express EphrinB2.^{26,27} The normal capillary network of the microvasculature composed of arterial capillaries expressing EphrinB2 and EphB4-expressing venous capillaries form regular junctions with each other, play an essential role in angiogenesis (See [Figure 2-A](#)). In KS, EphrinB2 is specifically induced and upregulated by HHV-8, while EphB4 is downregulated. Therefore, KS tumor cells express EphrinB2, but EphB4, is nearly absent in KS.²⁵

Figure 2-A: Normal capillary network of the microvasculature composed of arterial capillaries which express EphrinB2 and venous capillaries which EphB4. EphrinB2-EphB4 form regular junctions with each other and are key regulators of angiogenesis.



Evidence shows that EphrinB2 expression is necessary for KS cell viability by supplying survival signaling to KS cells. This was shown by knocking down EphrinB2 by small interfering ribonucleic acid (RNA), which led to KS cell death.²⁸ Consequently, antagonists to EphrinB2 interactions potentially have ways to intercede in growth in malignancies such as KS where EphrinB2 are expressed. In addition to EphB4, EphrinB2 can bind several other Eph receptors, which include EphB2 and EphA4 which are expressed in KS cells.²⁵ However, since EphB4 is expressed in the vessels, and its only ligand is EphrinB2, its function is highly predictable as an attractive antagonist and inhibitor of EphrinB2.

A soluble monomeric derivative of the extracellular domain of EphB4 (sEphB4) has been shown *in vitro* to antagonize EphB4/EphrinB2 signaling. sEphB4 blocks EphB4-induced phosphorylation of EphrinB2 in human umbilical vein endothelial cells (HUVEC).²⁹ EphB4-EphrinB2 blockade inhibits migration of endothelial cells, and disrupts angiogenesis, recruitment of pericytes, and perfusion of newly forming vessels.²⁹ sEphB4-HSA, a fusion protein composed of soluble EphB4 extracellular domain complex with albumin, which improves sEphB4's solubility, inhibits the invasion of KS cells across matrix proteins (see [Figure 2-B](#), and [Figure 2-C](#)). This inhibition is most prominent when KS cells are stimulated with growth factors that promote KS growth and migration, including VEGF.²⁵ sEphB4-HSA is very effective antagonist in *in vivo* KS mouse and non-primate models. Therefore, sEphB4-HSA would be an attractive choice for clinical investigation in KS.

Figure 2-B: EphrinB2-EphB4 interaction induces forward and reverse (bidirectional) signaling. sEphB4-HSA is an antagonist which will inhibit the EphrinB2-EphB4 complex, thereby blocking the forward and reverse (bidirectional) signaling).

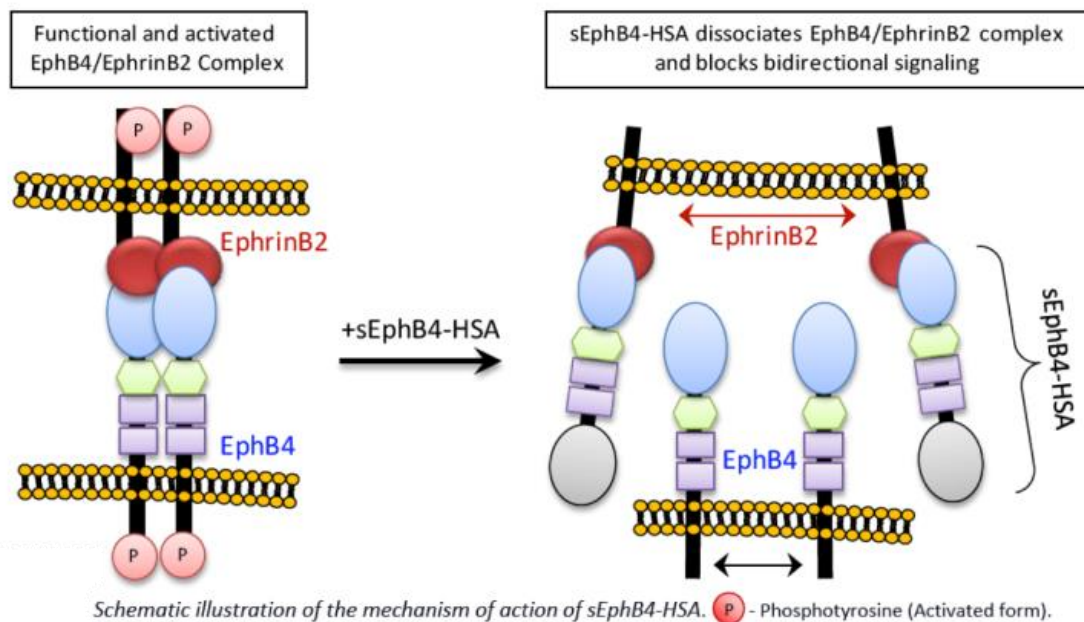
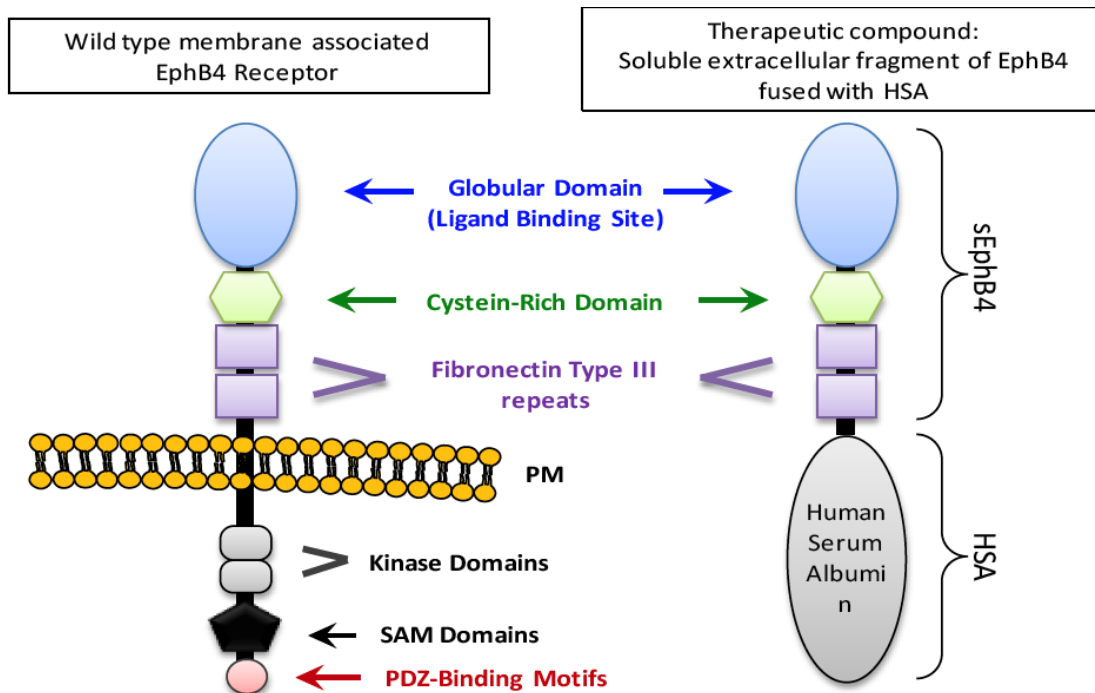


Figure 2-C: Schematic structure of EphB4 receptor (left) and sEphB4-HSA (right).



We hypothesize that KS is highly dependent on the VEGF-Notch-EphrinB2 pathway for development and progression, and sEphB4-HSA will be effective in patients with KS, clinically as well as at the tumor progenitor level. The goal of this study is to determine the

efficacy and toxicities of sEphB4-HSA in patients with KS. We will examine the sEphB4-HSA pharmacokinetics and pharmacodynamics including effects on the HHV-8, angiogenic pathways, immune function, tumor apoptosis, and proliferation by comparing pre-treatment baseline and post-treatment blood and tissue biopsy samples to clinical response. Correlative studies planned will further our knowledge regarding the pathophysiology of angiogenesis in KS as well as modulation of immune response via inhibition of EphrinB2.

One of the challenges for evaluating and comparing effectiveness of treatments for KS is determining the most appropriate measure of what is considered “effective” to the patient. Historically, KS response and effectiveness have been evaluated by the degree of decrease in tumor burden based on the changes in number, characteristics, size, and nodularity of lesions,³⁰ which then translates into complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). However, a patient’s view on effectiveness may be different than our traditional definitions; improvement in symptoms may not be captured by our protocol terminology. Responses (complete or partial) do not consistently correlate with improvement in QOL. Patients often have QOL benefit without an objective response; for example, patients may have improvement in lymphedema and lightening of skin without achieving a true PR by definition. Additionally, patients undergo multiple treatment regimens which can take a toll on a patient’s physical and emotional state, and psyche. While other symptoms such as diarrhea or weight loss related to KS are captured on adverse event (AE) reporting, considering that KS is a unique disease which can significantly impact QOL due to pain, limitations to ambulation, as well as distress related to disfigurement and social stigma, the FAHI+KS questionnaire will hopefully capture these concerns of patients with KS, and explore whether sEphB4-HSA improves overall QOL. sEphB4-HSA is a novel drug, and as we try to move into evaluating alternative treatments beyond chemotherapy drugs, which hopefully will have fewer toxicities, patients may be able to influence the delivery of clinical trials by converting their experience into worthwhile hypotheses for subsequent trials.

2.3 Study Agents

2.3.1 sEphB4-HSA

sEphB4-HSA is a fully human recombinant fusion protein composed of soluble EphB4 extracellular domain fused at the C-terminus with albumin upon expression as a single 128.7 kDa protein. The protein is expressed in recombinant Chinese hamster ovary (CHO) cells and produced using standard mammalian cell cultivation methods followed by chromatographic purification. The protein is administered in a sterile solution for intravenous (I.V.) infusion.

2.3.2 Non-clinical studies

sEphB4-HSA specifically binds to its ligand EphrinB2, a transmembrane protein of EphrinB family. sEphB4-HSA blocks the interaction between EphB4, a receptor tyrosine kinase (RTK), and its only ligand EphrinB2. *In vitro*, non-clinical studies showed that sEphB4 binds specifically to EphrinB2 and binds to EphrinB2-expressing cells with low nanomolar affinity. sEphB4-HSA blocks EphrinB2-induced EphB4 phosphorylation and EphB4 induced EphrinB2 phosphorylation. As a result, sEphB4-HSA inhibits EphB4-EphrinB2-mediated bidirectional

signaling, leading to inhibited endothelial invasion, binding to extracellular matrix proteins, and tube formation at low nanomolar effective concentration for 50% inhibition (EC50) values. In a Matrigel plug assay, sEphB4-HSA effectively reduced the angiogenesis induced by VEGF. Furthermore, sEphB4-HSA was shown to inhibit cell viability *in vitro* on a panel of susceptible cancer cell lines including KS.³¹

sEphB4-HSA has also demonstrated *in vivo* activity in multiple tumor xenograft models. In a therapeutic human colon cancer model, in which HT29 cells were implanted in athymic mice subcutaneously, sEphB4-HSA was active in a dose range of 5 mg/kg/dose to 20 mg/kg/dose. In this model, an approximate 30% to over 80% tumor growth inhibition was seen at these dose levels. In several other tumor types, sEphB4-HSA also induced significant tumor growth inhibition at 20 mg/kg/dose. In an orthotopic pancreatic tumor model, sEphB4-HSA not only inhibited the growth of primary tumor, but also significantly reduced the incidence of tumor metastasis into the major organs. sEphB4-HSA significantly inhibits tumorigenesis and/or tumor growth in Kras mutant oral cavity model, skin cancer model and PTEN knockout prostate cancer model. Furthermore, sEphB4-HSA inhibited KS tumor growth in a murine tumor xenograft model. Mice implanted with KS-SLK and KS- IMM, which represent nodular and late-stage KS tumors, were treated with intramuscular sEphB4-HSA, PBS (negative control), VEGF monoclonal antibody (Ab) (positive control), or combination sEphB4-HSA/VEGF monoclonal Ab. Relative to PBS, sEphB4-HSA inhibited tumor growth in both cell lines KS-SLK and KS- IMM by 15.9% and 37.2% of control, respectively. sEphB4-HSA was more active in SLK tumors compared with VEGF monoclonal Ab ($p < .01$). The combination of sEphB4-HSA and VEGF monoclonal Ab was superior to each alone in both tumor types, with tumor volumes of 12.1% for KS-SLK ($p < .001$) and 12.6% for KS-IMM ($p < .001$).³¹

2.3.3 Non-clinical toxicology

A human tissue cross-reactivity study was performed to evaluate the potential of sEphB4-HSA to bind to normal tissues. sEphB4-HSA was biotinylated in a manner which preserved its EphrinB2 binding activity. Biotinylated sEphB4-HSA was incubated at a concentration of 2.5 µg/mL with the human Food and Drug Administration (FDA) Standard Frozen Tissue Array consisting of 30 different human organ tissue specimens. EphrinB2-positive LTC tumors from xenograft studies were included as a positive control. Bound sEphB4-HSA was analyzed by streptavidin conjugated with HRP. There was no binding of sEphB4-HSA to the majority of human tissues except kidney, stomach, thyroid, and bone marrow, where focal binding to small areas was observed. In human kidney there was specific binding to renal tubules, whereas there was no binding to glomerulus or vessels. In stomach, there was specific binding to villi but not to surrounding tissue.³¹

The study was repeated for sEphB4-HSA binding to cynomolgus monkey tissues. Similar to human tissue array sEphB4-HSA showed cross reactivity to the renal tubule, but not organs such as spleen, liver, and lung.

2.3.4 Non-clinical toxicities

sEphB4-HSA was well-tolerated in cynomolgus monkeys when administered as a single I.V. injection at dose levels up to 30 mg/kg. In a 5-week multiple and different dose (5 weekly drug administration ranging from 0 to 30 mg/kg) toxicity study of sEphB4-HSA in the monkeys, all animals survived until their scheduled necropsy. No anatomical pathology findings were present in all animals at the end of the 5-week observation period, nor after the recovery period (7-week study). There were no test article-related changes in clinical observations including food consumption, body weights, electrocardiographs, blood pressure, ophthalmology, urinalysis, hematology, coagulation, and serum chemistry. No significant local injection site irritation related to I.V. injection of sEphB4-HSA was observed. Reproductive and developmental toxicity studies have not been conducted with sEphB4-HSA.³¹

2.3.5 Non-clinical pharmacokinetics

Non-primate pharmacokinetics studies show that the peak values primarily occur after administration of the first dose. Pharmacokinetic studies illustrate that peak values primarily occurred after administration of the first dose of sEphB4-HSA and decreased in the magnitude of the response over time. At 30 mg/kg, sEphB4-HSA has the half-life of 51.5 hours and maximum serum concentration of 789 mg/L (see [Table 2-D](#)).³¹

Table 2-D: Non-clinical pharmacokinetics

Dose (mg/kg)	n	C _{max} (mg/L)	AUC _{last} (mg/L·h)	AUC _{inf} (mg/L·h)	AUC _{inf, DN} (mg/L·h)	Half-Life (h)
30	12	789 (21.5)	17300 (18.5)	43000 (24.5)	1430 (20.0)	51.5 (28.4)
Definitions: C_{max} = serum concentration at 30 min post end of infusion AUC_{last} = area under the curve for C _{max} as a function of time (AUC) from start of 0.5 I.V. infusion to 168 hours post end of infusion AUC_{inf} = observed AUC from start of I.V. infusion to infinity AUC_{inf, DN} = observed AUC _{inf} normalized to a 1 mg dose						

2.3.6 Non-clinical immunogenicity

The amino acid sequences of monkey and human sEphB4 differ by four amino acids. Within the albumin domain, the difference between species is 40 amino acids. sEphB4-HSA was immunogenic in monkeys following I.V. administration (anti-drug antibody response ranged from 0.0 to 164 µg of antibodies per mL of serum). In the 5-week toxicity study, the immunogenicity appeared to impact the pharmacokinetics as the majority of the animals had lower serum concentrations of sEphB4-HSA after drug administration on Day 29 (after the fifth dose) compared to Day 1 (first dose). None of the monkeys in the 5-weeks toxicity study developed

reactions consistent with anaphylaxis after sEphB4-HSA. Anti-sEphB4-HSA antibodies were detected in 75% of animals exposed to the low drug dose (3 mg/kg), 100% of animals exposed to the middle drug dose (10 mg/kg), and 50% of animals exposed to the high drug dose (30 mg/kg). Immune response in monkeys was associated with decreased drug levels in sera. The majority of immune responses were against the albumin domain. No drug-neutralizing antibodies were detected in this study.³¹

2.3.7 Clinical pharmacokinetics, safety, toxicities and efficacy

A phase I trial in solid tumors has been completed in relapsed/refractory solid tumors (unpublished data). Human pharmacokinetic studies in patients at dose level 2.5 mg/kg, 5 mg/kg, 10 mg/kg, and 15 mg/kg have been done. Half-life of the protein increases with higher dose and ranges from 7-10 days. Weekly administration of the drug leads to drug accumulation (See [Figure 2-E](#) and [Table 2-E](#)).³¹

Figure 2-E: Pharmacokinetics of sEPHb4-HSA at 2.5 mg/kg, 5 mg/kg, 10 mg/kg, and 15 mg/kg

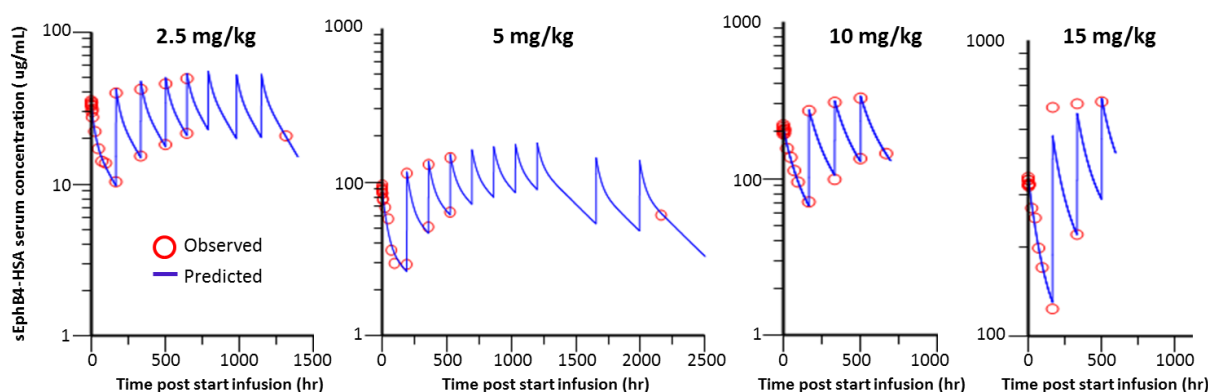


Table 2-F: Pharmacokinetics of sEPHb4-HSA at 2.5 mg/kg, 5 mg/kg, 10 mg/kg, and 15 mg/kg

Dose (mg/kg)	C _{max} (ug/mL)	AUC _{0-96h} (h*ug/mL)	AUC _{0-tlast} (h*ug/mL)	AUC _{0-∞} (h*ug/mL)	t _{1/2} (h)	CL (mL/h/kg)
2.5	46.4	2400	3270	4580	130	0.315
5.0	86.6	4980	7330	10600	157	0.283
10	188	11700	17300	30400	302	0.133
15	345	23500	23600	49000	226	0.132

Preliminary PK Parameters (Means) of sEphB4-HSA following a 1 Hour Intravenous Infusion

The phase I trial for solid tumors being conducted by VasGene has had no dose limiting toxicities reported for the whole trial, even for the highest dose level of 15 mg/kg weekly. After noting drug accumulation at weekly dosing, expanded cohorts

of 10 mg/kg and 15 mg/kg every 2 weeks were also tested without occurrence of dose limiting toxicity (DLT). Given it is a soluble protein cleared by the reticuloendothelial system no drug interactions are anticipated. Responses have been seen in patients with head and neck cancers (including 1 CR), cholangiocarcinoma, mesothelioma, and long stable disease in parotid gland carcinoma, and adenocarcinoma of the lung. Several participants have received 4 - 11 months of therapy indicative of safety when used for prolonged period of time.

Phase I safety has been accrued. As of July 2013, 13 participants with serious adverse events were accrued and summarized (See [Tables 2-G](#)).³¹

Table 2-G: Serious adverse events reported in phase I studies

Participant	Serious Adverse Events During the Reporting Period	Causality
1	Serious nausea and vomiting, progressing to hematemesis, admitted for treatment with resolution	Possibly related
2	Syncopal episode requiring admission with resolution	Unrelated
3	Shaking/chills/fever requiring admissions for observation with resolution	Unrelated
4	Death due to progression	Unrelated
5	Hospitalized for pain secondary to tumor burden	Unrelated
6	Hospitalized for hemorrhage from progression of tonsil tumor; off-study	Possibly related
7	Death due to progression	Unrelated

Data is still being cleaned before the study is formally presented.

2.3.8 Dosing in clinical trial

A phase I trial in refractory/relapsed solid tumors has been completed. The following dose levels were tested in the phase I trial: 2.5 mg/kg, 5 mg/kg, 10 mg/kg and 15 mg/kg administered weekly on Days 1, 8, 15, and 22; and 10 mg/kg, 15 mg/kg and 20 mg/kg administered every 14 days (2 weeks) on Days 1 and 15, by I.V. infusion for 60 minutes. The weekly dosing schema and schedule was chosen based on safety of weekly dosing in animal studies of doses up to 30 mg/kg. No DLTs were seen in this weekly dosing, however dose accumulation was noted. Cohorts consisting of 10 mg/kg, 15 mg/kg and 20 mg/kg, with administration every 14 days (2 weeks), were added to the phase I trial, given the dose accumulation noted with weekly dosing. The phase I maximum tolerated dose (MTD) has been reached with 2 of 6 participants, at 20 mg/kg administered every 14 days,

experiencing grade III hypertension that was not responsive to therapy, requiring dose reduction to 15 mg/kg every two weeks, and long half-life of 7 - 10 days. Therefore, the dose of 15 mg/kg every 2 weeks, administered on Days 1 and 15, was initially used as the starting dose in this Phase II trial.³¹

Based on updated data (unpublished communication with VasGene, 8/2018) from other trials with sEphB4-HSA (ClinicalTrials.gov ID NCT03146871), which take into consideration rates of hypertension and drug accumulation, other trials have been modified to use a dose of sEphB4-HSA of 10 mg/kg every 2 weeks. Based on this information combined with initial experience in this study, this trial is amended with protocol version 4.0 to use a dose of 10 mg/kg every 2 weeks, administered on Days 1 and 15 of each cycle. In addition, other unpublished studies (ClinicalTrials.gov ID NCT02717156 and NCT03049618), which dosed sEphB4-HSA at 10 mg/kg weekly, showed grade 3/4 hypertension in 3/21 and 3/19 participants, respectively. Given the updated information of drug accumulation in the context of an approximately 5-day half-life of sEphB4-HSA, the weekly dose will not be used in this trial.

2.4 Study Design and Rationale

The proposed trial will be a phase II multi-institutional safety and efficacy single-agent trial of sEphB4-HSA in patients with Kaposi sarcoma, with or without HIV seropositivity. In the proposed trial design, each cycle of sEphB4-HSA will be 28 days. Each cycle of the study drug includes administration of 2 doses of sEphB4-HSA at 10 mg/kg administered on Days 1 and 15. If participants have a CR, treatment will continue for 1 cycle past CR. Treatment may be resumed if KS returns off treatment after a CR. The participant will resume the previous dose that he/she was receiving when CR was achieved. Participants will receive a maximum of 12 cycles. Participants who achieve PR or SD may continue the treatment at the dose he/she was receiving when PR or SD was achieved, for a maximum of 12 cycles, if the participant and treating physician feel that it is beneficial to continue treatment.

2.5 Correlative Studies

sEphB4-HSA is designed to block EphrinB2 signaling upon which preclinical studies have demonstrated KS is critically dependent. Participants will be studied for pharmacokinetics and pharmacodynamics to better understand factors predictive of response to sEphB4-HSA, and to provide additional insight into action of sEphB4-HSA in humans. For this purpose, blood and tumor biopsies will be required in this study; specifically, a mandatory biopsy is required before and during treatment. While this biopsy will not directly benefit the study participant who is providing the samples, the specific correlative studies performed on the biopsy noted in this study were considered because they will scientifically advance the understanding of KS tumor growth via the EphB4-EphrinB2 angiogenic pathway, confirm projected mechanism of action of sEphB4-HSA treatment of KS, as well as aid in development of potentially predictive and/or surrogate biomarkers that may be used in future trials with sEphB4-HSA.

sEphB4-HSA is a fusion protein of soluble EphB4 and human serum albumin, which binds specifically to EphrinB2 that is expressed on KS tumor cells. sEphB4-HSA inhibits signaling from EphrinB2 in tumor cells leading to cell growth inhibition and cell death.

Human KS tumor tissue expresses EphrinB2. EphrinB2 expression is regulated by hypoxia, VEGF, Interleukin-8 (IL8) and several other factors. EphrinB2 physically binds VEGFRs and promotes VEGFR signaling.^{29,32} Inhibition of EphrinB2-EphB4 interaction disrupts the VEGF feedback pathway, and ultimately angiogenesis.³³

KS tumor cells have high expression of VEGFRs, specifically VEGFR2 and VEGFR3. In this pathway, VEGF induces the Notch ligand Dll4, which activates Notch. Activation of Notch, in turn, induces high expression of EphrinB2.²³⁻²⁵ In important feedback loops, VEGF signaling requires EphrinB2 activation, and VEGFRs physically bind EphrinB2.

Based on this, the most critical studies important to the development of sEphB4-HSA will be correlation of the following with response to therapy:

- The expression level of EphrinB2.
- Signaling downstream of EphrinB2, in particular the activated form of Src.
- Biological outcomes of EphrinB2 targeting, such as cell death, inhibition of cell proliferation, modulation of cytokines and chemokines, and signaling downstream of VEGFRs including pVEGFRs.

Obtaining a biopsy at baseline and prompt processing of the tissue provides the best opportunity to determine if the target is expressed and if it correlates with response. The opportunity to obtain a second biopsy on therapy allows determination of mechanism of efficacy such as decreased signaling downstream of EphrinB2 and other markers of tumor response (ki67, TUNEL, pVEGFRs, etc.). Obtaining the second biopsy could also be helpful for determining mechanisms of resistance to sEphB4-HSA.

As this is the first time that the drug will be studied in KS, tissue analysis at baseline and on therapy to predict biomarkers of response is critical. In addition, failure following response to determine mechanism of resistance can only be addressed from tissue analysis. Since cutaneous KS is typically an easily accessible tumor, it is an ideal candidate to study mechanisms of action and resistance to sEphB4-HSA therapy. In doing so, this study may elucidate rational combinations of sEphB4-HSA with other therapeutics when response is suboptimal.

Given that there has only been one human phase I trial using this agent and the small size of this study, the correlative studies and biomarkers in this study are exploratory. We anticipate that one of these markers, such as expression levels of EphrinB2, may be fully developed as an integrated biomarker in further studies, identifying patients who are more likely or less likely to benefit from treatment with sEphB4-HSA. See Appendices [II](#) and [III](#) for full details of the timing of blood and biopsies samples. See [Appendices VIII, IX, and X](#) for collection, shipping, and handling of the specimens.

2.5.1 sEphB4-HSA levels

As the phase I trial of sEphB4-HSA did not include participants with HIV or KS, this study will evaluate C_{max} and trough levels. The schedule of time points can be found in [Appendices I and II](#). All peripheral blood samples will be shipped to and stored at the AMC biorepository. sEphB4-HSA levels will be measured via Enzyme-Linked Immunosorbent Assay (ELISA) by Dr. Parkash Gill at USC Norris Hospital as noted in [Appendix X](#).

2.5.1.1 Trough sEphB4-HSA levels

Pre-dose blood levels will be drawn on Days 1 and 15 of Cycles 1 and 2. sEphB4-HSA levels will be measured via ELISA by Dr. Parkash Gill at USC Norris Hospital.

2.5.1.2 Cmax

On Day 1 of Cycle 1, a blood level will be drawn immediately prior to the end of the infusion. This will serve as the Cmax, or peak serum concentration of sEphB4-HSA. sEphB4-HSA levels will be measured via ELISA by Dr. Parkash Gill at USC Norris Hospital.

2.5.2 Pharmacodynamics

Peripheral blood and biopsies will be shipped to the AMC Biorepository, and then shipped to each respective center for specific correlative studies to be performed, unless otherwise noted. See [Appendix IX](#) for details on which centers will be performing each correlative study.

2.5.2.1 HHV-8

Initiation of KS starts with HHV-8 infection and transformation of human endothelial cells, eventually forming the neoplastic spindle-cell component of the KS. HHV-8 copy number in PBMC and plasma will be obtained before treatment, and Day 1 of Cycles 4, 7, and 10, and at treatment discontinuation. Real-time quantitative RT-PCR will be performed by the AMC Genomics Core labs at the University of North Carolina, Chapel Hill. The same assay will also investigate a set of known endothelial-specific mRNAs (targeted array).

2.5.2.2 VEGF-Notch-EphrinB2 angiogenic pathway

EphB4-EphrinB2 plays a central role in angiogenesis. sEphB4-HSA blocks the interaction between EphB4 receptor tyrosine kinase and its ligand EphrinB2 at the site of the tumor, inhibiting EphrinB2-induced EphB4 phosphorylation and EphB4 induced EphrinB2 phosphorylation, and EphB4-EphrinB2-mediated bidirectional signaling.^{29,32} Inhibition of this interaction disrupts the VEGF feedback pathway, and ultimately angiogenesis.³³ We hypothesize that KS is dependent on the VEGF-Notch-Ephrin2 pathway, and by interrupting this pathway, treatment with sEphB4-HSA will reduce cell markers of angiogenesis, cell cycling, and cell migration, and ultimately will lead to an increase in cell markers of cell death and apoptosis at the level of the tumor. Participants will have two tumor biopsies obtained before initiation of the study, and two biopsies obtained after one cycle of treatment. The tumor samples will be stored at the AMC Repository and then shipped to each respective center for specific correlative studies to be performed. The following correlative studies will be investigated on the biopsy samples

EphrinB2

EphrinB2 selectively marks arterial vessels and neovascularization sites. EphrinB2 is the only consistently expressed Ephrin ligand in KS cell lines. Infection of venous endothelial cells *in vitro* with HHV-8 results in a phenotype switch from EphB4 to EphrinB2.²⁸ In protein analysis, EphrinB2 was highly expressed in both KS cell lines and KS tissue.²⁵ EphrinB2 expression is required for KS cell viability, and EphrinB2 regulates biologic functions of cell migration, adhesion, and invasion in KS.^{25,28} Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

VEGFR-2

VEGFR-2 mediates almost all of the known cellular responses to VEGF and regulates vascular endothelial function.³⁴ VEGFR-2 is greatly expressed in Kaposi sarcoma cells and can promote subsequent transformation of KS cells.³⁵ HHV-8-induces upregulation of VEGFR2, and VEGFR2 expression is 3-5-fold higher in the tumor vasculature than in the normal vasculature.^{36,37} In endothelial cells, VEGF was shown to stimulate VEGFR2 gene expression via a positive feedback mechanism.³⁸ Blocking the VEGF-VEGFR2 interaction can abolish VEGF induced growth.³⁹ EphrinB2 also physically binds to VEGFR-2 and is required for VEGF signaling.²⁴ Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

VEGFR-3

VEGFR-3 is a protein encoded by the FLT4 gene. VEGFR-3 mediates response to VEGF-C and VEGF-D. VEGFR-3 is widely expressed in vascular endothelial cells. VEGFR-3 facilitates HHV-8 infection and fosters subsequent transformation of KS cells. Expression of VEGFR-3 is increased in KS spindle cells, and its ligand, VEGF-C, stimulates the migration and proliferation of KS cells *in vitro*.^{39,40} Histopathological studies indicate that HHV-8 latency-associated nuclear antigen (LANA) and VEGFR3 co-localize in nodular KS. In 2005, Zhang et al showed that a HHV-8 virion envelope-associated glycoprotein is able to activate VEGFR-3 on the microvascular endothelium and modulate endothelial cell migration and proliferation by an interaction between the $\alpha 3\beta 1$ integrin and the VEGFR-3 receptor.⁴¹ Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

Notch receptors 1, 3, 4

The Notch signaling pathway is evolutionarily conserved and proven to regulate and play a crucial role in growth, differentiation, and patterning processes. Several Notch receptors (Notch1, Notch4), ligands, and signaling pathway mechanisms have been identified in endothelial cells *in vitro* and *in vivo*, during development and tumor angiogenesis.⁴² VEGF can induce gene expression of Notch1 and its ligand, Delta-like 4 (Dll4), in human

arterial endothelial cells. Alterations in expression of Notch and its ligand have been shown to provide survival signal in HHV-8-transformed endothelial cells.^{43,44} In KS, lesions express elevated levels of Notch signaling components *in vivo* and *in vitro*, and KS lesions appear sensitive to inhibition of this pathway.^{23,45} Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

Jagged-1

Jagged-1 is a ligand for the receptor Notch-1. HHV-8 upregulates the expression of Jagged-1. Expression of HHV-8-encoded vFLIP induces Jagged-1 through an NFκB-dependent mechanism.⁴⁶ Jagged-1 stimulates Notch signaling in adjacent lymphatic endothelial cells and alters the expression of cell cycle-associated genes.⁴⁶ Jagged-1 is expressed in latently infected lymphatic endothelial cells with further induction during lytic phase of HHV-8.⁴⁴ Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

Dll4

Dll4 is a Notch ligand with expression in the endothelium of blood vessels. Dll4 shows a pattern of strong expression in the endothelial cells of tumor blood vessels compared to neighboring normal tissue vessels.⁴⁷ Dll4 expression is essential for arterial patterning and lymphatic sprouting.⁴⁸ New vessel “tip” cells form the guiding cells of endothelial sprouts and Notch signaling is essential for the specification of these cells; Dll4 expression confers the tip phenotype and suppresses it in neighboring receiving cells under physiological conditions.⁴⁹ Dll4-stimulated signaling results in the suppression of genes associated with the cell cycle in adjacent lymphatic endothelial cells, indicating a role for Notch signaling in inducing cellular quiescence in these cells.⁴⁶ Upregulation of Dll4 by HHV-8 could therefore change the expression of cell cycle components in neighboring uninfected cells during latent and lytic phases of viral infection. Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

pSrc

Several proteins function in the Eph signaling pathway. Among these proteins is pSrc, implicated in regulating cell morphology, attachment, and motility.⁵⁰ Stimulation of EphB4 in human microvascular endothelial cells leads to Src phosphorylation. Blocking Src prevented Akt phosphorylation induced by EphB4 activation and attenuated the migratory effect of EphrinB2.⁵¹ Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

pS6

pS6 is a marker of the PI3K/mTOR pathway activation. EphrinB2 induces the migration of endothelial cells through the PI3K signaling pathway. EphrinB2 promotes *in vivo* angiogenesis in adult mice, suggesting that it

contributes to adult angiogenesis.⁵² Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

pAkt

Stimulation with EphrinB2 leads to Akt phosphorylation in endothelial cells in which PI3 kinase is activated.⁵² Activation of EphB4 in human microvascular endothelial cells leads to Src phosphorylation; blocking Src with an inhibitor prevented Akt phosphorylation induced by EphB4 activation and attenuated the migratory effect of EphrinB2.⁵¹ Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

EphB4 receptor phosphorylation

Eph receptor signaling induced by Ephrin binding is initiated by autophosphorylation and Src family kinases-mediated phosphorylation of the intracellular tyrosine residues, resulting in the activation of the tyrosine kinase catalytic domain.⁵³⁻⁵⁵ Once the Eph receptors are phosphorylated, adaptor proteins can bind and initiate phosphorylation of downstream substrates. Preclinical studies showed that in HUVECs, pre-incubation of sEphB4 in the presence of sEphrinB2 suppressed EphB4 activation via phosphorylation. Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

2.5.2.3 Immune response

Some members of Ephs and Ephrins are also expressed in the lymphoid organs, and their physiological role in immune responses is still being elucidated.⁵⁶ T cells express EphBs and EphrinBs, both of which can provide co-inhibitory or co-stimulatory signals for T-cell proliferation.⁵⁷ Bidirectional signals via Eph receptors and Ephrins have been recognized as major forms of contact-dependent cell communications. EphrinB1 and EphrinB2 increased CD3-mediated murine T-cell proliferation. However, higher concentrations of EphrinB1 and EphrinB2 suppressed proliferation, a mechanism thought to be by phosphorylating EphB receptors.⁵⁸ Similarly, EphrinB2 knockdown expression in human mesenchymal stem cells (MSC) reduced MSC's ability to inhibit T-cell proliferation.⁵⁹ Therefore, there is evidence that EphB-EphrinB interactions play an important role in mediating activation and inhibition of activated T cells, which may be important for tumor suppression of, and immune response to HHV-8, as well as HIV in this population of patients.

sEphB4-HSA's effect on the immune response at the level of the tumor has not been well defined. However, it appears that sEphB4-HSA alters T-cell function. Preclinical studies show that sEphB4 can increase the migration of T cells into the tumor beds, possibly reinstating and activating T cell function. We hypothesize that the disruption of the EphrinB2-EphB4 interaction will activate T-cells and restore previously blocked T-cell function. This hypothesis will be examined with peripheral blood and

biopsy samples. Peripheral blood samples will be used for flow cytometric analysis, cytokine measurements, and *in vitro* studies. See [Appendices I and III](#) for full details of the blood sample collection times. Participants will have two tumor biopsies obtained before initiation of the study, and two biopsies obtained after one cycle of treatment. See [Appendices I and III](#) for full details of the biopsy collection times. The peripheral blood will be shipped to Dr. Jeffrey Bethony directly at room temperature.⁶⁰ See [Appendix IX](#) for shipping details. The tumor samples will be stored at the AMC Biorepository and then shipped to each respective center for specific correlative studies to be performed. See [Appendix VIII](#) shipping details. Biopsy samples will be used to perform cytokine assays and evaluate for tumor regression and immune infiltration. Immune studies will be performed as follows pending funding.

Peripheral blood

To measure the potential effects of sEphB4 on immune cell activity, blood samples will be examined using standard 10-color flow cytometry with Ki-67 as a marker of proliferation and standard markers for T (CD3, CD4, CD8, CD25, CD69, CCR5, CCR7, FOXP3), B (CD20), NK (CD56, CD16), NK-T (Va24, CD56, CD3, CD69), monocytes (CD14, HLA-DR, CD64), and neutrophils (FSC, SSC, CD15, CD16). Blood samples will be obtained within 14 days prior to Cycle 1, Day 1 of study drug (Baseline and Day 1 can be the same day), Day 15 of Cycle 1, and Day 1 of Cycles 2, 4, and 6 before study treatment dosing. Mononuclear cells from the blood will be Ficoll-purified and frozen for future functional studies if needed. These may include Enzyme-Linked Immunosorbent Spot (ELISPOT) or cytokine secretion assays using HHV-8 peptides as stimuli. Peripheral blood will be shipped overnight to Dr. Jeffrey Bethony at George Washington University Medical Center. Flow cytometry will be performed by Dr. Jeffrey Bethony at the George Washington University Medical Center.

Biopsy

To examine cells in the tissue, IHC will be used to detect cells expressing CD45, CD3, CD20, CD68, and CD163 as initial queries into the types of cells that may be preferentially recruited to the tumor site by sEphB4 treatments. Participants will have biopsies performed within 14 days prior to Cycle 1, Day 1 of study drug (Baseline and Day 1 can be the same day), as well as after one cycle of treatment (can be any time after Cycle 1, Day 15 dosing and before treatment on Cycle 2, Day 1). Immunohistochemistry will be performed at the AMC Core Pathology laboratories by Dr. Ethel Cesarman.

2.5.2.4 Apoptosis and proliferation

HHV-8 infection reprograms cellular gene expression upregulating proteins important in proliferation and apoptosis. These pathways regulate cell functions which include cell division, cellular stress response and cell death. We hypothesize sEphB4-HSA will alter and modulate various cellular

pathways important in proliferation and apoptosis. We will obtain the following immunohistochemistry staining on the biopsy samples. Participants will have two tumor biopsies obtained within 14 days prior to Cycle 1, Day 1 of study drug (Baseline and Day 1 can be the same day), as well as after one cycle of treatment (can be any time after Cycle 1, Day 15 dosing and before treatment on Cycle 2, Day 1). The tumor samples will be stored at the AMC Repository and then shipped to each respective center for specific correlative studies to be performed. The following correlative studies will be investigated on the biopsy samples:

LANA-1

LANA-1, latency-associated nuclear antigen, is a HHV-8 associated latent protein. LANA-1 is associated with cellular chromatin and stays on the chromosomes during cell division.⁶¹ It is one of the few proteins consistently shown to be expressed by in latent KS, and likely mediates the episomal replication of the HHV-8 genome.^{62,63} It has been showed to be expressed in KS lesions, but the LANA-1 levels are noted to increase throughout tumor progression.⁶⁴ Immunohistochemistry will be performed at the AMC Core Pathology laboratories by Dr. Ethel Cesarman.

K8.1

The K8.1 glycoprotein is a structural component of the KSHV particle and is thought to facilitate virus entry into the cell. It is a lytic viral antigen and rarely expressed in KS but may be found in a small subset of cases where lytic replication may contribute to KS pathogenesis. In these cases, it has been showed to be predominately expressed in infected KS cells and within virion envelopes. Immunohistochemistry will be performed at the AMC Core Pathology laboratories by Dr. Ethel Cesarman.

TUNEL

TUNEL detects DNA fragmentation by labeling the terminal end of nucleic acids. It is useful for identifying apoptosis. It has been used to confirm apoptosis in cells infected with HHV-8.⁶⁵ Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

CD31

CD31 is also known as platelet endothelial cell adhesion molecule (PECAM-1). CD31 plays a key role in removing aged neutrophils from the body. It is found on platelets, monocytes, neutrophils, some T-cells, and a large portion of endothelial cell intercellular junctions. It is a measure of angiogenesis. CD31 is highly expressed in Kaposi sarcoma and used routinely for the diagnosis of Kaposi sarcoma.⁶⁶ Immunohistochemistry will be performed by Dr. Parkash Gill at USC Norris Hospital.

2.5.2.5 HIV plasma viral loads and T-cell counts in participants with HIV

HIV plasma viral loads and CD4 and CD8 counts will be ordered routinely by each institution at regular intervals of 3 months. Labs will be drawn

before treatment, and on Day 1 of Cycles 4, 7, 10, and at treatment discontinuation. See [Section 8.0](#) and [Appendix I](#) for details of the collection times. Peripheral blood will be drawn; and viral loads and T-cell counts will be performed at the AMC site in which the participant is being treated.

2.5.2.6 Archive PBMCs, plasma, and tumor biopsies

Blood for PBMCs and plasma, and tissue samples will be collected and archived to be used in conjunction with samples collected from subsequent trials of sEphB4-HSA in KS for future studies including identification of biomarkers predictive of response. One of two tumor biopsies obtained before initiation of the study drug, and one of two biopsies obtained after one cycle of study drug will be saved for future studies. These biopsy specimens will be collected in RNAlater. Biopsies will be stored at the AMC Biorepository. Biopsy for KSHV and targeted endothelium gene transcription will be performed by Dr. Dirk Dittmer and the AMC Core Genomics Core labs at the University of North Carolina, Chapel Hill. Pending funding, RNA sequencing via NextGen sequencing will be performed by Dr. William Wachsman at the Moores UCSD Cancer Center Genomics and Bioinformatics Shared Resource.

2.6 Quality of Life Measure

The Functional Assessment of HIV infection (FAHI) instrument was adapted from the Functional Assessment of Cancer Therapy – General (FACT-G) questionnaire and found to have acceptable psychometric properties in HIV-infected persons.⁶⁷ The instrument detected changes in the health status of patients that were not detected through conventional efficacy measures. Individual items in the FAHI are scored into the following sub-scales: physical well-being, emotional well-being, functional well-being, social well-being, and cognitive functioning.

In this study, the FAHI plus 3 KS-specific questions will be used to evaluate health-related QOL measures. The KS-specific questions relate to pain, edema or swelling, and physical appearance.

The FAHI + KS instrument has previously been used in the AMC009/ECOG E1D96 study that randomized AIDS-KS participants to receive paclitaxel or liposomal doxorubicin. The treatment arms were combined for the QOL analysis which demonstrated improvements with treatment in cognitive functioning, social well-being, pain and swelling.¹⁸

Like previous AMC studies, each participant will serve as his/her baseline; this will account for variable baseline assessments. While the FAHI+KS questionnaire has not been validated in a large cohort of KS participants, and will not be in this phase II trial, as an exploratory study, the utilization of the same QOL instrument would facilitate comparisons between previous AMC studies. In addition, QOL assessments would provide meaningful education and empowerment for the patient, as well as allow us to examine and describe whether QOL adds to our understanding of sEphB4-HSA's clinical use. All participants, HIV and non-HIV positive, will be assessed via the FAHI+KS questionnaire. Non-HIV KS is typically cutaneous in nature and also associated with the virus KSHV. Patients with non-HIV KS are similarly concerned about appearance, and about spreading the disease

and KSHV virus; these issues are captured by the FAHI+KS questionnaire. sEphB4-HSA is a novel drug, and as we try to move into an era which evaluates alternative treatments beyond chemotherapy drugs, patients may be able to influence the delivery of clinical trials by converting their experience into constructive and productive hypotheses for subsequent trials and future clinical recommendations.

3.0 PARTICIPANT SELECTION

A rostered AMC investigator (Cancer Therapy Evaluation Program [CTEP]-registered physician investigator) must document that each protocol participant meets all stated eligibility criteria. Participating sites must have documentation that each eligibility requirement is satisfied prior to participant enrollment. In compliance with CTEP policy, no exceptions to eligibility criteria will be granted under any circumstance.

NOTE: Institutions may use this section of the protocol as an eligibility checklist for source documentation if it has been reviewed, signed, and dated before registration/randomization by the study investigator. If used as source documentation, this checklist must be printed, the investigator must check each item to document their assessment that the participant meets each eligibility criterion, and the completed checklist must be maintained in the participant's chart.

Participant ID Number: 096 - _____ - _____

Patient's Initials (F, M [optional], L): _____

NOTE: All questions regarding eligibility should be directed to the study chair.

3.1 Eligibility Criteria

- _____ 3.1.1 Participants may be treatment naïve, refractory to, or intolerant of one or more prior therapies, or treated with prior systemic treatment including but not limited to liposomal doxorubicin.
- _____ 3.1.2 Participants must have biopsy-proven KS involving skin, with or without visceral involvement.
- _____ 3.1.3 If HIV-positive, any CD4 count will be allowed on study.
- _____ 3.1.4 Age \geq 18 years.
- _____ 3.1.5 Eastern Cooperative Oncology Group (ECOG) performance status \leq 2 or Karnofsky performance score (KPS) \geq 50% ([Appendix IV](#)).
- _____ 3.1.6 Life expectancy of greater than 3 months.
- _____ 3.1.7 Participants must have normal organ and marrow function as defined below within 21 days of enrollment:

Absolute neutrophil count (ANC)	\geq 1,500/mcL*
Platelets	\geq 100,000/mcL
Total bilirubin	\leq 1.5 X upper limit of normal (ULN)
aspartate aminotransferase (AST) or serum glutamic-oxaloacetic transaminase (SGOT) / alanine aminotransferase (ALT) or serum glutamic pyruvic	\leq 2.5 X ULN

transaminase (SGPT)	
Creatinine within normal institutional limit for the reference lab OR creatinine clearance	≥ 60 mL/min/1.73 m ² as calculated by Cockcroft-Gault formula for participants with creatinine levels above institutional normal

*Participants may be receiving growth factor support to meet these criteria

- _____ 3.1.8 Participants must have cutaneous lesion(s) amenable to four (4) 5-mm tumor biopsies during the study (either 4 separate lesions measuring ≥ 5 mm each OR 2 separate lesions measuring ≥ 10 mm each) and at least five additional lesions measurable for assessment with no improvement over the past month.
- _____ 3.1.9 Females of childbearing potential (FCBP)[†] must have a negative serum or urine pregnancy test with a sensitivity of at least 25 mIU/mL within 14 days prior to enrollment and again within 24 hours prior to starting Cycle 1 of sEphB4-HSA. Further, they must either commit to continued abstinence from heterosexual intercourse or begin TWO acceptable methods of birth control: one highly effective method and one additional effective method AT THE SAME TIME during receipt of sEphB4-HSA, and 12 weeks after discontinuation of sEphB4-HSA. FCBP must also agree to ongoing pregnancy testing. Men must agree to use a latex condom during sexual contact with a FCBP even if they have had a successful vasectomy. See [Appendix XI](#): Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods.
- [†] A female of childbearing potential is a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).
- _____ 3.1.10 Documentation of HIV status. If participant is HIV positive, HIV-1 infection, as documented by any federally approved, licensed HIV rapid test performed in conjunction with screening (or ELISA test kit, and confirmed by Western blot or other approved test, or HIV rapid multispot antibody differentiation assay). Alternatively, this documentation may include a record demonstrating that another physician has documented the participant's HIV status based on either: 1) approved diagnostic tests, or 2) the referring physician's written record that HIV infection was documented, with supporting information on the participant's relevant medical history and/or current management of HIV infection.
- If the participant is HIV-negative, documentation of a negative result for any federally approved, licensed HIV rapid test within 4 weeks prior to study enrollment will suffice. If the initial rapid test is positive, further approved confirmatory test results must be present to document the subject's HIV status.
- _____ 3.1.11 If participant is HIV positive, participant must be on a stable antiretroviral regimen for at least 12 weeks prior to study enrollment.
- _____ 3.1.12 There should be no evidence for improvement in KS in the 3 months prior to study enrollment, unless there is evidence for progression of KS in the 4 weeks

immediately prior to study enrollment.

- ____ 3.1.13 Participants must, in the opinion of the investigator, be capable of complying with the protocol.

3.2 Exclusion Criteria

Participants who do not fulfill the criteria as listed in [Section 3.1](#) above, are ineligible. Additionally, the presence of any of the following conditions will exclude a participant from study enrollment:

- ___ 3.2.1 Inability to understand and inability to provide informed consent.
- ___ 3.2.2 Participants who are receiving any other investigational agents.
- ___ 3.2.3 Participants who have had anti-neoplastic treatment for KS (including chemotherapy, radiotherapy, local treatment including topical 5-FU, biological therapy or investigational therapy) within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study OR those who have not recovered from adverse events due to agents administered more than 4 weeks earlier.
- ___ 3.2.4 Participants with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
- ___ 3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to sEphB4-HSA or other agents used in study.
- ___ 3.2.6 Concurrent, acute, active infection, or treatment for infection, other than oral thrush, genital herpes, or long-term (i.e., > 1 month) suppression therapy after treatment and clearance of the infection episode (examples include *Coccidioides*, *mycobacterium avium* complex) within 14 days of enrollment.
- ___ 3.2.7 Participants for whom front-line cytotoxic therapy is indicated (i.e., symptomatic visceral or pulmonary KS or symptomatic KS impairing functional status).
- ___ 3.2.8 Concurrent neoplasia requiring cytotoxic therapy.
- ___ 3.2.9 Participant is ≤ 2 years free of another primary malignancy. Exceptions include the following:
 - Basal cell skin cancer
 - Cervical carcinoma in situ
 - Anal carcinoma in situ
- ___ 3.2.10 Any steroid treatment except for that required for replacement therapy in adrenal insufficiency, topical or injected testosterone for hypogonadism, or inhaled steroids for the treatment of asthma.
- ___ 3.2.11 Previous local therapy of any KS-indicator lesion unless the lesion has clearly progressed since that local 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.
- ___ 3.2.12 Female participants who are pregnant, lactating, or breastfeeding.
- ___ 3.2.13 Pregnant women are excluded from this study because sEphB4-HSA has not been tested in pregnant women and it could have potential teratogenic or abortifacient

effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with sEphB4-HSA, breastfeeding should be discontinued if the mother is treated with sEphB4-HSA.

- ___ 3.2.14 Participants with a recent history (< 6 months) of a major infarct including but not inclusive to bowel ischemia, cerebral vascular accident, transient ischemic attack, myocardial infarction, limb ischemia, or skin necrosis.
- ___ 3.2.15 Participants with a QTcF (Fridericia Correction Formula) > 480 ms on 2 out of 3 electrocardiograms (EKGs) (if first EKG is < 480, no need to repeat, if first EKG is > 480 repeat twice for a total of 3 EKGs).
- ___ 3.2.16 Participants with systolic blood pressure >120 mm Hg or diastolic blood pressure >80 mm Hg who are unwilling to start antihypertensives (see [Section 5.4](#) for recommendations for management of hypertension during the study), or participants with systolic blood pressure > 140 mm Hg, or diastolic blood pressure > 90 mm Hg, with or without use of antihypertensive medication (participants *may not* enroll onto the study until blood pressure (BP) is \leq 140/90 mm Hg with or without antihypertensive medication).
- ___ 3.2.17 Participants with a recent history (< 6 months) of a major bleed which will be defined as a symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarticular or pericardial, or intramuscular with compartment syndrome, and/or bleeding causing a fall in hemoglobin level 2 grams/dL or more or leading to transfusion of two or more units of whole blood or packed red cells.
- ___ 3.2.18 Participants on any dose of warfarin or on full dose anticoagulation with other agents (including low molecular weight heparin, antithrombin agents, antiplatelet agents and full dose aspirin) within 7 days prior to study enrollment; participants on prophylactic doses of low molecular weight heparin and low-dose anticoagulants are allowed.
- ___ 3.2.19 Cardiac related illnesses including, but not limited to:
 - Symptomatic congestive heart failure including participants with grade III/IV cardiac disease as defined by the New York Heart Association (NYHA) functional criteria
 - Unstable angina pectoris
 - Cardiac arrhythmia
- ___ 3.2.20 Proteinuria as defined as \geq 2+ on urine dipstick. If dipstick urinalysis shows \geq 2+ proteinuria, 24-hour urine for protein must be < 2 grams.
- ___ 3.2.21 Participants with diabetes mellitus with ketoacidosis or chronic obstructive pulmonary disease (COPD) requiring hospitalization in the preceding 6 months, or any other intercurrent medical condition that contraindicates treatment with sEphB4-HSA or places the participant at undue risk for treatment-related complications.

- ____ 3.2.22 Physical or psychiatric illness/social situations that in the estimation of the investigator would limit compliance with study requirements or place the participant at high risk of toxicity or non-compliance.

Physician Signature: _____ Date: _____

(Optional unless this section is used as an eligibility checklist)

3.3 Number of Participants to be Enrolled

3.3.1 Proposed sample size

This study will enroll a minimum of 6 participants and a maximum of 20 participants.

3.3.2 Accrual rate

Approximately 1 participant per month.

3.4 Participant Enrollment Procedures

Sites must have this protocol approved by their Institutional Review Boards (IRB) and be registered for study participation with the AMC Operations and Data Management Center (ODMC) before they may enroll participants.

After it has been determined that the participant is eligible and an informed consent form has been signed by the participant, the participant must be registered on-line via the AMC Advantage eClinical Internet Data Entry System (Advantage eClinical) Enrollment and data collection will occur via the AMC Internet Data Entry System.

The participating site will ensure a participant meets all eligibility criteria prior to completing the protocol-specific eligibility checklist in Advantage eClinical for enrollment. Participants will be enrolled on-line via Advantage eClinical 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 participant enrollment. If the on-line system is inaccessible, the site should notify the AMC ODMC (via email at amcpm@emmes.com or via phone at 301-251-1161) for further instructions.

4.0 TREATMENT PLAN

4.1 Agent Administration

Protocol agents will be administered on an outpatient basis. Appropriate dose modifications for sEphB4-HSA are described in [Section 5.0](#). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Each cycle of sEphB4-HSA will be 28 days. sEphB4-HSA is administered intravenously. Each cycle includes administration of 2 doses of sEphB4-HSA intravenously at 10 mg/kg administered on Days 1 and 15. With protocol versions earlier than 4.0, a 15 mg.kg dose level was used as dose level 1, and has been discontinued as discussed in [Section 2.3.8](#).

Study medications will be dispensed at study entry (Day 1) and at each subsequent study visit. sEphB4-HSA will be administered via intravenous infusion at approximately the same time each day.

Whenever possible, Cycle 1 Day 1 should be scheduled on a Monday due to timing of laboratory and biopsy samples. The doses should be calculated prior to or on Day 1 of each cycle; the dose administered should remain the same throughout a cycle but should be recalculated at the start of the next cycle. Doses must be adjusted if a participant's weight has changed more than 10% from baseline or the prior dose. Otherwise, doses may be adjusted in conjunction with institutional guidelines. When holidays or other scheduling conflicts exist, doses may be delayed, ideally no more than 1 day (see [Section 5.6](#) for additional guidance on management of dose interruptions).

If any participant develops major bleeding or any unexpected grade 4 or greater event on study, accrual will be held until the PI and sponsor assess the need for changes to protocol or consent. Dose modifications for other toxicity will be allowed and are described below. Participants requiring a delay of > 28 days (4 weeks) due to drug related toxicity will be removed from the study.

Table 4-A: Standard dosing

Regimen Description – INITIAL DOSE LEVEL					
Agent	Premedications/ Precautions	Dose	Route	Schedule	Cycle Length
sEphB4-HSA	No premedication*	10 mg/kg	I.V. over 1 hour**	Days 1 and 15	28 days (4 weeks)
*Reactions are not anticipated. If fevers, chills, or a mild allergic reaction occurs, acetaminophen and/or antihistamines (intravenously or orally) may be administered according to the institutional policy and guidelines. For subsequent doses, acetaminophen and/or antihistamines (intravenously or orally) are recommended prior to re-dosing of sEphB4-HSA.					
**Infusion time variation is allowed (1 hour ± 15 minutes)					

Each cycle of the study drug includes administration of 2 doses of sEphB4-HSA during a 28 day cycle. Doses will be administered on Days 1 and 15 but may be delayed up to 48 hours if necessary. If complete response (CR) is achieved, the participant will receive one cycle beyond CR. Treatment may be resumed if KS progresses off treatment after a CR. The participant will resume the previous dose that he/she was receiving when CR was achieved. Participants who achieve PR or SD may continue the treatment at the dose he/she was receiving when PR or SD was achieved, for a maximum of 12 cycles, if the participant and treating physician feel that it is beneficial to continue treatment. The maximum duration of treatment on study is twelve 4-week cycles.

4.2 General Concomitant Medication and Supportive Care Guidelines

Given it is a soluble protein cleared by the reticuloendothelial system, no drug interactions are anticipated with sEphB4-HSA. However, given this drug is still early in its development, the concurrent use of all other drugs, over-the-counter medications, alternative therapies, herbal medicines/tea, or grapefruit juice must be captured in the Concomitant Medications Form in Advantage eClinical. The Principal Investigator should be alerted if the participant is taking any agent known to affect or with the potential to affect CYP450 isoenzymes.

Participants MUST receive medically appropriate care and treatment for HIV infection, including antiretroviral medications. Due to known cytochrome P450 inhibitory effects of protease inhibitors, particularly for ritonavir, the case report form will particularly note the use of ritonavir for HIV management.

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the participant are allowed, provided their use is documented in the participant records and on the appropriate CRF. Use of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) is not permitted. Low-dose prophylactic heparin will be allowed.

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. Interactions of sEphB4-HSA with antiretroviral therapy and any other drug have not been studied, however drug-drug interactions with other common concurrent medications used in the HIV participants are not anticipated as this is a fusion protein of sEphB4-HSA and is degraded by the reticuloendothelial system.

No prophylactic or supportive care regimens are required for this drug. No significant irritation related to I.V. administration of sEphB4-HSA has been observed at the injection sites. Fever, chills, shakes, itching, rash, hyper- or hypotension, difficulty breathing have been noted in a previous study with this drug, but after evaluation were deemed unrelated to the drug. However, if any of these symptoms occur, slow or interrupt the infusion and administer supportive care, acetaminophen and/or antihistamines (I.V. or P.O.) as per institutional policy and guidelines.

Nausea or vomiting has been reported in patients receiving this agent. Administer supportive care according to the institutional policy and guidelines.

Rash and pruritus generally resolve when drug therapy is discontinued. No drug related significant skin reaction has been observed thus far.

4.2.1 Required medications

- If HIV positive, participants must be on stable antiretroviral therapy for a minimum of 12 weeks prior to study enrollment. Antiretroviral therapy with a minimum of three active drugs, which may include the HIV protease inhibitors, non-nucleoside reverse transcriptase inhibitors (NNRTI), nucleoside (and nucleotide) reverse transcriptase inhibitors (NRTI), integrase strand transfer inhibitors (INSTI), CCR5 antagonists, or fusion inhibitors, is strongly recommended.
- If HIV positive, chemoprophylaxis for *Pneumocystis jirovecii* pneumonia is required for all participants with a CD4 count ≤ 200 cells/mm³.
- Participants refusing antiretroviral therapy for HIV are ineligible to participate in this study.

4.2.2 Permitted medications

- Topical and/or antifungal agents are permitted for prophylaxis.
- Treatment maintenance or chemoprophylaxis with approved agents for opportunistic infections as clinically indicated is permitted.
- Regularly prescribed prescriptions such as antipyretics, analgesics, allergy medications, anti-depressants, sleep medications, oral contraceptives, megestrol acetate, testosterone, or any other medications which are not specifically prohibited will be allowed. This will include alternative therapies such as vitamins, acupuncture, and visualization techniques. Participants should, however, report the use of these therapies, and they will be recorded. However, aspirin or NSAIDS will not be permitted.
- Low dose prophylactic heparin will be permitted.

4.2.3 Prohibited medications

- Investigational drugs will be prohibited as will systemic cytotoxic chemotherapy or other KS-specific treatments.

4.3 Duration of Therapy

In the absence of treatment delays due to AE(s), treatment may continue for a maximum of 12 cycles or until one of the following criteria listed in [Section 4.5](#) applies. If CR is achieved, the participant will receive one cycle beyond CR. Treatment may be resumed if KS progresses off treatment after a CR, up to a maximum of 12 cycles. The participant will resume the previous dose that he/she was receiving when CR was achieved. Participants who achieve PR or SD may continue the treatment at the dose he/she was receiving when PR or SD was achieved for a maximum of 12 cycles, if the participant and treating physician feel that it is beneficial to continue treatment.

4.4 Duration of Follow-up

Participants who have PR or better will be followed up every three months, for up to one year, or until an earlier time of disease progression requiring additional treatment. All other participants will be followed for 4 weeks after completion of study treatment, removal from study treatment, or until death, whichever occurs first. Participants who do not achieve a PR or better and are removed from treatment early will be reported as off-treatment on the last date of agent administration. Participants removed from study treatment for unacceptable AE(s) will be followed until resolution or stabilization of the AE. Participants who withdraw from treatment for toxicity reasons should be followed until the toxicity resolves/returns to baseline, or for 4 weeks, whichever is later. In addition, participants who go off study treatment for reasons other than toxicity should be followed for at least 4 weeks after discontinuing drug.

4.5 Criteria for Removal from Treatment

Participants will be removed from study treatment when any of the criteria listed in [Section 5.0](#) and below applies. The reason for study treatment removal and the date of the participant's last treatment must be documented in the Off Protocol Treatment Form in Advantage eClinical. The date the participant was removed from study treatment must be documented in the source.

- Uncontrolled sustained hypertension, which will be defined as systolic BP > 140, and diastolic BP > 90, even with use of antihypertensive medications.
- Disease progression after completing 2 or more cycles (or at least after 4 doses of sEphB4-HSA).
- Dose delay of more than 28 days.
- Rapid progression of disease that is deemed to be life-threatening.
- Intercurrent illness that prevents further administration of treatment.
- Any participant who develops Multicentric Castleman's disease or a life-threatening systemic inflammatory syndrome will not receive further sEphB4-HSA and will be managed using the best judgment of the treating physician.
 - **These events require mandatory notification of a study chair within 24 hours which will result in immediate notification of all participating sites and a conference call to assess the safety of continued enrollment.**
- Participants who experience Steven-Johnson Syndrome or major allergic reaction.
- Participants who become pregnant or need to breast-feed.
- Participants who require systemic chemotherapy for the treatment of a malignancy other than KS.
- Participants who are noncompliant with respect to taking drugs, keeping appointments, or having tests required for the evaluation of drug safety and efficacy.
- Participants have the right to withdraw from the study at any time for any reason.

- The Investigator has the right to remove participants from the study for clinical reasons which he/she believes are life-threatening to the participant even if such reasons do not fall into the toxicity classifications discussed in [Section 5.0](#).
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the investigator.
- Unacceptable AE(s).

5.0 DOSING DELAYS/DOSE MODIFICATIONS

5.1 Dose Modifications for sEphB4-HSA

If any participant develops major bleeding or any unexpected grade 4 or greater event on study, accrual will be held until the PI and sponsor assess the need for changes to prophylaxis or changes to protocol or consent. In the event accrual is stopped, accrual will not resume until CTEP has reviewed the decision in the case where the PI and Sponsor feel no consent or protocol changes were needed. Dose modifications for other toxicities will be allowed and are described below. Participants requiring a delay of > 28 days (4 weeks) due to drug-related toxicity will be removed from the study.

When there is a dose modification, the participant should be notified of the change in dose.

Table 5-A: Dose modifications

LEVEL	DOSE	SCHEDULE
0	10 mg/kg	Days 1 and 15 (28 day cycle)
-1	7.5mg mg/kg	Days 1 and 15 (28 day cycle)
-2	5.0 mg/kg	Days 1 and 15 (28 day cycle)

5.2 Dose Modifications for Non-Hematologic Toxicity (EXCEPT hypertension)

See [Section 5.4](#) for dose modifications for hypertension.

Symptomatic grade 2 toxicity

If the participant experiences a new symptomatic grade 2 non-hematologic toxicity, sEphB4-HSA will be withheld until the toxicity has resolved to \leq grade 1. sEphB4-HSA will then be resumed at the same weekly dose. If the symptomatic grade 2 toxicity recurs despite optimal supportive care (e.g., fluids, electrolyte replacement, anti-diarrheal medications), sEphB4-HSA will be withheld until the toxicity has resolved to \leq grade 1, and the weekly dose may be reduced by one dose level. If symptomatic grade 2 toxicity recurs at the reduced dose, sEphB4-HSA treatment will be discontinued.

Grade 3 or 4 toxicity

If the participant experiences grade 3/4 non-hematologic toxicity, sEphB4-HSA must be withheld until the toxicity has resolved to \leq grade 1 or baseline. sEphB4-HSA will then be resumed at a weekly dose that is reduced by one dose level. If the grade 3/4 toxicity recurs at the lower dose (level -1) dosing, sEphB4-HSA treatment will be stopped. If any participant experiences Steven-Johnson Syndrome, major allergic reaction which requires epinephrine, grade 3 neuropathy lasting at least 4 weeks, or grade 4 non-hematological toxicity that does not resolve with optimal supportive care (e.g., fluids, electrolyte replacement, anti-diarrheal medications), sEphB4-HSA must be discontinued, and the participant taken off study.

See below for a summary of dose modifications for non-hematologic toxicities.

Table 5-B: Summary of dose modifications for non-hematologic toxicity (except for hypertension)

OCCURRENCE	SYMPTOMATIC GRADE 2	GRADE 3/4
1 st	Hold until \leq grade 1, then resume at same dose	Hold until \leq grade 1 or baseline, then reduce sEphB4-HSA by one dose level
2 nd	Hold until \leq grade 1 then reduce dose by one dose level. If the dose is a lowest level, sEphB4-HSA may be stopped	Stop treatment
3 rd	Stop treatment	N/A

5.3 Dose Modifications for Renal Toxicity

If any participant experiences renal failure or renal toxicity with $\text{CrCl} < 60 \text{ mL/min}$ (calculated by modified Cockcroft-Gault method or 24-hour collection method at the Investigator's discretion), sEphB4-HSA must be withheld until toxicity has resolved. Serum creatinine must be evaluated weekly until returns to baseline. If renal toxicity does not resolve within 7 days, and CrCl is $> 30 \text{ mL/min}$ (calculated by Cockcroft-Gault method or 24-hour collection method at the Investigator's discretion), then sEphB4-HSA may be restarted at the next lowest dose level. If renal toxicity recurs and is felt to be related to sEphB4-HSA, and/or with $\text{CrCl} < 30 \text{ mL/min}$ (calculated by Cockcroft-Gault method or 24-hour collection method at the Investigator's discretion), then sEphB4-HSA treatment will be stopped.

If any participant experiences proteinuria, defined as $\geq 2+$ proteinuria on urinalysis, the following will be done.

If urine dipstick results are:

- 1) Negative, trace, or 1+ protein: proceed with administration of study drug.
- 2) 2+ protein: Proceed with study drug and collect 24-hour urine within 3 days before next cycle
 - 24-hour proteinuria $< 2 \text{ g}$: Give study drug. Continue to follow by 24-hour urinary protein before each cycle. If urinary protein falls to $< 1 \text{ g}$, resume monitoring by dipstick method.
 - 24-hour proteinuria $\geq 2 \text{ g}$: Hold study drug and repeat 24-hour urine collection for proteinuria before the next planned dose.
 - o Repeat 24-hour proteinuria $< 2 \text{ g}$: Give study drug and continue to check 24-hour protein before each cycle. If protein decreases to $< 1 \text{ g}$, resume monitoring by dipstick urinalysis.
 - o Repeat 24-hour urine $\geq 2 \text{ g}$: Hold study drug and continue to check before each dose of planned treatment. Give study drug if protein decreases to $< 2 \text{ g}$. If not $< 2 \text{ g}$ after 3 planned doses, discontinue study drug.

- 3) 3+ proteinuria: Hold and check 24-hour urinary protein. Restart study drug if 24-hour urinary protein decreases to <2 g. If not <2 g after 3 planned doses, discontinue study drug.
- 4) Grade 4: Discontinue study drug.

5.4 Dose Modifications for Hypertension

Hypertension: this is an expected AE within the class of anti-neoplastic drugs affecting the VEGF pathway. In participants with a baseline BP $\geq 120/80$ mm Hg and cardiac risk factors (history of hyperlipidemia, smoking, diabetes, treatment for hypertension, African-American ethnicity),⁶⁷ hypertension should be anticipated and therefore may need more aggressive management *prior to* first dose of study drug. In these cases, it is recommended that an internal medicine specialist or cardiologist be consulted to assist in management of hypertension. BPs are required at all visits prior to drug administration. All BP measurements should be made after the patient has been in a sitting or supine position for 5 minutes or longer.

Unless the patient has congestive heart failure, significant peripheral edema and/or significant salt intake that is difficult to modify, ACE inhibitors, ARBs and calcium channel blockers (nifedipine and amlodipine) may be more effective than diuretics.⁶⁸

Grade 2 Hypertension (systolic BP 140 - 159 mm Hg or diastolic BP 90 - 99 mm Hg)

Participants with grade 2 hypertension as documented by two or more different measurements - that are at least 30 minutes apart and measured by a healthcare provider - must be initiated on antihypertensive therapy per the treating physician. Treatment with sEphB4-HSA may continue during this time. BP should be rechecked 3-5 days after initiation of an antihypertensive, or after approximately 5 half-lives to allow antihypertensive drug levels to achieve steady state (~5 days for an antihypertensive dosed daily and ~3 days for one dosed twice daily). If systolic blood pressure (SBP) is still ≥ 140 mm Hg and/or diastolic blood pressure (DBP) is ≥ 90 mm Hg, physician should increase the dose of the antihypertensive or add additional antihypertensive therapy as indicated. Continue to monitor BP and titrate antihypertensives every 3-5 days until target BP (<140/90 mm Hg) is achieved.

Grade 3 Hypertension (systolic BP ≥ 160 mm Hg or diastolic BP ≥ 100 mm Hg)

In the case of grade 3 hypertension as documented by two or more different measurements - that are at least 30 minutes apart and measured by a healthcare provider - treatment with sEphB4-HSA must be held until hypertension returns to target BP (<140/90 mm Hg). Participants should be initiated on antihypertensive therapy, or if already on antihypertensive therapy, participants should have their antihypertensive therapy titrated, per the treating physician. BP should be rechecked 3-5 days after initiation or titration of antihypertensive therapy, or after approximately 5 half-lives to allow antihypertensive drug levels to achieve steady state (~5 days for an antihypertensive dosed daily and ~3 days for one dosed twice daily). If SBP is still ≥ 140 mm Hg and/or DBP is ≥ 90 mm Hg, the antihypertensive dose should be increased, or additional antihypertensive therapy added as indicated. Continue to monitor BP and titrate antihypertensives every 3-5 days until target BP (<140/90 mm Hg) is achieved. sEphB4-HSA should be held until hypertension returns to target BP (<140/90 mm Hg) and patient is asymptomatic, at which point sEphB4-HSA

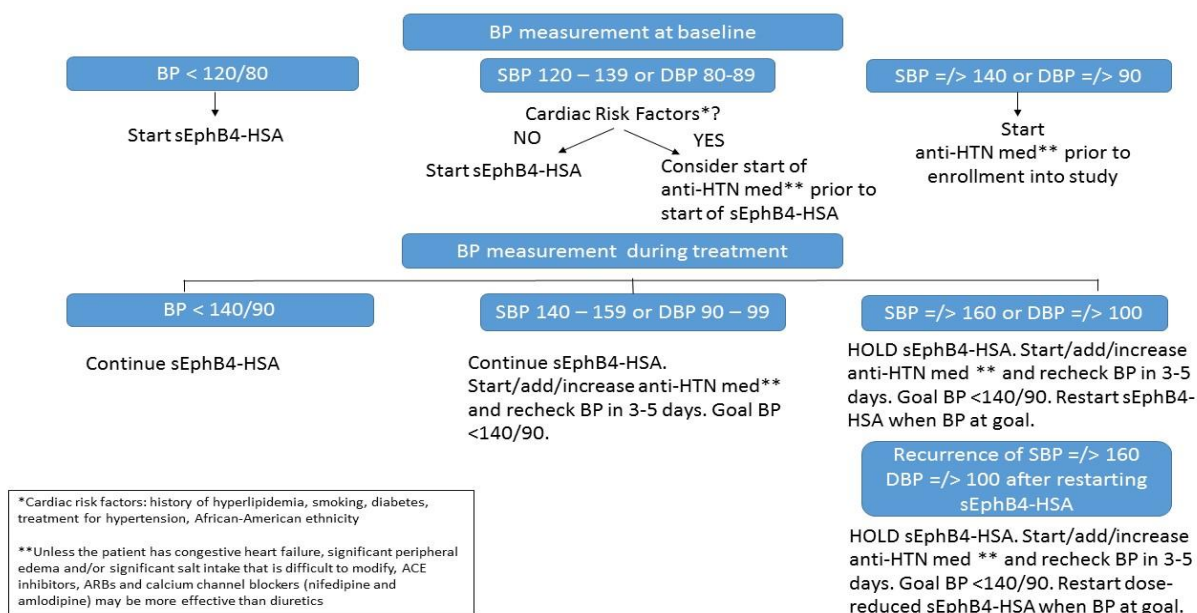
treatment can be resumed.

If after subsequent dosing of sEphB4-HSA, grade 3 hypertension recurs - as documented by two or more different measurements that are at least one hour apart and measured by a healthcare provider - hold sEphB4-HSA and titrate antihypertensives as above until target BP (<140/90 mm Hg) is achieved. When subsequent dosing of sEphB4-HSA is restarted, patient will require reduction of sEphB4-HSA by one dose level.

If patient has to hold treatment for 4 weeks due to hypertension, sEphB4-HSA treatment will be discontinued.

All participants must be educated about the hypertension side effect and instructed to contact the study team if their BP remains under poor control between visits. It is recommended participants keep a BP log, but this is up to the treating physician's discretion.

Hypertension Management Schema



5.5 Dose Modifications for Hematologic Toxicity

Grade 1 or 2

There will be no dose interruptions or reductions for any grade 1/2 hematological toxicity.

Grade 3 or 4 Thrombocytopenia

If the participant experiences grade 3/4 thrombocytopenia, defined as a platelet count < 50 x 10⁹/L, sEphB4-HSA must be withheld until the toxicity has resolved to ≤ grade 2 within 14 days (2 weeks). sEphB4-HSA treatment may then be resumed at dose that is reduced by one dose level. If ≤ grade 2 not reached within 14 days (2 weeks), then stop treatment. If the grade 3/4 thrombocytopenia recurs after dose reduction, sEphB4-HSA must again be withheld until the toxicity level returns to < grade 2. If the toxicity again resolves to < grade 2 within two weeks, sEphB4-HSA may be recommenced, with the dose again reduced by one dose level. If sEphB4-HSA is at the lowest dose level, stop treatment. If, at any time,

the grade 3/4 toxicity persists for longer than two weeks, sEphB4-HSA will be stopped permanently. If grade 3 or 4 thrombocytopenia recurs at dose level 1, treatment will be stopped permanently. A grade 4 hematological toxicity lasting more than 4 weeks will be recorded as an unacceptable toxicity.

Table 5-C: Summary of dose modifications for thrombocytopenia

OCCURRENCE	GRADE 3/4
1 st	Hold until \leq grade 2 within 14 days (2 weeks), then resume at dose reduced by one dose level. If \leq grade 2 not reached within 14 days (2 weeks), then stop treatment.
2 nd	Hold until \leq grade 2 within 14 days (2 weeks), then resume at dose again reduced by one dose level. If at lowest dose level, stop treatment. If \leq grade 2 not reached in 14 days (2 weeks), then stop treatment.
3 rd (after 2 nd dose reduction)	Stop treatment.

Grade 3 or 4 Neutropenia

If a participant experiences grade 3/4 neutropenia, defined as an ANC $< 1 \times 10^9/L$, treatment with sEphB4-HSA may be interrupted and growth factor (filgrastim or pegfilgrastim) should be started at standard doses. If growth factor has already been started, growth factor should be continued (NOTE: Only the ANC, but not the white blood cell (WBC) count, will be considered in determining toxicity for purposes of dosage modification (i.e., doses should not be interrupted, or treatment modified for a WBC $< 2.0 \times 10^9/L$ if the ANC is $\geq 1 \times 10^9/L$)).

If the ANC returns to \leq grade 2 within 1 week without any additional toxicity (i.e., fevers), treatment with sEphB4-HSA may continue without dose modification with continued growth factor support.

If the ANC fails to return to \leq grade 2 within one week with growth factor support, or if additional toxicity seen (i.e., fevers), or if \geq grade 3 neutropenia recurs in a participant on sEphB4-HSA with growth factor support, treatment with sEphB4-HSA should be held for one week. If ANC recovers to \leq grade 2 within two weeks and additional toxicities resolve, if any, restart at a reduced dose by one level. If \geq grade 3 neutropenia or fevers persists at 14 days (2 weeks) despite growth factor support, sEphB4-HSA will be stopped. If grade 3 or 4 neutropenia subsequently recurs but ANC recovers to \leq grade 2 within two weeks, restart at a reduced dose by one level.

Table 5-D: Summary of dose modifications for neutropenia

OCCURRENCE	GRADE 3/4
1 st	<ul style="list-style-type: none"> Hold sEphB4-HSA until ANC \leq grade 2

OCCURRENCE	GRADE 3/4
	<ul style="list-style-type: none"> • Add growth factor (filgrastim or pegfilgrastim) if not already on growth factor. <ul style="list-style-type: none"> ◦ If \leq grade 2 within 1 week, continue on study drug at current dose with growth factor. ◦ If not \leq grade 2 within 1 week, or additional infection-related toxicity seen (i.e., fever), hold sEphB4-HSA for another week and continue growth factor. ◦ If \leq grade 2 within 14 days (2 weeks) and infection-related toxicity, if any, resolves, restart sEphB4-HSA at reduced dose by one level. ◦ If not \leq grade 2 within 14 days (2 weeks), stop treatment.
2 nd	<ul style="list-style-type: none"> • Hold sEphB4-HSA until ANC \leq grade 2 • Add growth factor (filgrastim or pegfilgrastim) if not already on growth factor. <ul style="list-style-type: none"> ◦ If \leq grade 2 within 14 days (2 weeks), restart sEphB4-HSA at reduced dose by one level. ◦ If not \leq grade 2 within 14 days (2 weeks), stop treatment.
3 rd – after first dose reduction	Stop treatment

Grade 3 or 4 Anemia

No dose reductions will be performed for grade 3-4 anemia. If the participant develops anemia, participant may be transfused or receive an available recombinant human erythropoietin preparation (epoetin alfa; darbepoetin alfa) at the discretion of the investigator.

5.6 Dose Interruptions

Participants who have a delay in administration of a dose of the study drug of ≤ 48 hours should take the planned dose as soon as possible after the intended time of administration and continue on the same 28-day cycle (i.e., Day 15 of the same cycle will not change if Day 1 is delayed, or the next cycle start date Day 1 will not be delayed). Relevant evaluations due prior to the next sEphB4-HSA dose may be delayed accordingly.

For participants who have a delay in administration of study drug of >48 hours but ≤ 28 days (please refer back to [Sections 5.2](#), [5.3](#), and [5.5](#) for hold and stop parameters for non-hematologic and hematologic toxicities), the study drug administration may continue with the following instructions: If the Day 1 dose of any new cycle is delayed ≥ 48 hours, that cycle start date and subsequent treatment days and cycles will be reset. If the Day 15 dose of is delayed ≥ 48 hours, the following Day 1 dose of the next cycle will not be given until 14 days later. All relevant evaluations will be delayed accordingly.

Any dose interruption of more than 28 days requires discontinuation of protocol therapy.

6.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs ([Section 6.1](#)) and the characteristics of an observed AE ([Section 6.2](#)) will determine whether the event requires expedited (via CTEP-AERS) **in addition** to routine reporting (via Advantage eClinical).

The CTEP Version 5.0 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting beginning April 1, 2018. The CTEP Version 5.0 of the CTCAE is identified and located on the CTEP website at

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

All appropriate treatment areas should have access to a copy of the CTEP Version 5.0 of CTCAE.

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

6.1 Comprehensive Adverse Events and Potential Risks Lists

6.1.1 Adverse event list for sEphB4-HSA

Agent not supplied by CTEP: Please see the investigational brochure for the full details of potential risks.

Table 6-A: sEphB4-HSA expected toxicities:

System Organ Class	Adverse Event Term
General disorders and administration site conditions	Fatigue
Nervous system disorders	Headache
Vascular disorders	Hypertension

Table 6-B: Reported on trials using sEphB4-HSA but with the relationship to sEphB4-HSA still undetermined:

System Organ Class	Adverse Event Term
Gastrointestinal disorders	Nausea
	Vomiting
	Other (hematemesis)
General disorders and administration site conditions	Chills
	Vomiting
	Fever
Neoplasms, benign, malignant and unspecified (incl. cysts and polyps)	Tumor pain
Nervous system disorders	Syncope

Table 6-C: Risks not reported in prior trials of sEphB4-HSA but the occurrence of which will be monitored in this investigation:

System Organ Class	Adverse Event Term
Blood and lymphatic system disorders	Anemia
	Leukocytosis
Cardiac disorders	Left ventricular systolic dysfunction
	Ventricular arrhythmia
	Myocardial infarction
General disorders and administration site conditions	Infusion related reaction
Immune system disorders	Allergic reaction
Investigations	Platelet count decreased
	White blood cell decreased
	Lymphocyte count increased
Metabolism and nutrition disorders	Acidosis
	Tumor lysis syndrome
	Hyperkalemia
	Hyperuricemia
	Hyperphosphatemia
	Hypocalcemia
Musculoskeletal and connective tissue disorders	Arthralgia
	Generalized muscle weakness
	Myalgia
	Myositis
Nervous system disorders	Stroke
	Ischemia cerebrovascular
	Seizure
	Tremor
Renal and urinary disorders	Acute kidney injury
	Proteinuria
Respiratory, thoracic, and mediastinal disorders	Dyspnea
Skin and subcutaneous tissue disorders	Pruritus
	Rash
Vascular disorders	
	Hypotension
	Thrombosis

Note: sEphB4-HSA in combination with other agents could cause an exacerbation of any AE currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

6.2 Classification of AEs by Severity and Relationship to Study Drug Administration

- 6.2.1 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).
- 6.2.2 Life-threatening Adverse Event: Any AE that places the participant or participant, in view of the Investigator, at immediate risk of death from the reaction.
- 6.2.3 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.
- 6.2.4 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 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.
- 6.2.5 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.
- 6.2.6 Unexpected Adverse Event: Any AE that is not listed in available sources including the package insert, the Investigator’s Brochure, or the protocol.
- 6.2.7 CTEP Adverse Event Reporting System (CTEP-AERS): An electronic system for expedited submission of AE reports.
- 6.2.8 Attribution: The determination of whether an AE is related to a medical treatment or procedure. Attribution categories:
 - Definite – The AE is clearly related to the investigational agent.
 - Probable – The AE is likely related to the investigational agent.
 - Possible – The AE may be related to the investigational agent.
 - Unlikely – The AE is doubtfully related to the investigational agent.
 - Unrelated – The AE is clearly NOT related to the investigational agent.

6.3 Expedited Adverse Event Reporting

- 6.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), 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 ([Section 6.3.3](#)).

A 24-hour notification is to be made to AMC ODMC by telephone at 301-251-1161, only when Internet connectivity is disrupted. Once Internet connectivity is restored, a 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

- 6.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other email recipients.

- 6.3.3 Expedited reporting guidelines

All unacceptable toxicities should be reported no later than the end of the current cycle.

Use the NCI protocol number and the protocol-specific participant ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **grade 5 “General disorders and administration site conditions – Disease Progression”** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Table 6-D: Phase 1 and early phase 2 studies: Expedited reporting requirements for adverse events that occur on studies under an IND/IDE within 30 days of the last administration of the investigational agent/intervention ^{1, 2}

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the participant or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- “24-Hour; 5 Calendar Days” - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- “10 Calendar Days” - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

¹Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

 All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

 Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

² For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote “1” above applies after this reporting period.

Effective Date: May 5, 2011

6.3.4 SAE reporting to FDA and VasGene

All SAEs that occur in trial participants following treatment initiation require a report to VasGene within one working day of investigator's awareness of the event, or immediately if the event is life-threatening or fatal. Participating institutions will make this report by submitting the AE Form in Advantage eClinical. The AMC ODMC will manage all SAE report submissions to VasGene in accordance with its agreement with VasGene, using the applicable forms designated for this purpose.

VasGene will be responsible for reporting all serious and unexpected suspected adverse drug reactions (SUSAR) to the FDA for the IND for this trial.

6.4 Routine Adverse Event Reporting

All adverse events **must** be reported in routine study data submissions in the Adverse Event Form. **AEs reported through CTEP-AERS must also be reported in routine study data submissions.**

6.4.1 Clinical laboratory abnormalities

In assessing laboratory results, an abnormal laboratory value will be considered clinically significant and reportable as an adverse event if it is characterized by one or more of the following criteria:

1. Is judged by the investigator to have a causal relationship to the investigational agent
2. Requires clinical intervention or monitoring, such as: close observation, more frequent follow-up assessments, further diagnostic intervention, treatment/therapeutic intervention, or protocol therapy dose modification
3. Is associated with clinical signs or symptoms, which may suggest a disease and/or organ toxicity, or may represent a new condition or worsening of a baseline condition
4. Is associated with a serious adverse event, or is otherwise judged by the Investigator to be of significant clinical impact

Laboratory results that are proven erroneous by repeat testing will not be considered clinically significant.

In general, a laboratory abnormality that is not clinically significant will be consistent with CTCAE grade 1 (mild) or 2 (moderate) severity, as categorized by the relevant severity description in the Investigations System Organ Class (SOC) or Metabolism and Nutrition Disorders SOC. Investigators may not designate laboratory abnormalities that are consistent with grade 3 or greater severity as not clinically significant.

6.4.2 Timeline for routine adverse event reporting

AEs must be reported via Advantage eClinical if the AE began any time within 4 weeks 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 treatment discontinuation, the

event should be reported to the NCI through CTEP-AERS (see [Section 6.3](#)) within 24 hours of when the investigator learns of the event. This information may provide additional insight into the safety of sEphB4-HSA.

6.5 Secondary Malignancy

A secondary malignancy is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation, or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

6.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine adverse event reporting via CDUS unless otherwise specified.

7.0 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in the investigational brochure and [Section 6.0](#).

7.1 sEphB4-HSA, IND Agent (NSC # 782348)

sEphB4-HSA is an investigational agent supplied to AMC investigators by VasGene Therapeutics, Inc.

7.1.1 Classification

sEphB4-HSA is a fully human recombinant protein consisting of the extracellular domain of the human EphB4 receptor at the N-terminus and human albumin at the C-terminus. EphB4-HSA specifically binds to EphrinB2. EphrinB2 is the only ligand for the EphB4 receptor.

7.1.2 CAS registry number

N/A

7.1.3 Molecular formula

sEphB4-HSA is a monomeric recombinant protein with calculated polypeptide molecular weight of 123.3 kDa; the glycosylated form has a molecular weight of 128.7 kDa. It is a fusion protein consisting of the extracellular domain (aa 1 to 522) of human EphB4 receptor (sEphB4) and full length (aa 1 to 585) HSA.

7.1.4 Approximate solubility

sEphB4-HSA is a highly water-soluble polypeptide. The protein remains in solution at a concentration of 25 mg/kg for up to 12 months.

7.1.5 Product description

The molecular weight of EphB4-HSA is 128.7 kDa. This protein is expressed in recombinant CHO cells and produced using standard mammalian cell cultivation methods followed by chromatographic purification. The clinical trial product is a sterile solution for intravenous (I.V.) infusion.

7.1.6 Dosage forms

The final volume of the drug product is 10 mL. Each vial contains 250 mg of drug in a 10 mL volume. The vial is closed and sealed. The quantitative composition of the drug product is shown in the table below:

Ingredient	Amount
sEphB4-HSA	250.0 mg
Sodium chloride, USP	87.6 mg
L-Histidine, USP	2.3 mg
Sucrose, NF	1000 mg
Water for injection, USP	q.s. to 10 mL

7.1.7 Ingredients

The drug product contains sEphB4-HSA at 25 mg/mL in 10 mM L-histidine, 150 mM sodium chloride, and 10% sucrose, pH 7.4.

7.1.8 Packaging

The final container product is a clear solution. The color may vary from light straw yellow, to light bluish or light greenish sterile solution in 10 mL glass vials. Each vial contains 250 mg of drug in a 10 mL volume. The vial is closed with an appropriately sized stopper and seal.

7.1.9 Mode of action

sEphB4-HSA is a fully human fusion protein composed of soluble EphB4 extracellular domain fused at the C-terminus with albumin upon expression as a single 128.7 kDa protein. sEphB4-HSA specifically binds to its ligand EphrinB2, a single pass transmembrane protein of EphrinB family. sEphB4-HSA blocks the interaction between EphB4 RTK and its ligand EphrinB2.

7.1.10 How supplied

sEphB4-HSA is supplied in a single-use 10 mL vial. Each vial contains a concentrated solution with the equivalent of 250 mg of sEphB4-HSA (25 mg/mL).

7.1.11 Storage and stability

sEphB4-HSA is stable at 4°C for up to three years. Temperature excursions are allowed as long as the storage temperature does not fall below 2°C or rise above 8°C. Vials should be protected from light. Recommended safety measures for preparation and handling of sEphB4-HSA using standard practice. Gentle mixing prior to reconstitution is allowed, but vials are not to be shaken and not to have foam. The total dose to be administered will be diluted to a total volume of 200 mL with sterile normal saline and placed in a sterile mixing container. Any remaining drug product in the vial after dilution should be wasted. Once diluted in an I.V. bag for administration, sEphB4-HSA can be stored for up to 12 hours at room temperature/under room light, and 24 hours at 2° to 8°C in the refrigerator. Care must be taken to ensure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent.

7.1.12 Route and method of administration

A 0.2-micron filter should be used to administer this agent. sEphB4-HSA will be administered intravenously as a continuous infusion over 1 hour ± 15 minutes. A peripheral or central line is acceptable as are any standard types of I.V. tubing. It is not to be administered as an I.V. push or bolus injection. The I.V. line can be flushed. Recommended safety measures for handling of sEphB4-HSA using standard practice.

7.1.13 Potential drug interactions

Given it is a soluble protein cleared by the reticuloendothelial system, no drug interactions are anticipated.

7.2 Drug Supply, Distribution, and Pharmacy

sEphB4-HSA is being supplied by VasGene Therapeutics, Inc. The principal investigator (or their authorized designee) at each study site request study drug through designated VasGene personnel. Information on ordering study agent will be maintained in the password-protected section of the AMC Operations website for protocol AMC-096 (www.AIDSCancer.org).

Medication should be kept in a secure locked area at the study site in accordance with the Pharmaceutical Management Branch (PMB) guidelines and applicable regulatory requirements.

7.2.1 Drug orders, transfers, returns and accountability

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>]). Agent disposition (receipt, dispensing, transfer, return or authorized local destruction of un-dispensed agent) shall be documented on the NCI Investigational Agent (Drug) Accountability Record (DARF) or the NCI Investigational Agent Accountability Record for Oral Agents (Oral DARF) as appropriate. Electronic accountability systems may be used. Paper printouts of electronic DARFs must be identical to the NCI DARF. Electronic accountability system database limitations are not valid reasons for improper accountability documentation according to NCI policy. A waiver statement allowing use of electronic DARFs (eDARFs) has not been issued by the NCI and the NCI does not endorse any eDARF pharmacy package. Institutions that choose to use an electronic accountability system must ensure the database is capable of producing a paper printout that is identical to the NCI DARF. A separate DARF is required for each protocol using the same agent. The investigator will ensure that the drugs are used only in accordance with this protocol.

The study drug can be destroyed on site; however, Vasgene prefers to receive the study drug to discard. All drugs can be returned to Vasgene 3539 Casitas Avenue, Los Angeles, CA 90039. Please contact Vasgene at 323-221-7818 for any additional questions regarding drug returns.

This is an open label trial; thus, the investigational agent is not participant specific. The clinical supply can be used for any participants enrolled in the trial.

8.0 CLINICAL AND LABORATORY EVALUATIONS

Schedules shown in the Study Calendar below are provided in [Appendix I](#).

All signs, symptoms, HIV-related and AIDS-defining events (refer to [Appendix V](#)), death and toxicities must be documented. All signs, symptoms and laboratory results 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 CRFs.

All prescription medications taken within 14 days prior to study enrollment and during the intervals between each visit must be recorded. The duration of all anti-HIV medications and all opportunistic infection treatment and/or prophylaxis medications at the time of enrollment must also be recorded. As this drug is still early in its development, the concurrent use of all other drugs, over-the-counter medications, alternative therapies, herbal medicines/tea, or grapefruit juice must be captured in the clinic record and the Concomitant Medications Form in Advantage eClinical.

8.1 Screening/Baseline Evaluations

Baseline evaluations are to be conducted within 21 days prior to study enrollment, unless otherwise specified below. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. See [Appendix I](#) for full details of evaluations.

- 8.1.1 Biopsy diagnostic of KS at any time prior to study enrollment.
- 8.1.2 Informed consent at any time prior to study enrollment.
- 8.1.3 For HIV positive participants, documentation of HIV status at *any time* prior to the study enrollment. If the participant is HIV-negative, documentation of a negative result for any federally approved, licensed HIV rapid test within 4 weeks prior to study enrollment will suffice.
- 8.1.4 Chest X-ray to rule out pulmonary KS (must be done within 28 days of study enrollment). Chest X-ray is not required if a thoracic CT has been performed. Pulmonary involvement must be asymptomatic or minimally symptomatic and not require systemic cytotoxic therapy in the judgment of the investigator. Participants with a positive chest X-ray, thoracic CT or symptoms suggestive of pulmonary disease will have a chest CT performed at entry. This must be done within 28 days of study enrollment. 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.
- 8.1.5 A medical history within 21 days of study enrollment to include the following information:
 - Previous HIV-related and non-HIV-related diagnoses.
 - For HIV positive participants:
 - o Complete prior anti-HIV therapy, immune based therapy and prior anti-tumor therapy, including start dates of current anti-HIV therapy.

- o Assessment of adherence to antiretroviral therapy, participants must be on a stable antiretroviral regimen for at least 12 weeks prior to study enrollment.
 - o CDC HIV risk categories and review of history of AIDS-defining events (see [Appendix V](#)).
 - Any history of immunosuppressive therapy.
 - All prescription and non-prescription medications, and alternative therapies, herbal medicines/tea, or grapefruit juice taken within the preceding 14 days.
 - A signs and symptoms assessment prior to study enrollment, including history of weight change.
- 8.1.6 Laboratory studies must be obtained within 21 days (unless otherwise noted) prior to enrollment and will include the following:
- Complete blood count (CBC) with differential.
 - Serum chemistries, liver enzymes (AST (SGOT), ALT (SGPT), alkaline phosphatase), BUN, creatinine, electrolytes (sodium, potassium, chloride, bicarbonate, calcium, phosphorus, glucose) albumin, total protein, bilirubin (direct and indirect).
 - If HIV positive, T-lymphocyte subsets (CD4 and CD8) counts and percentages within 28 days prior to study enrollment.
 - If HIV positive, HIV-1 plasma RNA within 28 days prior to study enrollment.
 - Females of childbearing potential (FCBP)[†] must have a negative serum or urine pregnancy test with a sensitivity of at least 25 mIU/mL within 14 days prior to study enrollment and again within 24 hours of starting sEphB4-HSA, and must either commit to continued abstinence from heterosexual intercourse or begin TWO acceptable methods of birth control, one highly effective method and one additional effective method AT THE SAME TIME. FCBP must also agree to ongoing pregnancy testing. Men must agree to use a latex condom during sexual contact with a FCBP even if they have had a successful vasectomy. See [Appendix XI](#): Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods.
- [†] A female of childbearing potential is a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).
- Urinalysis
- 8.1.7 Biologic endpoints: peripheral blood for biologic endpoints as described in [Appendices I](#) and [III](#) will be collected at baseline, including blood for research purposes. The samples include blood for HHV-8 viral loads, immune studies, and archived research purposes. Screening and Day 1 of Cycle 1 blood samples can be

on the same day as long as the screening was done within 14 days of Day 1 of the study drug.

- 8.1.8 Complete physical exam including the following: vital signs (temperature, pulse, BP, and respiratory rate), height, weight, tumor assessment, and ECOG or KPS performance status. All BP measurements will be obtained after the participant has been sitting in a sitting or supine position for 5 minutes or longer.
- 8.1.9 Tumor assessments with photographic record must be performed prior to initiating treatment. These may be performed on Day 1, but no earlier than 7 days before initiating study treatment. Tumor assessments 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 as described in [Appendix VI](#).
 - Participants should have a sufficient number of non-indicator lesions available for biopsy of a size that will permit four 5-mm tumor biopsies during the study (two biopsies will be performed on Day 1 **prior to** treatment in Cycle 1 and two biopsies will be performed on Day 15 of Cycle 1 **after completion of one cycle**) (biopsies may be performed up until Day 1 of Cycle 2). The two biopsies can be performed on two separate non-indicator lesions measuring > 5 mm OR performed on one non-indicator lesion measuring >10 mm in size.
 - For participants with ≤ 50 total skin and oral lesions, all lesions must be evaluated for changes in number and characteristics. For participants with > 50 total skin and oral lesions, choose three representative areas, for evaluating change in lesion numbers and characteristics (preferably an area with ≥ 5 lesions). **These areas will be photographed as described in [Appendix VI](#).**

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.

- 8.1.10 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 ([Appendix VII](#)).
- 8.1.11 Functional assessment of HIV infection (FAHI) + Kaposi sarcoma (KS) QOL questionnaire ([Appendix XII](#)). Those who have HIV-negative KS will not have the HIV portion scored. This may be done within 14 days of starting the study drug up until Day 1 of Cycle 1.
- 8.1.12 Two 5-mm punch biopsy specimens (one stored in RNALater and the other formalin fixed) to be collected from a non-indicator lesion(s) on Day 1 of Cycle 1 prior to treatment. Biopsies may be obtained within 14 days prior to Day 1 Cycle 1. Two biopsies can be performed on two separate non-indicator lesions measuring >5 mm OR performed on one non-indicator lesion measuring >10 mm in size.

Screening and Day 1 samples can be on the same day.

8.1.13 EKG. Up to three EKGs to ensure participant has a QTcF < 480 ms per [Section 3.2.15](#).

8.1.14 Optional donation to the AIDS and Cancer Specimen Resource (ACSR) for any leftover tissue, or additional samples or tissues. (See [Appendix XIV](#) for ACSR Informed Consent Form and [Appendix XV](#) for shipping ACSR donations).

8.2 Evaluations during Treatment

Evaluations must be completed within 7 days prior to day 1 of each cycle, unless another window is noted. See [Appendix I](#) for full details. Dosing should occur on days 1 and 15 of each cycle but may be delayed up to 48 hours, if necessary. Doses delayed by more than 48 hours but less than 28 days due to toxicity or otherwise, require resetting the treatment schedule as outlined in protocol [Section 5.6](#). Evaluations will be completed as outlined below:

8.2.1 Cycle 1

Clinical assessment at Days 1 and 15 of Cycle 1 to include an assessment of the following unless otherwise specified.

8.2.1.1 KS Tumor Assessment: KS tumor assessments will occur on Day 1 of each cycle.

8.2.1.2 Photographic documentation as described in [Appendix VI](#) 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 to PR, 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 at that visit. Response of KS to treatment will be assessed as described in [Section 9.1](#).

8.2.1.3 A complete physical exam including: vital signs, weight, toxicity assessment (Day 15), and ECOG or KPS performance status. Vital signs must include blood pressure for evaluation of potential hypertension. Hypertension is a known and expected side effect of this drug. All BP measurements will be obtained after the participant has been sitting in a sitting or supine position for 5 minutes or longer. If the BP is elevated, BP will be repeated with another measurement that is at least 30 minutes apart to account for HP stabilization and variability in participants. If the BP is noted to be transiently elevated (decreases to baseline level during second measurement), the event will be documented in local source, but the BP will not be reported as an AE in Advantage eClinical. Please see [Section 5.4](#) for special considerations for participants with cardiac risk factors and specific instructions for participants who develop \geq grade 2 hypertension while on treatment.

8.2.1.4 If HIV-positive, all HIV-related and AIDS-defining events ([Appendix V](#)).

8.2.1.5 Assessment of adherence to antiretroviral therapy (if HIV positive).

- 8.2.1.6 Review of concurrent medications, including current anti-HIV drugs if HIV positive.
- 8.2.1.7 Review of signs and symptoms. Imaging, as appropriate, for suspected visceral KS or for follow-up of visceral KS is at the discretion of the investigator.
- 8.2.1.8 CBC with differential on Days 1 and 15 must be completed within 7 days prior to day 1 and day 15 of each cycle.
- 8.2.1.9 Blood chemistries: electrolytes (sodium, potassium, chloride, bicarbonate, calcium, phosphorus, glucose), total protein, albumin, liver enzymes (AST (SGOT), ALT (SGPT), alkaline phosphatase), BUN, creatinine, bilirubin (direct and indirect) on Days 1 and 15.
- 8.2.1.10 Urinalysis for proteinuria on Day 1.
- 8.2.1.11 Biologic endpoints: peripheral blood for biologic endpoints as described in [Appendices I](#) and [III](#) will be collected on Day 1 and Day 15 of Cycle 1. On Day 1, the samples include blood for HHV-8 viral loads, immune studies, and archived research purposes; screening and Day 1 blood samples can be on the same day as long as the screening was done within 14 days of Day 1 of the study drug. On Day 15, samples include blood for immune studies, and archived research purposes.
- 8.2.1.12 Biologic endpoints: peripheral blood for pharmacokinetics as described in [Appendices I](#) and [II](#) will be collected on Days 1 and 15 of Cycle 1. On Day 1, the samples include blood for pre-dose drug levels, as well as blood for drug levels immediately prior to the end of the infusion (C_{max}). On Day 15, the samples include blood for pre-dose drug levels.
- 8.2.1.13 If HIV positive, T-lymphocyte subsets (CD4 and CD8) on Day 1. Day 1 and screening may be the same as long as the screening was done within 28 days (4 weeks) of Day 1 of study drug.

If HIV positive, HIV-1 plasma RNA on Day 1. Day 1 and screening may be the same as long as the screening was done within 28 days (4 weeks) of Day 1 of study drug.
- 8.2.1.14 HHV-8 viral load on Day 1. Screening and Day 1 blood samples can be on the same day as long as the screening was done within 14 days of Day 1 of the study drug.
- 8.2.1.15 Two 5-mm punch biopsy specimens (one stored in RNAlater and the other formalin fixed) to be collected from a non-indicator lesion(s) on Day 1 of Cycle 1 prior to treatment. Biopsies may be obtained within 14 days prior to Day 1 of Cycle 1. Two biopsies can be performed on two separate non-indicator lesions measuring > 5 mm OR performed on one non-indicator lesion measuring > 10 mm in size. Screening and Day 1 samples can be on the same day. Then, two 5-mm punch biopsy specimens (one stored in RNAlater and the other formalin fixed) will be collected from a non-indicator lesion (s) on Day 15 of Cycle 1 after completion of one cycle

(two doses) of treatment (biopsies may be collected up until Day 1 of Cycle 2). Two biopsies can be performed on two separate non-indicator lesions measuring > 5 mm OR performed on one non-indicator lesion measuring > 10 mm in size.

8.2.1.16 Females of Childbearing Potential (FCBP) with regular, irregular or no menstruation must have a pregnancy test before study enrollment and on Day 1 (see [Appendix XI](#): Risks of Fetal Exposure, Pregnancy Testing Guidelines and Acceptable Birth Control Methods); the test must be done within 24 hours before starting the study drug. All participants must be counseled about pregnancy precautions, risks of fetal exposure, and other risks.

8.2.1.17 Functional assessment of HIV Infection (FAHI) + Kaposi sarcoma (KS) QOL questionnaire ([Appendix XII](#)). Those who have HIV-negative KS will not have the HIV portion scored. Screening and Day 1 questionnaire may be the same if the screening was done within 14 days of starting the study drug.

8.2.2 Cycle 2-12 Assessments (for dosing administered on Days 1 and 15)

Laboratory evaluations must be completed within 7 days prior to Day 1 and Day 15 of each cycle, while interval history and physical exam must occur within 7 days prior to study agent administration. See [Appendix I](#) for full details. Dosing should occur on days 1 and 15 of each cycle but may be delayed up to 48 hours if necessary. Doses delayed by more than 48 hours but less than 28 days due to toxicity or otherwise, require resetting the treatment schedule as outlined in protocol [Section 5.6](#). Clinical assessments to be done on Day 1 of each subsequent cycle to include assessment of the following at each visit unless otherwise specified:

8.2.2.1 KS tumor assessment to be completed on Day 1 of each cycle. Response of KS to treatment is described in [Section 9.1](#). Photographic documentation will be completed at each visit when the KS response category changes ([Appendix VI](#)).

8.2.2.2 A complete physical exam including: vital signs, weight, toxicity assessment, and ECOG or KPS performance status. See [Section 8.2.1.3](#) for BP measurement instructions. See [Section 5.4](#) for special considerations for participants with cardiac risk factors and specific instructions for participants who develop \geq grade 2 hypertension while on treatment. On Day 15, only vital signs including weight will be performed.

8.2.2.3 If HIV positive, assessment of adherence to antiretroviral therapy.

8.2.2.4 Review of concurrent medications, including antiretroviral therapy.

8.2.2.5 If HIV-positive, all HIV-related and AIDS-defining events ([Appendix V](#)).

8.2.2.6 Review of signs and symptoms. Imaging, as appropriate, for suspected visceral KS or for follow-up of visceral KS is at the discretion of the investigator.

- 8.2.2.7 CBC with differential on Days 1 and 15.
- 8.2.2.8 Serum chemistries: electrolytes (sodium, potassium, chloride, bicarbonate, calcium, phosphorus, glucose), total protein, albumin, liver enzymes (AST (SGOT), ALT (SGPT), alkaline phosphatase), BUN, creatinine, bilirubin (direct and indirect) on Days 1 and 15.
- 8.2.2.9 Urinalysis for proteinuria on Day 1.
- 8.2.2.10 Biologic endpoints: peripheral blood for biologic endpoints described in [Appendices I](#) and [III](#) will be collected at Day 1 of Cycles 2, 4, 6, 7, and 10. The samples include blood for immune studies on Day 1 of Cycles 2, 4, and 6 to be done before study treatment dosing. Given this will be a fresh blood sent at room temperature overnight, if the blood for immune studies cannot be collected on Monday-Wednesday (i.e., the dose is administered on Thursday or Friday), this blood work can be obtained the Monday AFTER the dose was given. Blood for HHV-8 viral loads and archived research purposes on Day 1 of Cycles 4, 7, and 10, and at treatment discontinuation.
- 8.2.2.11 Biologic endpoints: peripheral blood for pharmacokinetics as described in [Appendices I](#) and [II](#) will be collected on Day 1 and 15 of Cycle 2; the samples will be drawn pre-dose.
- 8.2.2.12 If HIV positive, T-lymphocyte subsets (CD4 and CD8) to be done every three cycles (Day 1 of Cycle 4, 7, and 10), and at treatment discontinuation.
- 8.2.2.13 If HIV positive, HIV-1 plasma RNA to be done every three cycles (Day 1 of Cycles 4, 7, and 10), and at treatment discontinuation).
- 8.2.2.14 FAHI+KS quality of life questionnaire on Day 15 of Cycle 4 (questionnaire may be performed up until Day 15 of Cycle 5). Those who have HIV-negative KS will not have the HIV portion scored. See [Appendix XII](#) for KAH1+KA questionnaire.

8.3 Treatment Discontinuation

At the time of treatment discontinuation, all evaluations should be completed as soon as possible. AEs must be reported to the AMC Operations and Data Management Center if the AE began any time within 4 weeks 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 treatment discontinuation, the event should be reported to the NCI through CTEP-AERS (see [Section 6.3](#)) within 24 hours of when the investigator learns of the event. This information may provide additional insight into the safety of sEphB4-HSA.

The following procedures should be performed upon discontinuation of study drug:

- CBC with differential.
- Serum chemistries: liver enzymes (AST [SGOT], ALT [SGPT], alkaline phosphatase), BUN, creatinine, electrolytes (sodium, potassium, chloride, bicarbonate, calcium,

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

- Complete physical examination including: vital signs, weight, ECOG or KPS performance status, signs and symptoms review, and toxicity evaluation.
- Review of antiretrovirals and adherence to antiretroviral therapy (if applicable).
- Review of AIDS-defining events.
- KS tumor assessment with photographic records. Response of KS to treatment with sEphB4-HSA will be assessed as described in [Section 9.1](#).
- KS staging ([Appendix VII](#)).
- Biologic endpoints: peripheral blood for biologic endpoints including HHV-8 viral load and peripheral blood for archive research purposes.
- If HIV positive, CD4 and CD8 counts and percentages, quantitative HIV-1 plasma RNA.
- Urinalysis.
- If the participant had documentation of visceral disease while on study, re-evaluation of visceral disease, if not already done while on study. This should be done before the post-treatment evaluation.
- Permanent discontinuation of drug will be documented.

8.4 Post-treatment Evaluation

All participants must be seen 4-6 weeks after discontinuation or completion of study treatment (counting from Day 28 of the final cycle). If participants are unable to return to the clinic, attempts should be made to obtain follow-up information from the treating physician regarding performance status, tumor status, toxicity, and subsequent care. Participants who withdraw for toxicity reasons should be followed until the toxicity resolves/returns to baseline, or for at least 4 weeks, whichever is later. In addition, participants who go off treatment for reasons other than toxicity should be followed for at least 4 weeks after discontinuing drug.

The following procedures should be performed during the post-treatment evaluation visit:

- CBC with differential.
- Serum chemistries: liver enzymes (AST [SGOT], ALT [SGPT]), alkaline phosphatase, BUN, creatinine, electrolytes (sodium, potassium, chloride, bicarbonate, calcium, phosphorus, glucose), total protein, albumin, bilirubin (direct and indirect).
- Complete physical examination including: vital signs, weight, ECOG or KPS performance status, signs and symptoms review, and toxicity evaluation (AEs must be reported per [Section 6.4](#)).
- Review of AIDS-defining events.
- KS tumor assessment with photographic records if there are any changes from the treatment discontinuation visit. Response of KS to treatment with sEphB4-HSA will be assessed as described in [Section 9.1](#).

- KS staging ([Appendix VII](#)).
- If the participant had documentation of visceral disease while on study, documented re-evaluation of visceral disease.
- FAHI+KS quality of life questionnaire ([Appendix XII](#)). Those who have HIV-negative KS will not have the HIV portion scored.
- At a participant's final study visit the Off-Study Summary Form will be completed.

Telehealth follow-up visits are permitted in the event an in-person visit is restricted and a response evaluation is not required at that visit. The complete physical examination will be deferred (i.e., vital signs, physical); however, symptoms review, and AIDS-defining events review will be conducted via teleconference. If there is any concern of progression or symptoms related to progression of KS, an in-person visit is required to follow the telemedicine visit within 2 weeks.

8.5 Follow-up Visits

All participants achieving PR, or CR will have additional physical exams repeated every 3 months (counting from, Day 28, of the final cycle), for up to 1 year or until an earlier time of disease progression requiring additional treatment. AEs must be reported to the AMC Operations and Data Management Center if the AE began any time within 4 weeks 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 CTEP-AERS (see [Section 6.3](#)) within 24 hours of when the investigator learns of the event. This information may provide additional insight into the safety of sEphB4-HSA.

The following procedures should be performed during the follow-up visits:

- Complete physical examination including: vital signs, and signs and symptoms review.
- Review of AIDS-defining events.
- KS Tumor Assessment (without photographs).
- At a participant's final study visit the Off-Study Summary Form will be completed.

Telehealth follow-up visits are permitted in the event an in-person visit is restricted. The complete physical examination will be deferred (i.e., vital signs, physical); however, symptoms review, and AIDS-defining events review will be conducted via teleconference. If there is any concern of progression or symptoms related to progression of KS, an in-person visit is required to follow the telemedicine visit within 2 weeks.

9.0 MEASUREMENT OF EFFECT

All participants will be evaluated for response by physical examination on Day 1 of every cycle (± 3 days). See [Appendix I](#) for the KS Tumor Assessment schedule.

CTEP-registered physician investigators and CTEP-registered advanced practice clinicians who are non-physician investigators (i.e., NP or PA) may perform toxicity and response assessment per local licensure requirements.

Evaluable for toxicity:

All participants will be evaluable for toxicity from the time of their first treatment with sEphB4-HSA. For the primary toxicity endpoint, we will report toxicities occurring during the first two cycles of therapy.

Evaluable for objective response:

Only those participants who have measurable disease present at baseline, have received at least two cycles of therapy, and have had their disease re-evaluated will be considered evaluable for response. These participants will have their response classified according to the definitions stated below (Note: Participants who exhibit objective disease progression prior to the end of Cycle 1 will also be considered evaluable.). For the primary efficacy endpoint, we will report the clinical response (CR or PR) rate after 2 or more cycles of treatment. Participants with KS which progress prior to the end of Cycle 2 will be considered to have treatment failure.

9.1 Definition of Response

Response and progression will be evaluated in this study using the ACTG response criteria.

9.1.1 Complete response: CR is defined as the absence of any detectable residual disease, including tumor-associated edema, persisting for at least 4 weeks. In participants, in whom 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 participants known to have had visceral disease, an assessment at restaging with appropriate endoscopic or radiographic procedures should be made.

9.1.2 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: Participants with residual tumor-associated edema or effusion who otherwise meet the criteria for complete response will be classified as having a PR.

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

progressive disease.

9.1.4 Progressive disease (PD) is defined as follows:

For participants with ≤ 50 cutaneous lesions

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

9.1.5 Non-cutaneous progression

Progressive disease 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 participant’s normal activities. Progressive visceral disease, for measurable and evaluable disease, should be analogous to non-KS response criteria.

9.1.6 Recurrent disease

Recurrent disease is defined as the appearance of tumor following documentation of a complete remission.

9.1.7 Time to response

Time to response is defined as time from the first dose of chemotherapy until documentation of first response.

9.1.8 Time to progression

Time to progression is defined as time from initiation of chemotherapy to documentation of first progression.

9.1.9 Response duration

Response duration is defined as the time from first documentation of response to documentation of first progression.

10.0 STATISTICAL CONSIDERATIONS

10.1 Study Design and Endpoint

This is a multi-institutional, Phase II single-agent trial of sEphB4-HSA in participants with KS. Participants will be administered sEphB4-HSA via intravenous infusion. Each cycle of sEphB4-HSA will be 28 days (4 weeks). Each cycle of the study drug includes administration of 2 doses of sEphB4-HSA at 10 mg/kg administered on Days 1 and 15. The primary endpoint simultaneously evaluates clinical response and toxicity after a minimum of 2 cycles of therapy with sEphB4-HSA in participants with KS. Participants may continue on study protocol as long as their KS is continuing to respond or is clinically stable on study medication for up to 12 treatment cycles. If CR is achieved, the participant will receive one cycle beyond CR. Treatment may be resumed if KS progresses off treatment after a CR. The participant will resume the previous dose that he/she was receiving when CR was achieved. Participants who achieve PR or SD may continue the treatment at the dose he/she was receiving when PR or SD was achieved, for a maximum of 12 Cycles, if the participant and treating physician feel that it is beneficial to continue treatment. Treatment will be discontinued if the participant develops unacceptable toxicity or develops one of the protocol-defined reasons for treatment discontinuation. Participants who show PR or better disease will be followed every 3 months for up to 1 year, or until an earlier time of disease progression requiring additional treatment. All participants will be followed for 4 weeks after treatment completion.

All participants who receive any amount of the study drug will be evaluable for toxicity. Toxicities will be tabulated by type and grade, according to dose cohort. Toxicity will be based on CTCAE version 5.0.

Unacceptable toxicities include all of the below which are deemed to be *at least probably* attributed to the study drug:

- Grade 4 hematological toxicity (thrombocytopenia, neutropenia on growth factor) that lasts > 4 weeks
- Steven-Johnson Syndrome
- Major allergic reaction which requires epinephrine
- Grade 4 non-hematological toxicity that does not resolve with optimal supportive care (e.g., fluids, electrolyte replacement, anti-diarrheal and titration of antihypertensive medications)
- Grade 3 neuropathy for at least 4 weeks

Primary endpoint analysis plan

The observed proportions of participants experiencing clinical response and unacceptable toxicity will be calculated, as well as 95% confidence intervals. This will be done for the primary endpoint related to clinical response and unacceptable toxicity after a minimum of 2 cycles of therapy at the 10 mg/kg every two weeks dose level, and separately for response and toxicity at later cycles. Participants treated prior to version 4 dosed at 15 mg/kg every two weeks (n=3) will be combined with those treated at the revised dose level in analyses; a sensitivity analysis excluding these participants will also be performed to investigate the

impact of these participants on the primary outcomes. Adverse events will be tabulated according to type and severity. The Kaplan-Meier method will then be used to estimate the distribution of time to response, time to relapse, and time to death. Time to response is defined as time from the first dose of treatment until documentation of first response. Time to progression is defined as time from initiation of chemotherapy to documentation of first progression. Response duration is defined as the time from first documentation of response to documentation of first progression.

10.2 Sample Size/Accrual Rate

A total of 20 evaluable participants will be enrolled. The design was developed by considering the joint probability of clinical response and toxicity simultaneously (Jin H. Alternative designs of phase II trials considering response and toxicity, *Contemp Clin Trials* 28 (2007): 525-53). The null hypothesis for defining the unacceptable region based on clinical response rate and toxicity rate simultaneously and the alternative hypothesis for defining the region for a promising agent are as follows:

- $H_0: P_{\text{resp}} \leq 0.30 \text{ OR } P_{\text{tox}} \geq 0.30$
- $H_1: P_{\text{resp}} \geq 0.60 \text{ AND } P_{\text{tox}} \leq 0.10$

In a single stage design, 20 participants will be evaluated for clinical response and toxicity. Toxicity will be defined as: unacceptable toxicities as defined in [Section 10.1](#). All unacceptable toxicities that are possibly, probably, or definitely attributed to protocol therapy will be counted as a toxicity.

The null hypothesis will be rejected if there are at least 9 responders, and no more than 3 participants who experience unacceptable toxicities. This design has power of 0.82 and a significance level of 0.11. The planned accrual rate will be one participant per month.

10.3 Stratification Factors

N/A

10.4 Analysis of Secondary Endpoints

Descriptive statistics and graphical displays will be used to evaluate correlation between response and trough levels and pharmacodynamics of sEphB4-HSA. Depending on the number enrolled, the association between clinical response and changes in trough levels, pharmacodynamic endpoints, including indicators of changes in VEGF-Notch-EphrinB2 angiogenic pathway, viral copy number, protein expression and activation status, and finally immune cell counts, and activation status will be investigated using the nonparametric Wilcoxon rank sum test.

10.5 Analysis of Exploratory Endpoints

Using the FAHI+KS questionnaire, pain and swelling improved in AIDS-KS patients treated with paclitaxel or pegylated liposomal doxorubicin on AMC009/ECOG E1D96 protocol.¹⁸ Significant improvements in social well-being and cognitive functioning were also reported. Thus, there is an interest in assessing QOL changes after baseline in this study using the same instrument to facilitate comparisons with the previous study.

In the FAHI+KS questionnaire, 5 measures of overall QOL will be scored: physical well-being, emotional well-being, functional and global well-being, social well-being, and

cognitive functioning. The questionnaire will be collected at baseline, cycle 4, and end of treatment. General estimating equations will be used to evaluate changes in the 5 measures of overall QOL over time adjusting for intra-participant variation. There are three supplemental statements regarding participant perception of KS-specific symptoms: pain, swelling. Agreement with each of these statements is graded using a Likert scale from 0 to 4. Responses to these statements may be dichotomized to reflect positive or negative impact of these symptoms. General estimating equations using a log-binomial model will be used to assess changes in these symptoms over time.

10.6 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first treatment with sEphB4-HSA.

10.7 Evaluation of Response

All participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each participant will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).

All of the participants who met the eligibility criteria (with the possible exception of those who received no study medication) will be included in the main analysis of the response rate. Participants in response categories 4-9 will be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

All conclusions will be based on all eligible participants. Sub-analyses may then be performed on the basis of a subset of participants, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses will not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding participants from the analysis will be clearly reported. The confidence intervals will also be provided.

11.0 ROLE OF DATA MANAGEMENT

11.1 CRF Instructions

Access to the internet data entry system for this study, Advantage eClinical, and instructions for recording of study data on CRFs will be provided by the AMC ODMC at www.AIDSCancer.org. Participating institutions are responsible for submitting data and/or data forms via Advantage eClinical in accordance with the AMC Data Entry Guide and specific form instructions, within the timelines specified by the AMC's Standards of Procedure for Site Performance Measures.

11.2 Data Quality

It is the responsibility of the AMC ODMC to assure the quality of data for the study (See [Appendix XIII](#), AMC Data and Safety Monitoring Plan). This role extends from protocol development to generation of the final study database.

11.3 Data Monitoring

This study will be monitored in compliance with AMC policies and by the CDUS Version 3.0. Cumulative protocol- and participant-specific CDUS data will be submitted electronically to CTEP on a quarterly basis. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site

(<http://ctep.cancer.gov/reporting/cdus.html>).

The AMC ODMC is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.0 ETHICAL AND REGULATORY CONSIDERATIONS

12.1 IRB Approval and Informed Consent

The principles of Institutional Review Board (IRB) approval and informed consent described in the Food and Drug Administration (FDA) regulations (21 CFR Part 50 and 56) and Department of Health and Human Services (DHHS) regulations for the Protection of Human Subjects regulations (45 CFR Part 46) must be followed. IRB approval of the protocol and the informed consent form must be given in writing.

The sponsor's designee (AMC ODMC) must receive a copy of the letter of approval from the IRB, which specifically approves the protocol and informed consent, before participant enrollment. The IRB must also approve any significant changes to the protocol and documentation of this approval must be sent to the AMC ODMC. The IRB must review the research project at least once every 365 days during the duration of the project. Continuing approval of the project must also be given in writing and provided to the AMC ODMC.

Records of all study review and approval documents must be kept on file by the Investigator and are participant to inspection during or after completion of the study. AEs must be reported to the IRB according to local procedures. The IRB should receive notification of completion of the study and final report 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.

Written informed consent will be obtained from the participant. The nature, significance, and risks associated with the study must be explained to the participant. The informed consent will describe the purpose of the study, the procedures to be followed, the risks and benefits of participation, all risks of the investigational agent(s) and/or study participation as listed in the model informed consent form, and all other elements of informed consent as required by regulation. A copy of the consent form will be given to the participant to keep.

In addition, any institution(s) conducting research according to the guidelines of this protocol is required to adhere to local and national laws and regulations governing the confidentiality and disclosure of health information.

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 Investigator before implementation. All amendments require approval by the IRB/IEC of the treating institution. A copy of the written approval of the IRB/IEC must be sent to the ODMC.

12.3 Women and Minorities

This study is being conducted by the NCI-sponsored AIDS Malignancy 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 participants 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 participants on this trial will reflect the constitution of the respective populations.

Table 12-A: Accrual targets

Ethnic Category	Sex/Gender				
	Females		Males		Total
Hispanic or Latino	2	+	11	=	13
Not Hispanic or Latino	1	+	6	=	7
Ethnic Category: Total of all participants	3	+	17	=	20
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	0	+	1	=	1
Black or African-American	3	+	8	=	11
Native Hawaiian or other Pacific Islander	0	+	1	=	1
White	1	+	6	=	7
Racial Category: Total of all participants	4	+	16	=	20

(A1 = A2) (B1 = B2) (C1 = C2)

12.4 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. This trial is sponsored by VasGene and supported by NCI and will comply with the NCI requirement for CTEP registration accordingly, while using AMC applications and processes for site registration and data entry. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at <https://ctepcore.nci.nih.gov/iam>. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (*i.e.*, clinical site staff requiring write access to the electronic data entry system for this protocol or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at <https://ctepcore.nci.nih.gov/rcr>.

RCR utilizes five-person registration types.

- IVR: MD, DO, or international equivalent,
- NPIVR: advanced practice providers (*e.g.*, NP or PA) or graduate level researchers (*e.g.*, PhD),
- AP: clinical site staff (*e.g.*, RN or CRA) with data entry access to CTSU and/or AMC applications (*e.g.*, Roster Update Management System [RUMS], OPEN, Rave; Advantage eClinical for this protocol),
- Associate (A): other clinical site staff involved in the conduct of NCI-sponsored trials, and
- Associate Basic (AB): individuals (*e.g.*, pharmaceutical company employees) with limited access to NCI-supported systems.

Table 12-B: CTEP investigator registration documentation requirements

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✓	✓		
Financial Disclosure Form	✓	✓	✓	
NCI Biosketch (education, training, employment, license, and certification)	✓	✓	✓	
HSP/GCP training	✓	✓	✓	
Agent Shipment Form (if applicable)	✓			
CV (optional)	✓	✓	✓	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL; the AMC DTL template will be used for this study; see [Section 12.5](#)).

Additional information can be found on the CTEP website at <https://ctep.cancer.gov/investigatorResources/default.htm>. For questions, please contact the RCR **Help Desk** by email at RCRHelpDesk@nih.gov.

12.5 Protocol Registration and Delegation of Tasks Log

Each site must complete a protocol-specific registration packet, including an AMC DTL using the provided AMC template or local equivalent. The Clinical Investigator (CI) is required to review and sign the DTL prior to the site receiving an approved site registration status and enrolling participants to the study. The AMC DTL template is provided in the protocol registration packet for this protocol, located on the AMC Operations web site at www.AIDSCancer.org. Any individual at the enrolling site on a participating roster may initiate the site DTL. Instructions on completing the DTL are embedded in the AMC DTL template.

The AMC DTL must be updated contemporaneously as personnel are added or removed and/or study roles and delegated tasks change. Changes must be approved by the CI, and documented by his/her initials and date, before they are implemented.

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APPENDIX I: SCHEDULE OF EVALUATIONS

Baseline evaluations are to be conducted within 21 days prior to start of protocol therapy, unless otherwise specified. Scans and x-rays must be done ≤ 28 days prior to the start of therapy. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. Please note, hypertension is a known and expected side effect of this drug. Please see [Section 5.4](#) for special considerations for participants with cardiac risk factors (history of hyperlipidemia, smoking, diabetes, treatment for hypertension, African American ethnicity) and specific instructions for participants who develop \geq grade 2 hypertension while on treatment.

	Eligibility	Screening/ Baseline	Cycle 1 Day 1	Cycle 1 Day 15	Cycles 2 – 12				Treatment Discont.	Post Treatment Eval.	Follow Up Visits
					Day 1		Day 15				
Informed Consent	X ^a										
Documentation of HIV status		X ^a									
KS Biopsy (Diagnostic)	X ^a										
Complete Medical History		X									
Complete Physical Exam ^s , Complete Review of Signs and Symptoms, and Review of AIDS- defining events		X	X ^g	X ^h	X ^h		X ^h		X	X	X
ECOG or KPS Performance Status	X	X	X ^g	X ^h	X ^h				X	X	
Toxicity Assessment		X ^{bb} (hyper- tension only)	X ^{bb} (hyper- tension only)	X ^{h, bb}	X ^{h, bb}				X	X	
EKG		X ^v									
KS Tumor Assessment, Photographic Record		X ^d	X ^g		X ^{h,r}				X	X ^r	X ^x
Concomitant Medication Review		X	X ^g	X ^h	X ^h						
Assess Adherence to Antiretroviral Therapy	X ^u	X	X ^g	X ^h	X ^h				X		
Review of current and prior anti- HIV therapy (if HIV positive), prior immune based therapy and prior anti-tumor therapy		X	X ^g		X ^h				X		
FAHI+KS Quality of life questionnaire (see Appendix XII)		X ^k	X ^k				X ^m (Cycle 4 only)			X	
KS staging		X ^b							X	X	
CBC with Differential, Serum Chemistries ^l		X	X ^h	X ^h	X ^h		X ^h		X	X	
CD4 and CD8 Count (T Cell Subsets), and HIV-1 plasma		X ^b	X ^l		X ^h (Cycle 4, 7,				X		

	Eligibility	Screening/ Baseline	Cycle 1 Day 1	Cycle 1 Day 15	Cycles 2 – 12				Treatment Discont.	Post Treatment Eval.	Follow Up Visits
					Day 1		Day 15				
RNA, if HIV-positive					10 only)						
HHV-8 Viral Loads (see Appendix III)		X ^{k, y}	X ^{k, y} (predose)		X ^{aa} (Cycle 4, 7, 10 only)				X		
Serum or Urine HCG Pregnancy Test ^c		X ^c	X ^q								
Education and counseling guidance		X ^g									
Dispense sEphB4-HSA			X	X	X		X				
Urinalysis		X	X ^w		X ^w				X		
Peripheral blood sEphB4-HSA levels (see Appendix II)			X (predose and immediately prior to end of infusion)	X (predose)	X ⁿ (predose)		X ⁿ (predose)				
Peripheral blood (for immune studies) (see Appendix III)		X ^{k, z}	X ^{k, z} (predose)	X	X ^o (predose)						
Peripheral blood (for PBMCs for Research) (see Appendix III)		X ^{k, y}	X ^{k, y} (predose)	X	X ^{aa} (Cycle 4, 7, 10 only)				X		
5-mm KS Biopsy (formalin-fixed), and 5-mm KS Biopsy (RNA later)		X ^k	X ^k (predose)	X ⁱ (after Day 15 dose)							
Chest X-ray ^f , CT Scan ^p		X ^b									
ACSR Donation (Screening or Day 1, optional with consent)		X ^j									

^a To be done any time prior to study enrollment

^b To be done ≤ 28 days prior to study enrollment

^c To be done ≤ 14 days prior to study enrollment

^d To be done ≤ 7 days prior to study enrollment

^e Pregnancy test to be done on females of childbearing potential

^f Participants with a positive CXR or minimal symptoms suggestive of pulmonary involvement of KS will have a CT thorax performed. CXR not required if a thoracic CT has been performed

^g Baseline assessments can be done on Day 1, or < 14 days prior to first dose of study drug

^h Labs may be done up to 7 days prior to days 1 and 15 of each cycle; interval history and physical exam must be done up to 7 days prior to study agent administration.

ⁱ To be done after Cycle 1, Day 15 dosing. Can be done any time after treatment on Cycle 1, Day 15 and before treatment of Cycle 2, Day 1

^j To be done at any time from time of study enrollment to Day 1, if the participant consents

^k To be completed within 14 days prior to Cycle 1, Day 1 of study drug. Baseline and Day 1 can be the same day

- ¹ Baseline and Day 1 can be the same (as long as done within 4 weeks of treatment)
- ^m To be administered anytime between Cycle 4, Day 15 and Cycle 5, Day 15
- ⁿ After Cycle 1, pre-dose blood for sEphB4-HSA trough levels only to be drawn days 1, 15 of Cycle 2.
- ^o Only on Day 1 of Cycles 2, 4, and 6; to be done before study treatment dosing. Given this will be a fresh blood sent at room temperature overnight, if the blood for immune studies cannot be collected on Monday-Wednesday (i.e., the dose is administered on Thursday or Friday), this blood work can be obtained the Monday AFTER the dose was given.
- ^p Only needed if symptomatic or specific findings on CXR
- ^q To be done within 24 hours of first dose of study drug
- ^r Photographs to be obtained when the KS response category changes
- ^s Includes vital signs (temperature, pulse, BP, respiratory rate), height (baseline only), weight. For cycles 2-12, only vital signs and weight are to be collected on Day 15.
- ^t Includes liver enzymes (AST (SGOT), ALT (SGPT), alkaline phosphatase), BUN, creatinine, electrolytes (sodium, potassium, chloride, CO₂, calcium, phosphorus, glucose), total protein, albumin, bilirubin (direct and indirect)
- ^u Participant must be on a stable antiretroviral regimen for at least 12 weeks prior to study entry.
- ^v QTcF (Fridericia Correction Formula) > 480 on 2 out of 3 EKG's (if first EKG is < 480, no need to repeat; if first EKG is > 480, repeat twice for a total of 3 EKG's)
- ^w Negative, Trace, or 1+ protein: continue with sEphB4-HSA; ≥ 2+, see [Section 5.3](#) for dosing changes
- ^x No photographs needed.
- ^y Must be obtained on Sunday-Thursday.
- ^z Must be obtained on Monday-Wednesday.
- ^{aa} If scheduled day is Fri-Sat, please obtain sample 7 days before or after scheduled day to be mailed express overnight on a Sunday-Thursday.
- ^{bb} Hypertension is an expected adverse event within the class of anti-neoplastic drugs affecting the vascular endothelial growth factor (VEGF) pathway. In participants with a baseline blood pressure (BP) ≥ 120/80 mm Hg and cardiac risk factors (history of hyperlipidemia, smoking, diabetes, treatment for hypertension, African-American ethnicity),⁶⁷ hypertension should be anticipated and therefore may need more aggressive management *prior to* first dose of study drug. In these cases, it is recommended that an internal medicine specialist or cardiologist be consulted to assist in management of hypertension. See [Section 5.4](#) for specific instructions for titration of antihypertensive drugs for those with cardiac risk factors, as well as specific instructions for participants who develop ≥ grade 2 hypertension while on treatment. See [Section 8.2.1.3](#) for BP measurement instructions.

APPENDIX II: SEPHB4-HSA LEVELS

sEphB4-HSA Peripheral Blood	Sample	C1, D1	C1, D15	C2 D1	C2, D15		Special Handling	Where to Send
Pre Dose	Peripheral blood – yellow top tube(s) with 5-10 cc of blood.	X	X	X	X		Separate into plasma on site. Store at -80°C.; shipped in batches Sun- Thurs.	See Appendix X
Cmax (immediately prior to the end of the infusion)		X						

APPENDIX III: BIOMARKERS AND CORRELATIVE STUDIES

Sample	Collected in	Screening or C1, D1	C1, D15	Subsequent Cycles D1	Treatment Discont.	Special Handling	Where to Send
5-mm skin biopsy	RNA Later	X (prior to first dose)	X (after completion of D15)			Shipped overnight express on cold packs and shipped Sun-Thurs.	See Appendix VIII
	Formalin-fixed	X (prior to first dose)	X (after completion of D15)			Store at ambient temperature and shipped overnight Sun-Thurs.	
Peripheral Blood for immune studies	10 mL of peripheral blood (10 mL lavender top tube)	X (prior to first dose)	X	X (Cycles 2, 4, 6, to be done before study treatment dosing)		Must be shipped at room temperature; Ship overnight express immediately (Mon-Wed) See Appendix IX for specimens that are to be collected Thurs-Sun.	George Washington University Medical Center See Appendix IX
Peripheral blood for PBMCs for Research (archived)	8.5mL of peripheral blood (one 8.5 mL yellow top tube)	X (prior to first dose)	X	X (Cycles 4, 7, 10)	X	Ship overnight express immediately (Sun-Thurs). See Appendix VIII for specimens that are to be collected Fri-Sat.	See Appendix VIII
HHV-8 viral load	8.5 mL of peripheral blood (one 8.5 mL yellow top)	X (prior to first dose)		X (Cycles 4, 7, 10)	X	Ship overnight express immediately (Sun-Thurs); See Appendix VIII for specimens that are to be collected Fri-Sat.	See Appendix VIII

APPENDIX IV: PERFORMANCE STATUS SCALES

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 V: DEFINITION OF AIDS INDICATOR CONDITIONS

- Aspergillosis, invasive *
- *Bartonella henselae* infection, disseminated (bacillary angiomatosis, peliosis hepatitis) *
- 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 (pulmonary¹ or extrapulmonary) *
- *Mycobacterium*, other species or unidentified species, disseminated or extrapulmonary *
- Nocardiosis, pulmonary, brain or disseminated *
- *Pneumocystis jirovecii* pneumonia (new or recurrent diagnosis)
- Progressive multifocal leukoencephalopathy *
- *Salmonella* septicemia, recurrent *
- Toxoplasmosis of brain *
- Wasting syndrome due to HIV *
- New Diagnosis *

APPENDIX VI: PHOTOGRAPHIC RECORD

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 (described in [Section 8.1.9](#)). The five markers lesions must be labeled in the photographs #1 - #5. The same lesions must be consistently labeled throughout the trial. 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 of the 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 (e.g., the face).

In participants with > 50 cutaneous lesions, photographs will be taken of the three representative areas (each with ≥ 5 lesions), defined at study enrollment and used for clinical assessment of response.

Photographic documentation will be completed at each visit when the KS response category changes (as described in [Section 8.2.2.1](#)). For example, if a participant's KS response category changes from no response 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.

Photographs will be stored electronically under the participant ID number and back-up electronic storage will be kept.

APPENDIX VII: KS STAGING CRITERIA

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

T₀ = tumor confined to skin, lymph nodes and/or minimal oral disease.

T₁ = any tumor falling under the "Poor Risk" criteria.

S₀ = no history of OI or thrush, no "B" symptoms, and Karnofsky Performance status \geq 70.

S₁ = 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 ACTG Oncology Committee.

¹ Minimal oral disease is nonnodular KS confined to the palate.

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

APPENDIX VIII: AMC BIOREPOSITORY SPECIMEN PREPARATION AND SHIPPING INSTRUCTIONS

Regimen: Required study specimens are included in [Appendices I](#) and [III](#). Unless otherwise specified, specimens will be banked at the AMC Biorepository, which will serve as a tissue source site for processing samples for all AMC sites, for future testing at AMC core labs, UCSD and VasGene Therapeutics, Inc.

Assays:

- Blood for plasma for HHV-8 viral load
- Blood for peripheral blood mononuclear cells (PBMCs) and plasma; archived for future research
- Tissue (skin) biopsies; formalin-fixed and RNAlater biopsies

GENERAL

- A. To ship blood specimens, use a diagnostic shipper approved for a volume of at least 30 cc. The use of the SAF-T-PAK STP 210 diagnostic cardboard shipper is recommended. These shippers may be ordered at the SAF-T-PAK website: 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 Sunday through Thursday** as an **OVERNIGHT PRIORITY** shipment. Specimens are **NOT ACCEPTED ON SATURDAYS OR SUNDAYS** in the AMC biorepository or ACSR.

B. SPECIMEN PREPARATION, PACKAGING, AND SHIPMENT

INSTRUCTIONS FOR PERIPHERAL BLOOD SPECIMENS (SUNDAY – THURSDAY)

From study participant, draw:

- One 8.5 cc (mL) yellow top (acid citrate dextrose [ACD]) tube for HHV-8 viral load and one 8.5 cc (mL) yellow top (ACD) tube for archived PBMCs and plasma for research at baseline/Day 1 of Cycle 1, Days 1 of Cycles 4, 7, and 10, and at treatment discontinuation.

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

- AMC Protocol # 096
- AMC Participant ID#
- Date and time of collection
- Specimen type, i.e., WB=Whole Blood
- Specimen purpose: HHV-8 and future studies

Specimen shipment

- Seal the tops of the tube(s) with parafilm.
- Place the sealed tube(s) into bubble wrap (provided in STP-210 kit).
- Tape around the bubble wrap so that the roll stays together and the tubes cannot fall out or break.
- Place absorbent material sheet around the bubble wrapped tubes and slip into a biohazard poly-bag and “self-seal.”
- Place poly-bag containing tubes into the white TYVEK bag and seal.
- Place the TYVEK bag into the STP-210 diagnostic cardboard shipper. Seal the cardboard shipper with clear packing/shipping tape.
- Affix the FED-EX airbill on blank side of the shipper making sure that it is marked “FED-EX PRIORITY OVERNIGHT.”
- Mark “OTHER” in the air bill under “Packaging.” Please use the FedEx # available on the AMC member’s only website.
- Under air bill section “Special Handling” indicate “YES-SHIPPERS DECLARATION NOT REQUIRED.”
- Place “From/To” information onto areas provided on the shipper.

BLOOD SPECIMENS should be shipped by overnight express at room temperature to:

AMC Network Resources Laboratory
Jeffrey Bethony, PhD
George Washington University Medical Center
2300 I Street NW
Washington, DC 20037

- 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 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.

Specimens should be sent by 24-hour shipment at room temperature. If collecting on a Friday through Saturday where 24-hour shipment is not possible, please see Section below for instructions.

INSTRUCTIONS FOR PERIPHERAL BLOOD SPECIMENS (FRIDAY – SATURDAY)

Specimens for future research and HHV-8 (i.e., fresh whole blood) must be mailed overnight to the AMC Biorepository to arrive Monday-Friday. Therefore, participants who are scheduled for the study drug on a Friday or Saturday (thereby making the blood for future research and HHV-8 viral loads scheduled for Friday or Saturday), alternative timing of blood draws will have to be made.

To accommodate this issue, peripheral blood for future research and HHV-8 can be drawn and overnight shipped **up to 7 days before or after the scheduled date; samples must be drawn on a Sunday through Thursday.**

SEE PART B: SPECIMEN PREPARATION, PACKAGING, AND SHIPMENT FOR FULL DETAILS OF SHIPPING

Correlative studies shall be sent to:

The AMC biorepository will send the specimens to centers performing each correlative study as stated below:

- *Samples for HHV-8 viral load* will be shipped to the AMC Genomics Core labs at the University of North Carolina, Chapel Hill.
- The archived PBMCs and plasma samples will be stored at the AMC biorepository until otherwise noted.

INSTRUCTIONS FOR PREPARATION OF TISSUE SAMPLES

From study participant, obtain:

Skin biopsy; formalin-fixed. Biopsy will be obtained at baseline/Day 1 of Cycle 1, and after completion of Day 15 of Cycle 1 (and before Day 1 of Cycle 2)

Skin biopsy; RNeasy. Biopsy will be obtained at baseline/Day 1 of Cycle 1, and after completion of Day 15 of Cycle 1 (and before Day 1 of Cycle 2)

Tissue specimens (punch biopsies) should be placed (a) into no more than 10 mL buffered formalin and (b) 1 mL RNeasy (provided in batches of 20 to each site by the AMC Genomics Core Lab).

*NOTE: Specimens can only be accepted **Monday through Friday**. Therefore, specimens can only be shipped **Sunday-Thursday** for delivery the next day. Samples in RNeasy are to be shipped on cold packs and samples in formalin are to be shipped in ambient temperature.

TISSUE specimens should be shipped by overnight express to:

AMC Network Resources Laboratory
Jeffrey Bethony, PhD
George Washington University Medical Center
2300 I Street NW
Washington, DC 20037

Correlative studies shall be sent to:

The AMC biorepository will send the biopsy specimens to centers performing each correlative study as stated below:

- *EphrinB2, VEGFR-2, VEGFR-3, Notch 1/3/4, Jagged-1, Dll4, pSrc, pS6, pAkt, pEphB4, TUNEL, CD31*: To be performed by Dr. Parkash Gill at USC. *Immune response with CD45, CD3, CD4, CD20, CD68, CD163; K8.1; and LANA-1*: To be performed by AMC Core Pathology laboratories (Dr. Ethel Cesarman). *Archived tissue in RNAlater for KSHV and endothelial gene transcription*: To be performed by the AMC Genomics Core labs at the University of North Carolina, Chapel Hill. Mail to: Dr. Dirk Dittmer. Remaining tissue will then be mailed to Dr. Jeffrey Bethony after completion of studies.

C. RECORD OF SPECIMENS

This study will track specimens via GlobalTraceSM, a component of the AMC Advantage eClinical system. The GlobalTraceSM shipment manifest must accompany all specimen shipments.

APPENDIX IX: COLLECTION AND SHIPPING INSTRUCTIONS FOR PERIPHERAL BLOOD FOR IMMUNE RESPONSE (T CELL FUNCTION) TO SEPHB4-HSA

MATERIALS (PER PARTICIPANT)

From study participant, draw:

- One lavender top (EDTA) tube, 10 mL at baseline/Day 1 of Cycle 1, Day 15 of Cycle 1, and Days 1 of Cycle 2, 4, and 6.

PROCEDURE

- Samples need to be shipped OVERNIGHT EXPRESS at ambient temperature to George Washington University Medical Center.

SHIPPING PROCEDURES

- Samples need to be shipped OVERNIGHT EXPRESS at ambient temperature to George Washington University Medical Center. Samples should be sent Monday - Wednesday. Please notify George Washington University Medical Center (see contact below) one week prior to anticipated collection of samples to ensure proper handling. For samples that are collected Thursday through Sunday, please notify George Washington University Medical Center two weeks in advance so that arrangements can be made for alternative collection dates. Of note, it has been written into the protocol that if a drug is being administered on Thursday through Sunday (and therefore pre-dose samples will be obtained on a Thursday or Friday), the blood samples for immune studies may be obtained and shipped the Monday AFTER treatment has been administered.
- Please use the FedEx # available on the AMC member's only website to ship specimens.
- Shipments can be sent to:

George Washington University Medical Center
Ross Hall, Room 118
2300 I, Street, NW
Washington, DC 20037

Email: amc-bio@emmes.com **RECORD OF SPECIMENS**

Each sample must be labeled. Unlabeled samples will be discarded. Each sample should be labeled using a Sharpie pen with the following information:

Protocol #: AMC-096
11-digit AMC participant #
Study cycle #/Day#
Date and time of collection
Specimen Type: Whole Blood
Specimen Purpose:

This study will track specimens via GlobalTraceSM, a component of the AMC Advantage eClinical system. The GlobalTraceSM shipment manifest must accompany all specimen shipments.

APPENDIX X: SPECIMEN PREPARATION AND SHIPPING INSTRUCTIONS OF SEPHB4-HSA TROUGH AND CMAX LEVELS

Regimen: Required study specimens are included in [Appendix II](#).

AMC sites enroll consented participants in Advantage eClinical.

The pharmacokinetic samples will be stored at the AMC Biorepository. The AMC Biorepository will then ship samples to Dr. Parkash Gill. See address below.

MATERIALS (PER PARTICIPANT)

From study participant, draw:

- Peripheral blood on Day 1 of Cycle 1 (pre-dose and immediately before the end of infusion), Day 15 of Cycle 1 (pre-dose), and Days 1 and 15 of Cycle 2 (pre-dose).

A. PREPARATION OF PLASMA

It is preferable that separation occurs as soon as possible.

Materials

- 15 mL conical centrifuge tubes (sterile)
- 1.5 mL screw top tubes
- 1, 5 mL and 10 mL serologic pipettes (sterile)
- NUNC tubes
- Alcohol-saturated, control rate freezer container

Preparation of Plasma Samples

- The 8.5 mL tubes of whole blood in acid citrate dextrose should be rotated gently two or three times before being centrifuged. Do not transfer before centrifugation.
- The cells are separated by centrifugation at 500 g for 10 minutes.
- 0.5 mL aliquots of plasma are removed and put into separate 1.5 mL screw top tubes (NUNC) and transferred to liquid nitrogen storage at -70°C.

B. GENERAL

Plasma specimens may be shipped in batches monthly. To ship blood specimens, use a diagnostic shipper approved for a volume of at least 30 cc. The use of the SAF-T-PAK STP 210 diagnostic cardboard shipper is recommended. These shippers may be ordered at the SAF-T-PAK website 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 Sundays through Thursday** as an **OVERNIGHT PRIORITY** shipment. Specimens are **NOT ACCEPTED ON SATURDAYS, OR SUNDAYS**.

C. SPECIMEN PREPARATION, PACKAGING, AND SHIPMENT

BLOOD SPECIMENS

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

- AMC Protocol #096
- AMC Participant ID#
- Date and time of collection, including indication of C_{max}, pre-dose with day and cycle number
- Specimen type: i.e., Plasma
- Specimen purpose: sEPHb4 trough and C_{max} levels

*****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.**

Mail to:

AMC Network Resources Laboratory
Jeffrey Bethony, PhD
George Washington University Medical Center
2300 I Street NW
Washington, DC 20037

Correlative studies shall be sent to:

The AMC Biorepository will ship plasma samples to Dr. Parkash Gill to perform ELISA.

APPENDIX XI: RISKS OF FETAL EXPOSURE, PREGNANCY TESTING GUIDELINES AND ACCEPTABLE BIRTH CONTROL METHODS

It is unknown if sEphB4-HSA has teratogenic effects. Therefore, precautions below should be taken during this study.

Female participants:

- Urine pregnancy tests will be conducted as follows:
 - o < 14 days before start of the study drug
 - o Day 1 of Cycle 1
- If pregnancy or a positive pregnancy test does occur in a study participant, study drug must be immediately discontinued.
- Pregnancy testing and counseling must be performed if a participant misses her period or if her pregnancy test or her menstrual bleeding is abnormal. Study treatment must be discontinued during this evaluation.
- Females must agree to abstain from breastfeeding during study participation and for at least 28 days after study drug discontinuation.
- Further, they must either commit to continued abstinence from heterosexual intercourse or begin TWO acceptable methods of birth control: one highly effective method and one additional effective method AT THE SAME TIME during receipt of sEphB4-HSA, and 12 weeks after discontinuation of sEphB4-HSA. FCBP must also agree to ongoing pregnancy testing, if needed.
- The following are examples of highly effective and additional effective methods of contraception:
 - o Highly effective methods:
 - Intrauterine device (IUD)
 - Hormonal (birth control pills, injections, implants)
 - Tubal ligation
 - Partner's vasectomy
 - o Additional effective methods:
 - Male condom
 - Diaphragm
 - Cervical Cap

Male participants:

- Counseling about the requirement for complete abstinence or condom use during sexual contact with a pregnant female or a female of childbearing potential and the potential risks of fetal exposure to sEphB4-HSA must be discussed before receiving the trial drug.
- If pregnancy or a positive pregnancy test does occur in the partner of a male study participant during study participation, the investigator must be notified immediately.

APPENDIX XII: THE FUNCTIONAL ASSESSMENT OF HIV (FAHI) + KAPOSI SARCOMA (KS) QUALITY OF LIFE QUESTIONNAIRE

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	<u>PHYSICAL WELL-BEING</u>	Not at all	A little bit	Some- what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4
B1	I have been short of breath	0	1	2	3	4
B8	I am bothered by a change in weight	0	1	2	3	4
BMT6	I get tired easily	0	1	2	3	4
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
L2	I have been coughing	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

<u>EMOTIONAL WELL-BEING/ LIVING WITH HIV</u>		Not at all	A little bit	Some- what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4
HI1	I am unhappy with my appearance	0	1	2	3	4
HI2	It is hard to tell other people about my infection	0	1	2	3	4
HI4	I worry about spreading my infection	0	1	2	3	4
HI5	I am concerned about what the future holds for me	0	1	2	3	4
B7	I worry about the effect of stress on my illness	0	1	2	3	4
HI10	I am embarrassed by my illness	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

<u>FUNCTIONAL AND GLOBAL WELL-BEING</u>		Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
B4	I feel sexually attractive	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
HI6	I feel motivated to do things	0	1	2	3	4
HI11	I am hopeful about the future	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

<u>SOCIAL WELL-BEING</u>		Not at all	A little bit	Some- what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
HI3	I have people to help me if I need it	0	1	2	3	4
Q1	<i>Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box <input type="checkbox"/> and go to the next section.</i>					
GS7	I am satisfied with my sex life	0	1	2	3	4

<u>COGNITIVE FUNCTIONING</u>		Not at all	A little bit	Some- what	Quite a bit	Very much
L1	My thinking is clear	0	1	2	3	4
H18	I have trouble concentrating	0	1	2	3	4
H19	I have trouble remembering things	0	1	2	3	4

<u>ADDITIONAL QUESTIONS RELATED TO KAPOSI SARCOMA</u>		Not at all	A little bit	Some- what	Quite a bit	Very much
1	Pain has interfered with my normal work or activities	0	1	2	3	4
2	I am satisfied with my physical appearance	0	1	2	3	4
3	I have had swelling in my face, arms or legs	0	1	2	3	4

APPENDIX XIII: AMC DATA AND SAFETY MONITORING PLAN

(Version 8.0 • July 16, 2020)

Introduction

The AIDS Malignancy Consortium (AMC) Data and Safety Monitoring Plan (DSMP) outlines the measures employed by the group to monitor the safety of participants and ensure the data validity and integrity for all clinical trials it conducts. This includes methods to: 1) monitor the progress of trials and the safety of participants; 2) comply with regulatory requirements for adverse event (AE) reporting; 3) processes for trial termination or temporary suspension and major modifications; and 4) plans for ensuring data accuracy and protocol compliance. As the AMC conducts protocols of varying research phase, region of conduct, IND sponsor (AMC investigator, CTEP, or industry-sponsored) and clinical data entry system use, this plan addresses broad processes applying to the range of trial designs and requirements. Refer to the individual AMC protocol to identify the applicable study characteristics for the relevant requirements described in this plan.

Monitoring the Progress of Trials and the Safety of Participants

Routine and expedited AE reporting

All AMC protocols that collect safety data adhere to the *National Cancer Institute (NCI), Cancer Therapy Evaluation Program (CTEP) Guidelines: Adverse Event Reporting Requirements* (https://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm), as applicable to the clinical protocol. AEs are to be recorded in the source documents, assessed by a clinical investigator for the AE reporting criteria, and promptly reported in the clinical data entry system as required by each protocol. For AMC trials conducted under a CTEP IND and AMC trials conducted within the U.S., all AEs that meet the NCI's expedited reporting requirements are reported to the NCI via the CTEP Adverse Event Reporting System (CTEP-AERS) web application, either directly or through integration with Medidata Rave where this system is employed for AMC protocols. Use of this system ensures notification to the protocol chair and Investigational Drug Branch (IDB) at CTEP, as required for trials conducted under a CTEP IND, and a uniform expedited reporting and safety review process for AMC domestic trials. The system may also be programmed to include sponsor notification as required for trials with industry support. Alternate process for expedited AE reporting to the AMC protocol chairs and AMC Operations and Data Management Center (ODMC) within the clinical data entry system (AdvantageEDC or Advantage eClinical only) may be defined in the protocol for select trials (international studies and The ANCHOR Study).

All serious adverse events (SAEs) received by the AMC ODMC will be reviewed by the AMC medical monitor at the AMC ODMC for consideration of individual participant safety, safe trial conduct, data reporting quality for AE term selection, and appropriate application of the regulatory criteria for seriousness, expectedness, and relatedness to the investigational therapy. If alternate procedures are followed for SAE review, the process for adequate medical monitoring will be defined in the AMC protocol and the Transfer of Regulatory Obligations (TORO) with the sponsor. AMC medical monitor review includes review of the CTEP-AERS report before CTEP submission for IDB review (if applicable), or review of the SAE report in the data entry system for trials not using CTEP-AERS for expedited reporting. The IND sponsor or its designee will issue the determination as to whether the AE requires IND safety reporting to FDA as a serious and unexpected suspected adverse drug reaction (SUSAR). For protocols not conducted under an IND,

in the event of disagreement between the reporting physician and the AMC medical monitor regarding the relationship of the AE to the investigational agent(s) (i.e., determination of whether the attribution is unrelated or unlikely, or possible, probable, or definite), the AMC medical monitor will provide the final determination of the relationship. IND safety reporting to FDA is performed by CTEP for trials conducted under a CTEP IND; IND safety reporting is performed by the sponsor or sponsor's designee (AMC ODMC or other party defined in the study agreement or TORO) for IND studies sponsored by AMC investigators or industry sponsors.

Expedited reporting to the Institutional Review Board (IRB)

For trials subject to local IRB review, the site principal investigator is responsible for ensuring that expedited AE reports for its trial participants and any unanticipated problems that affect the local institution only are submitted to the local IRB of the reporting institution, per the local IRB's requirements for such reporting. For studies reviewed by the NCI CIRB, the protocol chair will render a determination as to whether a SAE or other problem constitutes a trial-wide unanticipated problem that requires reporting to the NCI CIRB; reporting will occur per the NCI CIRB's standards of procedure.

To comply with investigator notification requirements for IND studies under 21 CFR 312.32 and 312.55, IND safety reports from all trials the AMC conducts and reports from external sponsors investigating the same agents are made available to all investigators upon receipt from the sponsor or its designee, either via the password-protected section of the AMC Operations web site (AMC trials subject to local IRB review only) or the CTSU website (trials subject to CIRB review/CTEP IND agents). The site clinical investigator responsible for the applicable AMC protocol(s) is responsible for reviewing any IND safety reports received and documenting submission to the IRB of record (if required by local policy) within the timeline defined by the Clinical Trials Monitoring Branch (CTMB) audit guidelines.

Procedures for monitoring trial progress and pharmacovigilance

For trials using AdvantageEDC or Advantage eClinical for clinical data entry, the AMC ODMC provides on demand tabular listings of all reported AEs and SAEs on a participant level to the protocol chair and co-chair(s) for review via the password-protected section of the AMC Operations web site, www.AIDScancer.org. For trials using OPEN and Medidata Rave for clinical data collection, data listing will be made available using that system. Summary reports of AEs by frequency and relationship to the investigational agent(s) are provided to all AMC investigators and their staff. It is the responsibility of each site to provide trial-specific AE listings to their respective IRB, if required by its policies. For blinded studies, the AE and SAE listings are reviewed and tabulated without treatment assignment.

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 Scientific Working Group (SWG) during scheduled conference calls (monthly SWG calls and as required, protocol-specific monitoring conference calls). Summary accrual, summary AE, and individual SAE reports are provided to SWG leadership and protocol chairs to monitor participant safety during these monthly calls.

The AMC medical monitor reviews listings of all reported AEs on a quarterly basis for assuring compliance with the protocol requirements for AE reporting and the identification of any safety concerns (individual AE or increased frequency/severity of expected AEs) for the agents under

investigation. Findings from these reviews are communicated to the protocol chairs and all AMC investigators, and posted to the AMC Operations web site.

Data and Safety Monitoring Board Review (DSMB) review

The AMC has formed an independent Data and Safety Monitoring Board (DSMB) for AMC trials and for the ANCHOR Study. As required by NCI policy, the AMC requires DSMB review for all phase III randomized trials. All other clinical trials that the AMC initiates will be reviewed by the AMC ODMC and AMC Statistical Center during protocol development to issue a recommendation as to whether the study requires DSMB oversight, which will require the approval of the AMC Executive Committee. This determination will be based on the phase of the study, experimental design, risk posed by the investigational approach, extent of data available on the safety of an investigational agent, risk posed by the natural course of the health condition under research, and the categories of vulnerable populations involved. The involvement of a DSMB in reviewing an AMC protocol will be identified in each clinical protocol as approved CTEP and, as applicable, the NCI CIRB.

Regarding the composition of the AMC DSMB, voting members usually include physicians, statisticians, an ethicist, and a patient advocate. All voting members have no other affiliation to the AMC, and are appointed by the AMC Executive Committee with the approval of the OHAM Director. Nonvoting members are the AMC group statistician, the protocol statistician, an AMC ODMC staff member, two representatives (normally a clinician or statistician) from CTEP, and the grant program directors from the NCI Office of HIV and AIDS Malignancy (OHAM).

The DSMB reviews all applicable AMC studies in accordance with the National Cancer Institute's Policy for Data and Safety Monitoring. Confidential reports of all trials under review 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 DSMB charter. This report addresses specific toxicity issues and any other concerns about the conduct of the trial, as defined by the protocol plan for DSMB review. The report may contain information for the DSMB to render determinations for participant safety, early trial termination, results reporting, or continuing accrual or follow-up.

The results of each DSMB meeting are summarized in a formal report sent by the DSMB chair to the AMC 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 or designee is then responsible for notifying the protocol chair and relevant SWG 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 OHAM program directors and the NCI division director or designee must be informed of the reason for the disagreement. The protocol chair, relevant SWG 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 protocol amendment will be required prior to any implementation of a change to the study.

Following a DSMB meeting, the DSMB's recommendations are provided to all AMC investigators and staff. It is each site principal investigator's responsibility for conveying this information to its local IRB as relevant for its protocol participation. For trials reviewed by the NCI CIRB, the AMC

ODMC will support notification to the CIRB as required per its procedures.

Cohort trial reviews not subject to DSMB review

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 protocol statistician determine whether these criteria have been met based on a review of all safety data for the protocol-defined evaluation period. If applicable 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 protocol statistician determine whether these criteria have been met.

Plans for Assuring Compliance with Requirements Regarding AE Reporting

The protocol chair, AMC group chair, and the AMC ODMC share responsibility in assuring that participating investigators comply with applicable regulatory and protocol requirements for AE reporting. The AMC site principal investigator certifies compliance with NCI and FDA requirements for trial conduct by signing the site subaward agreement for the grant and the AMC Adherence Statement for site membership; clinical investigators also certify compliance in completing the protocol signature page for each protocol active at the site, and Form FDA-1572 for CTEP investigator registration, and also for AMC IND studies sponsored by AMC investigators or industry sponsors. Protocol compliance with AE identification, assessment and reporting requirements is assessed by the AMC ODMC using several methods: 1) programmed system checks and messages to instruct the site to complete routine and/or expedited reporting when certain criteria are reported in the clinical data entry system; 2) programmed data reports provided to the protocol chairs that identify reports requiring expedited AE reporting; 3) remote review of data entry or data reports to ensure compliance with protocol and NCI AE reporting requirements; 4) AMC medical monitor review described in the section above; and, 5) routine site audits by reviewing the site's source documentation.

The clinical data entry systems used for AMC studies include the Oncology Patient Enrollment Network, OPEN for enrollment, and Medidata Rave for clinical data entry for enrolled participants; trials activated before September 1, 2020 or that involve only AMC international sites may be reported in AdvantageEDC/Advantage eClinical, a web-based data entry and enrollment system. These data entry systems are programmed to notify the site investigator, protocol chair, AMC medical monitor, and AMC ODMC via email in the event that a site reports an AE that meets expedited reporting criteria to NCI and/or FDA. Additional reporting conditions may be programmed depending on the sponsor reporting requirements of a given protocol (e.g., adverse events of special interest [AESI]). If the site does not follow with an expedited report, the AMC ODMC contacts sites to request compliance with reporting requirements. Additionally, the protocol chair, AMC ODMC, and the AMC medical monitor review reported AEs on a routine basis to identify AEs reported by sites that require expedited reporting. The protocol chair, AMC SWG chairs, AMC group chair, and IND sponsors have general oversight for assuring that routine and expedited adverse reporting requirements are met by the responsible parties.

For studies monitored by CTEP using the Data Mapping Utility (DMU), cumulative protocol- and patient-specific data will be submitted weekly to CTEP electronically via the DMU. For trials monitored by the NCI's Clinical Data Update System (CDUS), AE information is transmitted electronically to NCI on a quarterly basis. For trials monitored by NCI's Clinical Trials Monitoring Service (CTMS), AE information is transmitted electronically to NCI every two weeks.

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 temporary or permanent suspension of a trial, or major modification to the protocol is under consideration, the protocol chair will convene the AMC ODMC, AMC Statistical Center, and SWG chair by conference call to discuss the options. Suspension actions will also be reviewed by the AMC Executive Committee for program oversight and direct communication of the action with the OHAM program directors. For phase III trials, closure decisions are typically rendered by the AMC DSMB; if the trial in question is under AMC DSMB oversight but rendered by the AMC investigators, the AMC DSMB will be notified of the suspension and the reason. For phase I and II trials, the protocol chair also has the option of asking the DSMB to review the study. The AMC ODMC will inform the CTEP Protocol Information Office (PIO), with copy to OHAM Directors, when studies are temporarily or permanently closed. In the event of major trial modification, CTEP 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 clinical site staff into the applicable clinical data entry system for the trial. 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. Submitted data entry forms are reviewed for compliance with the protocol and data entry instructions according to the AMC ODMC's standards for data quality processes. AMC ODMC staff routinely interacts with site staff to resolve any data submission problems.

In accordance with NCI guidelines, the AMC ODMC conducts audits 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 written corrective and preventative action plan to correct deficiencies. If needed, a repeat site audit 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 to implement remedial action(s) for 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 XIV: ACSR INFORMED CONSENT

Study Title for Study Participants: Collecting Blood and Tissue Sample Donations for Research for HIV/AIDS-Related Cancers

Official Study Title: Biospecimen Collection and Donation to the AIDS and Cancer Specimen Resource (ACSR)

What is the usual approach to donate blood and/or tissue to the ACSR?

You are being asked to donate blood and/or tissue for future research. You are being asked to donate your blood and/or tissue samples to the ACSR because you have HIV infection and are being considered for participation in an AIDS Malignancy Consortium (AMC) clinical trial. The AMC works with the ACSR to collect donated samples from persons with HIV infection for research studies. People who do not take part in an AMC clinical trial can also donate samples to the ACSR.

What are my other choices if I do not take part in this study?

It is your choice to donate or not donate your blood and/or tissue samples. You may still take part in the AMC clinical study if you choose not to donate blood or biopsy samples to the ACSR.

You may also choose to donate:

- Blood but not tissue, or
- Tissue but not blood.

What is the AIDS and Cancer Specimen Resource (ACSR)?

The ACSR is a biorepository (biobank) that collects human biological specimens (samples) from persons who have HIV or cancers related to HIV/AIDS. The ACSR stores the samples and some of the donor's medical information for use by researchers in future research studies. The National Cancer Institute (NCI) has set up the ACSR to assist researchers locate samples needed for their studies.

The ACSR has an independent research panel that approves researchers' requests to use the ACSR's stored samples for research studies. The ACSR only gives samples and medical information to researchers after their projects have been approved. Researchers may use the samples to study cancers and other diseases associated with HIV disease. This information may help us learn more about the causes of HIV-related diseases and cancers and to develop better ways to screen, diagnose, and treat them.

Why is this study being done?

The purpose of this study is to collect samples for the ACSR for future research studies. Researchers may study samples from the ACSR in combination with hundreds or thousands of other samples to explore how biologic or genetic factors may be related to HIV-related diseases and cancer. The information might help doctors in the future to identify who will or will not benefit from treatment. The samples may be used to learn more about how HIV-related diseases and cancers develop. The samples may also lead to new tests or discoveries. Finally, researchers may use the samples to study the genetic material from your cancer tissue and compare it to the material from your normal tissue (blood) to try to find the differences that exist. These studies could make

it possible to identify many of the changes that are associated with diseases such as cancers. It may also help us tailor treatments to a patient's unique genetic make-up and/or to the genetic markers of the tumors.

What extra tests and procedures will I have if I take part in this study?

1. If you agree to donate blood, the medical team will draw about 2 tablespoons of blood to give to the ACSR. This takes about 10 minutes.
2. If you agree to donate tissue, your leftover tissue biopsy material will be donated to and stored by the ACSR.
3. Some of your clinical information will be released to the ACSR and entered into their database. The information given to the ACSR will not include your name or any information that could personally identify you.

We will only give the ACSR tissue that is left over after making decisions about your treatment or diagnosis. The study doctor will not take any extra biopsies just for the ACSR.

We cannot tell you right now what future research these samples would be used for. Instead, we are asking that you give approval to give your samples for future testing without contacting you again. The results of whatever research is done on your samples will *not* be told to you or your doctor. The results of the tests will *not* be placed in your study records.

How long will ACSR keep my samples?

Your blood and/or tissue sample will be stored until it is used for research. The samples may be stored indefinitely.

What possible risks can I expect from taking part in this study?

Blood Draw: The risks of drawing blood include temporary discomfort from the needle stick, bruising, and, rarely, infection.

Confidentiality: The ACSR will receive study samples with code numbers. There will be no personal identifiers on the samples. Then the samples will be re-labeled with a barcode and stored for future testing. While the ACSR and researchers who study ACSR samples will have no information that could identify you, there is a risk that someone could use information from genetic studies to trace your samples back to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information. In some cases, this information could be used to make it harder for you to get or keep a job. There are laws against misuse of genetic information, but they may not give full protection. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

Let your study doctor know of any questions you have about these possible risks. You can ask the study doctor questions about side effects at any time.

What possible benefits can I expect from taking part in this study?

This study is unlikely to help you. This study may help us learn things that may help people in the future.

The information may help to identify those who are at increased risk and those who may benefit from targeted treatment and screening. In turn, these studies could help find ways to prevent or improve treatments for HIV-related diseases and AIDS-related cancers.

Can I stop taking part in this study?

Yes, you may withdraw your samples from the ACSR at any time. You may contact your AMC study coordinator if you would like to withdraw your samples. The coordinator can ask in writing that your sample be removed from research use and that any identifiable sample and information still in their possession be destroyed. However, if any research has already been done using some of your samples, the data will be kept and analyzed as part of those studies.

What are my rights in this study?

Taking part in this study is your choice. No matter what decision you make, and even if your decision changes, there will be no penalty to you. You will not lose medical care or any legal rights.

For questions about your rights while in this study, call the _____ (*insert name of center*) Institutional Review Board at _____ (*insert telephone number*). (*Note to Local Investigator: Contact information for patient representatives or other individuals at a local institution who are not on the IRB or research team but take calls regarding clinical trial questions can also be listed here.*)

What are the costs of taking part in this study?

There will be no cost to you for donating your samples to the ACSR. You will not be paid for taking part in this study.

What happens if I am injured or hurt because I took part in this study?

If you are injured or hurt as a result of taking part in this study and need medical treatment, please tell your study doctor. The AMC will not offer to pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance, you would be responsible for any costs.

If you feel this injury was a result of medical error, you keep all your legal rights to seek payment for injury even though you are in a study.

Who will see my medical information?

Your privacy is very important to us and the researchers will make every effort to protect it. Your information may be given out if required by law. For example, certain states require doctors to report to health boards if they find a disease like tuberculosis. However, the researchers will do their best to make sure that any information that is released will not identify you. Some of your health information, and/or information about your specimen, from this study will be kept in a central database for research. Your name or contact information will not be put in the database.

There are organizations that may inspect your records. These organizations are required to make sure your information is kept private, unless required by law to provide information. Some of these organizations are:

- The AIDS Malignancy Consortium (AMC)

- The Institutional Review Board, IRB, is a group of people who review the research with the goal of protecting the people who take part in the study.
- The Office for Human Research Protections and the National Cancer Institute in the U.S.

To protect your privacy, the AMC does not keep identifying information that links study participants to specific samples. As a result, the AMC and ACSR will not be able to link the results from studies that use your samples back to you. Thus, information, including genetic information, that researchers may obtain in studies that use your samples may not be directly linked to you and will not be placed in your medical record. However, some clinical and basic information obtained confidentially from the AMC will be attached with these data. It is possible that findings may one day help, for example, people of the same race or sex as you. It also is possible that genetic factors might come to be associated with people who have HIV and cancer through these kinds of studies.

Where can I get more information?

You may visit the NCI Web site at <http://cancer.gov/> for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at 1-800-4-CANCER (1-800-422-6237).

Who can answer my questions about this study?

You can talk to the study doctor about any questions or concerns you have about this study or to report side effects or injuries. Contact the study doctor _____ (*insert name of study doctor[s]*) at _____ (*insert telephone number*).

Please circle your answer to show whether or not you would like to take part in each option:

1. I agree to donate my blood to the ACSR for future research that may be used to learn about, prevent, diagnose, or treat HIV-related diseases and cancer.
YES NO
2. I agree to donate my blood to the ACSR for future research that may include genetic testing to learn about, prevent, diagnose, or treat HIV-related diseases and cancer.
YES NO
3. I agree to donate some of my tissue biopsy material that is not required for my treatment or diagnosis to the ACSR for future research that may be used to learn about, prevent, diagnose, or treat HIV-related diseases and cancer.
YES NO
4. I agree to donate some of my tissue biopsy material to the ACSR for future research that may include genetic testing to learn about, prevent, diagnose, or treat HIV-related diseases and cancer.
YES NO

My Signature Agreeing to Take Part in the Study

I have read this consent form or had it read to me. I have discussed it with the study doctor and my questions have been answered. I will be given a signed copy of this form. I agree to take part in the optional study.

Participant's signature: _____

Date of signature: _____

Signature of person(s) conducting the informed consent discussion: _____

Date of signature: _____

APPENDIX XV: AIDS AND CANCER SPECIMEN RESOURCE (ACSR) SPECIMEN PREPARATION AND SHIPPING INSTRUCTIONS

A. GENERAL

To ship blood specimens, use a diagnostic shipper approved for a volume of at least 30 cc. The use of the SAF-T-PAK STP 210 diagnostic cardboard shipper is recommended. These shippers may be ordered at the SAF-T-PAK website: 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 Wednesday** as an **OVERNIGHT PRIORITY** shipment. Specimens are **NOT ACCEPTED ON FRIDAYS OR SATURDAYS** in the ACSR.

B. SPECIMEN PREPARATION, PACKAGING, AND SHIPMENT

BLOOD SPECIMENS

Draw two 8.5 cc (mL) yellow top (acid citrate dextrose [ACD]) tubes from study participant. With a black, water resistant, sharpie pen, label each specimen with the following information:

- AMC Protocol # 096
- AMC Participant ID#
- Date and time of collection
- Specimen type, i.e., WB=Whole Blood, P=Plasma, S=Serum, or Tissue
- Specimen purpose: Donation

Specimen shipment

- Seal the tops of the two 8.5 cc yellow tops with parafilm.
- Place the two sealed tubes into bubble wrap (provided in STP-210 kit).
- Tape around the bubble wrap so that the roll stays together and the tubes cannot fall out or break.
- Place absorbent material sheet around the bubble wrapped tubes and slip into a biohazard poly-bag and “self-seal.”
- Place poly-bag containing tubes into the white TYVEK bag and seal.
- Place the TYVEK bag into the STP-210 diagnostic cardboard shipper. Seal the cardboard shipper with clear packing/shipping tape.
- Affix the FED-EX air bill on blank side of the shipper making sure that it is marked “FED-EX PRIORITY OVERNIGHT.”
- Mark “OTHER” in the air bill under “Packaging.” Please use the FedEx # available on the AMC member’s only website.

- Under air bill section “Special Handling” indicate “YES-SHIPPERS DECLARATION NOT REQUIRED.”
- Place “From/To” information onto areas provided on the shipper.

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

AMC Network Resources Laboratory
 Jeffrey Bethony, PhD
 George Washington University Medical Center
 2300 I Street NW
 Washington, DC 20037
 Phone: 202-590-8342
 Fax: 202-994-5056
 Email: jbethony@gwu.edu

- 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 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.

INSTRUCTIONS FOR BLOOD SPECIMENS COLLECTED ON THURSDAY OR FRIDAY

Preparation of plasma and mononuclear cells

Refer to the ACSR’s standard operating procedure (SOP) on Separation of Plasma and Mononuclear Cells on the AMC Operations web site for instructions on preparing plasma and PBMC aliquots. It is preferable that separation occurs as soon as possible. If necessary, whole blood in ACD (yellow top tubes) can be held at room temperature for no more than 24 hours.

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.**

PREPARATION OF TISSUE SAMPLES

Tissue specimens to be fresh frozen should be placed in OCT and then on dry ice immediately. The specimens may stay on dry ice until being transferred to a -80°C freezer.

Tissue specimens for donation may be batched for shipping after storage in -80°C freezer. ***NOTE:** Specimens can only be accepted Monday through **Thursday**. Therefore, specimens can only be shipped **Sunday-Wednesday** for delivery the next day. Shipping frozen tissue requires the use of packaging acceptable for dry ice and Class 9 label with weight of dry ice written on package.

TISSUE specimens should be shipped by overnight express to:

AMC Network Resources Laboratory
Jeffrey Bethony, PhD
George Washington University Medical Center
2300 I Street NW
Washington, DC 20037
Phone: 202-590-8342
Fax: 202-994-5056
Fax: (202) 994-5056

C. RECORD OF SPECIMENS

This study will track specimens via GlobalTraceSM, a component of the AMC Advantage eClinicalSM system. The GlobalTraceSM shipment manifest must accompany all specimen shipments.