**NU Study Number**: NU 18U10

# A Phase II study of sEphB4-HSA in metastatic castration-resistant prostate cancer


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**Study Intervention(s):** sEphB4-HSA

**IND Number:** 143436

**IND Holder:** Maha Hussain, MD

**Support for drug**: VasGene Therapeutics Inc

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**LIST OF ABBREVIATIONS**

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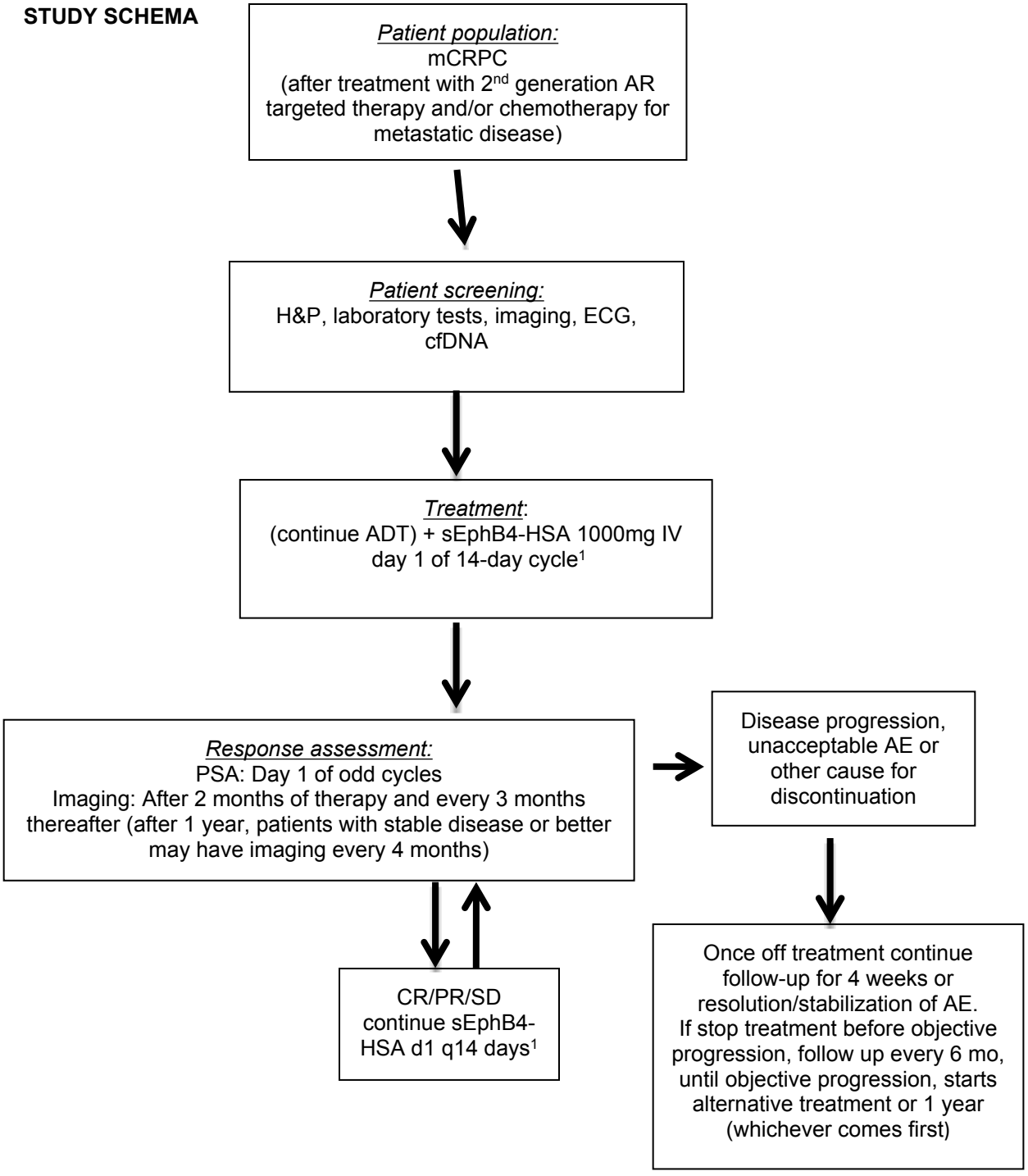
| ADT | Androgen Deprivation Therapy |
| AE | Adverse Event |
| ALT | Alanine Aminotransferase |
| ALC | Absolute Lymphocyte Count |
| AR | Androgen Receptor |
| AST | Aspartate Aminotransferase |
| BUN | Blood Urea Nitrogen |
| CBC | Complete Blood Count |
| CMP | Comprehensive Metabolic Panel |
| CR | Complete Response |
| CT | Computed Tomography |
| CTC | Circulating tumor cell |
| cfDNA | Cell Free DNA |
| CTCAE | Common Terminology Criteria for Adverse Events |
| DLT | Dose Limiting Toxicity |
| DSMC | Data and Safety Monitoring Committee |
| DSMP | Data and Safety Monitoring Plan |
| ECOG | Eastern Cooperative Oncology Group |
| H&PE | History & Physical Exam |
| IV (or iv) | Intravenously |
| MTD | Maximum Tolerated Dose |
| mCRPC | Metastatic Castration Resistant Prostate Cancer |
| NCI | National Cancer Institute |
| ORR | Overall Response Rate or Objective Response Rate |
| OS | Overall Survival |
| PBMCs | Peripheral Blood Mononuclear Cells |
| PD | Progressive Disease |
| PFS | Progression Free Survival |
| PO (or p.o.) | Per os/by mouth/orally |
| PR | Partial Response |
| SAE | Serious Adverse Event |
| SD | Stable Disease |
| SGOT | Serum Glutamic Oxaloacetic Transaminase |
| SPGT | Serum Glutamic Pyruvic Transaminase |
| WBC | White Blood Cells |

**STUDY SCHEMA**




1. Beginning at Cycle 7, cycle length will be 21 days and patients will return for treatment on Day 1 of each cycle.

**NU Study Number**: NU 18U10

**STUDY SUMMARY**

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| **Title** | A phase II study of sEphB4-HSA in metastatic castration-resistant prostate cancer (mCRPC) |
| **Version** | September 18, 2020 (Amendment 6) |
| **Study Design** | A phase II, single-arm, open-label, three center study |
| **Study Center(s)** | Northwestern University – Robert H. Lurie Cancer Center<br>University of Chicago<br>University of Southern California |

**Background and rationale:**

*There is a critical need for novel targets in lethal prostate cancer*. Prostate cancer is the third most common cause of cancer death for men in the United States [1]. Although 90% of men who receive androgen deprivation therapy (ADT) for metastatic disease initially respond, the development of metastatic castration-resistant prostate cancer (mCRPC) is almost universal within 2-3 years [2]. Standard therapy for mCRPC includes receptor (AR) targeted agents (i.e. abiraterone and enzalutamide), microtubule inhibiting chemotherapy (docetaxel and cabazitaxel), and in select circumstances sipuleucel-T or radium-223. The median overall survival (mOS) benefit for each of these therapies is 2-5 months and mOS is about 3 years for patients with mCRPC [3-9]. Unfortunately, response rates, progression free survival (PFS), and mOS drops substantially with subsequent lines of therapy. PSA response rate in patients receiving enzalutamide after abiraterone and docetaxel is 10-15% [10]. Median OS for 2$^{nd}$, 3$^{rd}$, and 4$^{th}$ line therapy is 21, 11, and 5 months, respectively [11].

*Ephrins and their receptors are potential targets for therapy in mCRPC*. Ephrin receptors and their membrane-localized ligands induce bidirectional signaling and facilitate tumor-stroma interactions. The ephrin pathway impacts a multitude of cellular processes such as cell survival and proliferation, neoangiogenesis, and immune development and trafficking [12]. EphB4-ephrinB2 interaction can lead to T-cell suppression with immune evasion and EphB4 interacts with PI3K/AKT and MAPK pathways [12-15]. Dysregulation of the Ephrin/Eph receptor pathway in prostate cancer cells can promote cell migration, invasion, and metastases [16]. Expression of EphB4 is increased in prostate cancer tissue and cell lines and retained in castration resistant states but not commonly expressed in benign prostate tissue [17, 18].

*EphB4 inhibition can cause tumor regression in preclinical aggressive prostate cancer models*. EphB4 is induced by molecular alterations implicated in aggressive CRPC development, including loss of the tumor suppressor genes, PTEN and TP53, and activation of the PI3K pathway [17]. Experiments from Dr. Parkash Gill at the University of Southern California and Dr. Abdulkadir at Northwestern University show its inhibition with a soluble EphB4-albumin fusion antagonist led to tumor regression in 10 of 10 MYC+/PTEN loss/P53 loss mice with prostate cancer. It also induced tumor cell death, and induced immune cell infiltration. PTEN and TP53 loss are frequent molecular aberrations in CRPC [19].

*An EphB4 antagonist (sEphB4-HSA) was safe and tolerable in a phase I trial in advanced solid tumors.* Four of 70 patients had a partial or complete response and 50% showed stable disease. There were no grade 4 or 5 related adverse events. The most common grade III toxicities were hypertension (27%), fatigue (16%), nausea (4%). Grade I/II toxicities included hypertension, fatigue, weight loss, and nausea. A dose limiting toxicity of QTC prolongation occurred in one patient at 10mg/kg IV in the weekly dosing cohort and of hypertension at 20mg/kg IV in the every 2 weeks cohort. A maximum tolerated dose was not reached.

*Study proposal and hypothesis:* We propose study evaluating the efficacy, safety, and tolerability of sEphB4-HSA in patients with mCRPC with evaluation of correlative biomarkers. Our hypothesis is EphB4-EphrinB2 pathways are active and pathogenic in aggressive mCRPC, and are a therapeutic target.

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| **Objectives** | **Primary Objective:**<br>1. To assess the efficacy of sEphB4-HSA in patients with mCRPC as measured by confirmed PSA response rate<br><br>**Secondary Objectives:**<br>1. Safety and tolerability of sEphB4-HSA in patients with mCRPC according to NCI CTCAE v 5.0<br>2. Time to PSA progression<br>3. Overall response rate in patients with measurable disease using RECIST 1.1 (soft tissue) and PCWG3 (bone) criteria.<br>4. Radiological progression free survival (rPFS) using RECIST 1.1 (soft tissue) and PCWG3 (bone) criteria.<br><br>**Exploratory Objectives:**<br>1. Molecular changes associated with EphB4 and ephrinB2 expression in tumor specimens.<br>**2.** Association of response with molecular biomarkers including aberrations in the PI3K pathway, MYC and TP53.<br>3. To assess immune cell infiltration of tumors in biopsies<br>4. To assess circulating immune changes associated with treatment. |
| **Endpoints** | **Primary endpoint:**<br>1. PSA response by PCWG3 criteria<br><br>**Secondary endpoints:**<br>1. Toxicity rate and severity by CTCAE v 5.0<br>2. Time to PSA progression by PSA response criteria (PCWG3)<br>3. Overall response by RECIST 1.1 (soft tissue) and PCWG3 (bone) criteria.<br>4. rPFS by RECIST 1.1 (soft tissue) and PCWG 3 (bone) criteria.<br><br>**Exploratory endpoints:**<br>1. IHC staining for expression of EphB4 and ephrinB2 in tumor samples and correlate the level of alteration with the MYC, PTEN/PI3K, AR, and p53 pathways.<br>2. Analysis of cfDNA for mutations in PI3K pathway, MYC or TP53.<br>3. IHC for CD3, CD4, CD8, NK cell markers and PD-L1. |
| **Sample Size** | 15 patients in stage one + 10 patients in stage 2 for a total of 25 evaluable patients.<br>Approximately 4-8 eligible patients are seen per month with an anticipated at least 1-2 patient enrolled per month. We expect it will take 18 months to accrue our study population. |

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| **Diagnosis & Key Eligibility Criteria** | **Inclusion Citeria**<br>• Patients must have pathologically confirmed diagnosis of prostate adenocarcinoma<br>• Patients must have metastatic (M1) disease as evidenced by soft tissue and/or bony metastases on CT or MRI scan or technetium bone scan.<br>• Patients must have castration resistant disease with disease progression despite castrate levels of testosterone (testosterone < 50 ng/dL)<br><br>Exclusion Criteria<br>• Patients who have had radiotherapy ≤14 days prior to entering the study are not eligible.<br>• Patients who have had systemic therapy for prostate cancer ≤21 days or 5-half lives (whichever is shorter) are not eligible.<br>*Note: Patients can receive a stable dose of bisphosphonates for bone metastases, including zoledronic acid, or denosumab before and during the study as deemed appropriate by the treating physician.*<br>• Patients receiving any other investigational agents are not eligible.<br><br>See section 3.0 for complete list |
| **Treatment Plan** | sEphB4-HSA will be administered as an IV infusion over 60 minutes on day 1 of each cycle (1 cycle = 14 days) along with continued androgen deprivation therapy (ADT). Beginning at Cycle 7, cycle length will be 21 days and patients will return for treatment on Day 1 of each cycle. Patients may continue to receive sEphB4-HSA treatment until no longer clinically benefiting (PCWG3), unacceptable toxicity, treatment delay ≥4 weeks, or prohibitive illness/change in patient's condition, or patient decides to withdraw from study. Response assessment with PSA will occur on Day 1 of odd cycles (every 28 days). Imaging will occur at baseline and 2 months after starting therapy; thereafter, imaging will be conducted every 3 months. After 1 year, imaging can be done every 4 months for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated. Once off treatment continue follow-up for 4 weeks or resolution/stabilization of AE ≤ Grade 1. If off treatment before progressive disease, patients will be followed every 6 months, until objective progression or starts alternative therapy for a maximum of 1 year. |

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| **Statistical Methodology** | To estimate **preliminary efficacy** we will assess PSA response rate (PR and CR) with a **Simon two stage minimax design**. We assume the undesirable overall response rate (null hypothesis) to be approximately 10% or less, and the alternate hypothesis suggesting success to be approximately 30% or more. Fifteen patients will be added in the first stage. If 2 or more respond, then an additional 10 patients will be added for a total of 25 evaluable patients. If not, we will stop the study after the first stage. The study will be assessed by the DSMC for excess toxicity at this point in time and stopped if DSMC finds excessive toxicity. If the second stage is completed, five or fewer successes will suggest failure and six or more responses a success of the entire study. This design has a Type I error rate of less than 5% and 80% or greater power. It has a 55% chance of stopping early after the first stage if the true response rate is 10% or less, and a total expected long run average sample size of n=19.5.<br><br>To **estimate time to PSA progression, rPFS, and overall survival**, we will use Kaplan Meier methods. To estimate overall response rate in patients with measurable disease using RECIST 1.1 (soft tissue) and PCWG3 (bone) criteria, and radiological progression free survival (rPFS) using RECIST 1.1 (soft tissue) and PCWG3 (bone) criteria we will use exact point estimation.<br><br>All adverse events (AEs) will be summarized using frequencies and percentages. Data on type, timing, frequency and attribution of AEs will be included. |

# 1.0 INTRODUCTION – BACKGROUND & RATIONALE

## 1.1 Disease background

Prostate cancer causes an estimated 26,730 deaths in 2017, making it the third most common cause of cancer death in the United States [20]. Metastatic disease is initially treated with androgen deprivation therapy with or without the addition of docetaxel or abiraterone [21, 22]. Although 90% of men with metastatic prostate cancer who receive androgen deprivation therapy (ADT) initially respond, the development of castration-resistant prostate cancer (CRPC) is common within 2-3 years, with a subset of patients following an even more aggressive course [1, 2]. For patients with metastatic CRPC (mCRPC) standard of care treatments include second generation AR targeted therapy (i.e. abiraterone and enzalutamide), chemotherapy (docetaxel and cabazitaxel), and in select circumstances sipuleucel-T or radium-223. The median overall survival (OS) benefit for each of these therapies is less than 6 months, and median OS is under 3 years for patients with mCRPC [3-9]. PSA response rate in patients receiving enzalutamide after abiraterone and docetaxel is 10-15% [10]. These results highlight the critical need to advance the understanding of disease biology and identify novel therapeutic targets for mCRPC.

## 1.2 Ephrins background & overview

Research investigating active pathways in aggressive prostate cancers has identified ephrins and their receptors (receptor tyrosine kinases) as potential targets for therapy in advanced or aggressive prostate cancer. Ephrin receptors and their membrane-localized ligands induce bidirectional signaling with downstream effects on several pathways including MAPK, PI3K/AKT, and VEGF. They are involved in multitude of cellular processes such as cell survival and proliferation, neoangiogenesis, and immune development and trafficking [12]. Additionally, ephrins impact tumor microenvironment and immunogenicity, such that ephrin-B2 on mesenchymal stromal cells interacts with EphB4 on T cells leading to T-cell suppression and potential immune evasion [14, 15]. EphB4 interacts with the PI3K/AKT and MAPK pathways that modulate androgen receptor (AR) signaling [12, 13]. Dysregulation of the Ephrin/Eph receptor pathway in prostate cancer cells can promote cell migration, invasion, and metastases [16].

The expression of EphB4 in human tumors has been analyzed with an immunostaining assay. Fresh frozen tumor samples and, when possible, adjacent normal tissues were analyzed for EphB4 expression using the EphB4-specific monoclonal antibody MAb131. EphB4 expression was induced in many of the epithelial cancers analyzed. For example, EphB4 is not expressed in the normal bladder and colon, but is highly expressed in the bladder and colon tumors. EphB4 expression was observed in breast, head and neck, ovarian, and prostate cancers. EphB4 gene amplification was analyzed in head and neck cancer, lung cancer, and esophageal cancer. In esophagus squamous cell carcinoma, 9 of 15 (60%) patients had a gene copy number between 4 and 20. Similarly, in esophagus adenocarcinoma, 5 of 8 (62%) had gene copy number ranging from 4 to 20. In lung cancer, more than three copy numbers were found in 6, 9, and 23 percent of adenocarcinoma, small cell lung carcinoma, and squamous cell carcinoma, respectively (unpublished data). Nearly 25% of head/neck carcinoma also has gene amplification

These observations suggest the ephrin pathway has an oncogenic role and may be a potential therapeutic target.

## 1.3 Summary of pre-clinical findings of EphB4 in prostate cancer

Expression of EphB4 protein is increased in most prostate cancers and is retained after exposure to ADT, but is not commonly expressed in benign prostate tissue. In one study, 66% of prostate tumors analyzed had increased EphB4 protein expression, compared to 15% of benign prostate tissue [17, 18].

EphB4 is induced by molecular alterations implicated in CRPC development including loss of tumor suppressors PTEN and TP53, and activation of the PI3K pathway [17]. The genomic signature of MYC activation, PTEN loss, and TP53 loss in prostate cancer has been associated with aggressive disease as demonstrated by a shorter time to disease relapse after primary treatment, and increased risk of death [23]. Experiments from Dr. Parkash Gill at the University of Southern California and Dr. Sarki Abdulkadir at Northwestern University showed prostate cancer PTEN knockout mouse models had high EphB4 expression and its inhibition with the soluble EphB4-albumin fusion antagonist, sEphB4, led to tumor regression in 10 of 10 mice (Figure 1). Additional experiments from Dr. Abdulkadir's group at Northwestern showed that sEphB4 induced tumor cell death and immune infiltration in genomically complex transgenic MYC+Pten-Tp53- prostate tumors (Figure 2). Additional preclinical studies show that sEphB4 antagonist also causes inhibition of angiogenesis, and cytotoxic T cell and NK cell recruitment. EphB4 inhibition also led to inhibition in PC3 prostate cancer cell line and C4-2B orthotopic mouse models. These preclinical data support therapeutic targeting of the ephrinB2-EphB4 interaction may lead to disease response in aggressive prostate cancer.

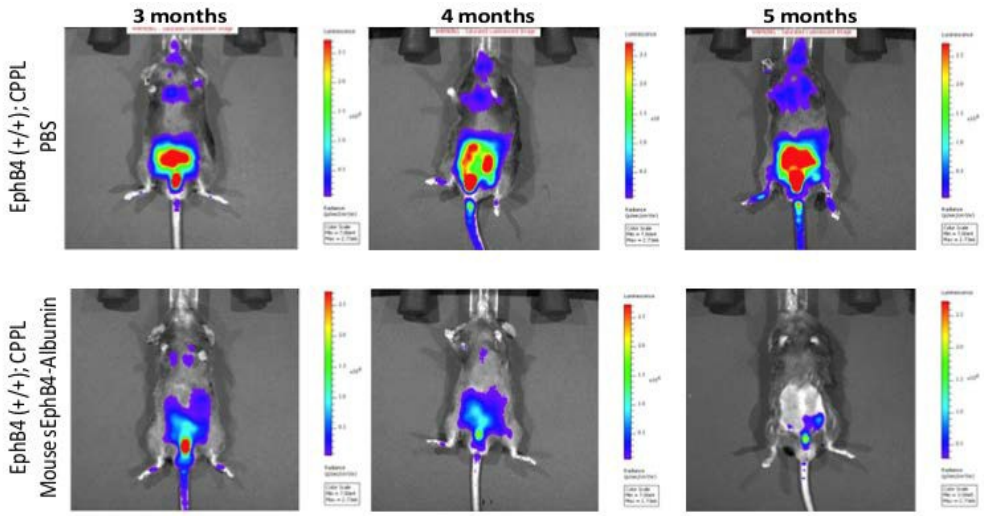


**Figure 1**. Mouse sEphB4-albumin causes regression of PTEN knock out prostate tumor (unpublished data, courtesy Dr. Sarki Abdulkadir's lab at Northwestern University and Dr. Parkash Gill's lab at University of Southern California)

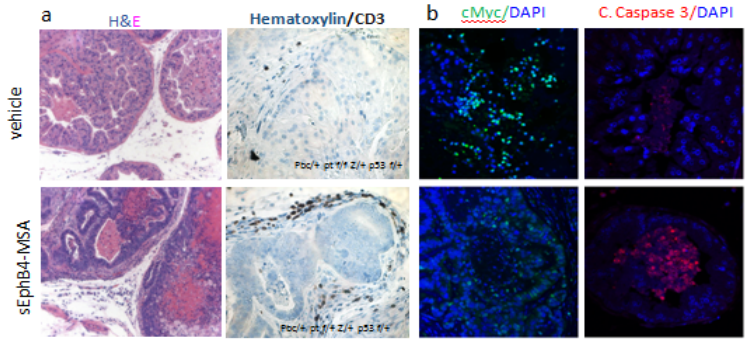


**Figure 2.** Transgenic mice (Myc/Pten/p53) treated with 20mg/kg sEphB4-MSA or vehicle (PBS) a week for 4 weeks.
a) Representative images of hematoxylin and eosin (H&E) and CD3 staining from sEphB4-MSA treated or PBS treated transgenic mice. b) Reduced c-Myc and increased cleaved caspase-3 expression in transgenic mice treated with PBS and sEphB4-MSA

### 1.4 sEphB4-HSA

The soluble decoy EphB4 receptor-human serum albumin fusion protein (sEphB4-HSA) is a fully human fusion protein with a soluble EphB4 extracellular domain that specifically binds ephrin-B2. Thus drug blocks ephrin-B2 binding, bidirectional signaling, and their downstream effects on angiogenesis and tumor growth *in vitro* and *in vivo.*

*In vitro* studies demonstrate that sEphB4 binds specifically to EphrinB2 and to EphrinB2-expressing cells with low nanomolar affinity. sEphB4-HSA blocks EphB4 binding to EphrinB2-expressing cells, 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 ($EC_{50}$) 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. In cell line and animal models, sEphB4 produces complete inhibition of PI3K signaling measured by phosphoS6 and phosphoAkt. In addition by downregulating PI3K beta, sEphB4 markedly reduces androgen receptor expression in a PTEN knock prostate cancer model with concurrent major reduction in tumor volume (Gill unpublished data).

In addition to the aforementioned studies in prostate cancer sEphB4-HSA demonstrated *in vivo* activity in multiple tumor xenograft models, including orthotopic HT29 colon cancer, A549 lung cancer, and orthotopic PANC265 pancreatic cancer in which it also significantly reduced the incidence of tumor metastases. Furthermore, sEphB4-HSA significantly inhibits tumorigenesis and/or tumor growth in several spontaneous tumor models including Kras mutant oral cavity and skin cancer models.

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 30mg/kg) toxicity study of sEphB4-HSA in cynomolgus 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 adverse dose-dependent effects observed.

### 1.5 Summary of clinical trial findings

In addition to these preclinical studies, the sEphB4-HSA has shown safety and tolerability in a first-in human phase I trial of 70 patients with advanced solid tumors. The study included a dose escalation phase and dose expansion phase at the maximum tolerated dose (MTD) or recommended phase II dose (RP2D). Dose levels tested were 2.5, 5, 10 and 15 mg/kg IV infusion weekly and 10, 15, and 20 mg/kg IV infusion every 2 weeks. No dose limiting toxicity was observed, except one patient with grade 3 hypertension not responsive to therapy in a patient at 20 mg/kg dose requiring dose reduction to 15 mg/kg and one patient with grade 3 QTC prolongation at 10mg/kg weekly. There were no grade 4 or 5 treatment related adverse events. The most common grade 3 toxicities were hypertension (27%), fatigue (16%), nausea (4%). Grade 1-2 toxicities included hypertension (48%), fatigue (35%), weight loss (13%), nausea (6%), and vomiting (5%). A maximum tolerated dose was not reached. Pharmacokinetic studies showed a half-life of 306 hours. Based on these findings, the recommended phase II dose was established as 10mg/kg weekly and 20mg/kg every 2 weeks. Four of 70 patients had a partial or complete response and 50% showed stable disease [24]. The median number of cycles received was 2 (range 1-19). Several patients have received 4-11 months of therapy indicative of safety when used for prolonged period of time.

Clinical trials are planned to investigate its use as neoadjuvant therapy in genitourinary malignancies (NCT02767921) and metastatic bladder cancer in combination with checkpoint inhibitor ant-PD1 (NCT02717156).

## 1.6 Rationale for the Current Study

A need for improving therapeutic options in mCRPC, extensive preclinical results showing the relevance of the EphB4/EphrinB2 pathway in mCRPC along with clinical data demonstrating the clinical safety of the soluble decoy EphB4 receptor-human serum albumin fusion protein (sEphB4-HSA) provide the foundation for the present proposal of a phase II study to explore preliminary efficacy and safety of sEphB4-HSA in patients with progressive mCRPC who have previously received a second generation AR targeted therapy (i.e., abiraterone or enzalutamide) and chemotherapy (docetaxel or cabazitaxel).

The patient population was chosen because this group of patients is in need for effective therapeutic approaches as they have advanced lethal prostate cancer and limited further therapies capable to improve survival. Additionally, based on preclinical models, EphB4 inhibition maybe more relevant in patients with more aggressive CRPC.

Patients will be maintained on ADT per standard of care and receive the study drug at the recommended phase II dose. Phase I studies showed the safety and tolerability of the sEphB4-HSA in patients with advanced cancers but the drug has not been specifically evaluated for patients with mCRPC or in combination with ADT.

## 1.7 Rationale for Exploratory Studies

Our preclinical data showed that EphB4 inhibition by sEphB4-MSA profoundly inhibited tumor growth in mouse model of Myc over expression and PTEN loss. In addition, we have also found that sEphB4-MSA induces T-Cell infiltration and expression of PD-L1 in tumors suggesting a role for immune cell mediated tumor regression. From this evidence, we would like to explore the effect of sEphB4-HSA in advanced prostate cancer patients as a single treatment or in combination with other agents. The rationale for obtaining archival pathologic specimen and peripheral blood to assess molecular features of the patient's disease is to gather correlative data that provides insight into the activity of sEphB4-HSA in this setting if response is seen. Results of this study will provide foundational data to further explore sEphB4-HSA therapeutic potential in other clinical settings of advanced prostate cancer as single agent and in combination with other agents, e.g. if immune cell infiltration associated with PD-L1 is seen, this can provide a rationale for combination with immune checkpoint therapy.

### 1.7.1 The role of EphB4 and ephrinB2 in mCRPC and correlation with outcomes

Mechanistically, sEphB4-HSA acts by binding to Ephrin B2 and preventing it's binding to EphB4. Therefore it is important to evaluate the expression of both binding partners and correlate response to therapy if activity of the drug is seen.

### 1.7.2 The role of molecular biomarkers (PI3K pathway, P53, and MYC expression) and their correlation with clinical response of sEphB4.

Our preliminary in vitro and in vivo studies have identified a relationship between PTEN/PI3K, Myc and p53 pathway to EphB4 expression and the response to EphB4 target therapy. Proposed correlation studies are geared towards understanding the various genomic alterations that can predict efficacy of sEpHB4. To this end, we will perform IHC on available tissue for EPHB4, EFNB2, AR, PTEN, p-AKT, and MYC and analyze genomic alterations that are described in the results of molecular analysis reports from standard of care tests (i.e., results from standard of care NGS testing/ genetic testing/ genomic testing, etc.). We will also utilize cfDNA results to analyze the changes in the alteration level at baseline and after treatment.

# 2 OBJECTIVES

## 2.1 Primary Objective:

2.1.1 To estimate the efficacy of sEphB4-HSA in patients with mCRPC as measured by confirmed PSA response rate.

## 2.2 Secondary Objectives:

2.2.1 The safety and tolerability of sEphB4-HSA in patients with mCRPC according to NCI CTCAE v 5.0 (See Appendix II).

2.2.2 To assess the time to PSA progression.

2.2.3 To assess overall response rate in patients with measurable disease using RECIST 1.1 (soft tissue) and PCWG3 (bone) criteria.

2.2.4 To assess radiological progression free survival (rPFS) using RECIST 1.1 (soft tissue) and PCWG3 (bone) criteria.

## 2.3 Exploratory Objectives:

2.3.1 To explore molecular changes associated with EphB4 and ephrinB2 expression in tumor specimens (primary and/or metastatic tissue).

2.3.2 To explore association of response with molecular biomarkers including aberrations in the PI3K pathway, MYC and TP53.

2.3.3 To assess immune cell infiltration of tumors in archival tissue, if tissue is available.

2.3.4 To assess circulating immune changes associated with treatment.

# 3 PATIENT ELIGIBILITY

The target population for this study are patients with mCRPC who have progressive disease despite castrate levels of testosterone (<50 ng/dL) and have received at least one standard first-line therapy for metastatic castrate resistant prostate cancer, which can include therapy with a 2$^{nd}$ generation AR targeted therapy (abiraterone or enzalutamide) or chemotherapy (docetaxel or cabazitaxel). This study will be a multicenter trial conducted at Northwestern University and will serve as the lead site and coordinating center for the study. Participating sites include University of Chicago and University of Southern California.

A total of 25 evaluable subjects will be needed for this trial. Approximately 4-8 potentially eligible patients are seen per month, and it is anticipated that at least 1-2 per month will be accrued (once all sites are up and running).

Eligibility will be evaluated by the study team according to the following criteria. Eligibility waivers are not permitted. Subjects must meet all of the inclusion and none of the exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered. Please refer to Section 11 for complete instructions regarding registration procedures.

## 3.1 Inclusion Criteria

3.1.1 Patients must have a pathologically confirmed diagnosis of prostate adenocarcinoma

3.1.2 Patients must have metastatic (M1) disease as evidenced by soft tissue and/or bony metastases on CT or MRI scan or technetium bone scan.

3.1.3 Patients must have castration resistant disease with disease progression despite castrate levels of testosterone (testosterone ≤ 50 ng/dL)

3.1.4 Patients must have received and progressed on at least one second generation AR targeted therapy for castration resistant disease irrespective of prior chemotherapy. No more than 3 prior treatment therapies for castration resistant disease (life prolonging) are permitted. Prior therapy can include:

- Second generation AR targeted therapy [i.e. abiraterone, enzalutamide, or other new antiandrogen (ODM-201, apalutamide)]
- Chemotherapy (docetaxel and/or cabazitaxel).

3.1.5 Documented progressive mCRPC based on at least one of the following criteria:

- PSA progression defined as 25% increase over baseline value with an increase in the absolute value of at least 2.0 ng/mL that is confirmed by another PSA level with a minimum of a 1 week interval and a minimum PSA of 2.0 ng/mL.
- Progression of bidimensionally measurable soft tissue or nodal metastasis assessed within one month prior to registration by a CT scan or MRI. (Please refer to Section 6.1.2 for definition of measurable disease.)
- Progression of bone disease (evaluable disease) (new bone lesion(s)) by bone scan.

3.1.6 Serum testosterone < 50 ng/dL. Patients must continue primary ADT with an LHRH analogue (agonist or antagonist) if they have not undergone orchiectomy.

3.1.7 Patients must be age ≥ 18 years.

3.1.8 Patients must have an ECOG performance status of 0-2 (See Appendix I Section 12.1)

3.1.9 Patients must have adequate organ and bone marrow function as defined below within 2 weeks prior to registration:

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| Absolute Neutrophil Count | ≥ 1,000/mcL |
| Hemoglobin | ≥ 9 g/dL* |
| Bilirubin | ≤1.5 x institutional upper limit of normal (ULN) except for unconjugated hyperbilirubinemia or Gilbert's Syndrome, who can have total bilirubin <3.0 mg/dL. |
| AST/ALT | ≤ 3 x ULN (≤ 5 x ULN if liver metastases present) |
| Serum Creatinine | ≤ 2.0 X ULN (upper limit of normal) or creatinine clearance ≥ 30 mL/minute (using Cockcroft/Gault formula) |
| Platelet | ≥100,000 |

* Transfusion is allowed as long as patients have not received prior transfusion ≤28 days from registration.

3.1.10 Patients must use a condom during treatment and for 3 months after the last dose of study treatment when having sexual intercourse. Female partners of male subjects should also use a highly effective form of contraception if they are of

childbearing potential (See Appendix III Section 12.3). Subjects should not donate sperm throughout the study and for 3 months following the last dose of treatment.

3.1.11 Patients must have the ability to understand and the willingness to sign a written informed consent prior to registration on study.

**3.2 Exclusion Criteria**

3.2.1 Patients who have received more than 3 prior treatment therapies (life prolonging) for mCRPC are not eligbile.

3.2.2 Patients who have had radiotherapy ≤14 days prior to entering the study are not eligible.
*Note: Palliative radiation therapy is allowed.*

3.2.3 Patients who have had systemic therapy for prostate cancer ≤21 days or 5-half lives (whichever is shorter) are not eligible.

*Note: Patients can receive a stable dose of bisphosphonates for bone metastases, including zoledronic acid, or denosumab before and during the study as deemed appropriate by the treating physician. Patients must continue Androgen Deprivation Therapy.*

3.2.4 Patients receiving any other investigational agents are not eligible.

3.2.5 Patients with Small cell carcinoma of the prostate are not eligible.
Note: Neuroendocrine differentiation is permitted. If there is doubt about this and it is clinically indicated then a biopsy should be obtained to document histological differentiation.

3.2.6 Patients who have a history of allergic reactions attributed to compounds of similar chemical or biologic composition to sEphB4-HSA are not eligible.
AND
Patients who have had prior exposure to compounds of similar chemical or biologic composition to sEphB4-HSA are not eligible.

3.2.7 Patients who have an uncontrolled intercurrent illness including, but not limited to any of the following, are not eligible:

- Ongoing or active infection requiring systemic treatment
- Symptomatic congestive heart failure (New York Heart Association Class III or IV congestive heart failure)
- Unstable angina pectoris
- Serious cardiac arrhythmia

3.2.8 Patients with uncontrolled hypertension (defined as systolic BP ≥ 160 mmHg or diastolic BP ≥ 95mmHg) are not eligible.
*Note: Patients with a history of hypertension are allowed provided blood pressure is controlled by anti-hypertensive treatment*

3.2.9 Patients with Electrocardiogram (ECG) with QT interval (QTc) >480 msec are not eligible.

3.2.10 Patients with other malignancy that has progressed or has required active systemic treatment in the last 3 years.

*Note: Patients with basal cell carcinoma of the skin, squamous cell carcinoma of the skin that has undergone potentially curative therapy or carcinoma in situ or non-muscle invasive bladder cancer are not excluded.*

3.2.11 Patients with known active CNS metastases and/or carcinomatous meningitis are not eligible.

*Note: A scan to confirm the absence of brain metastases is not required. Subjects with previously treated brain metastases may participate provided they are stable, i.e. without evidence of progression for at least 4 weeks by repeat imaging (note that the repeat imaging should be performed during study screening), without requirement of steroid treatment for at least 4 weeks prior to randomization and with any neurologic symptoms resolved or have returned to baseline of prior treatment for brain metastasis*

3.2.12 Patients with spinal cord compression are not eligible unless considered to have received definitive treatment for this and evidence of stable disease for 28 days.

3.2.13 Patients who underwent major surgery ≤14 days of starting study treatment or have not recovered from effects of surgery are not eligible.

3.2.14 Patients with a history of significant thromboembolic events, (including recurrent DVT, CVAs, or recurrent pulmonary embolism) are not eligible.

3.2.15 Patients with a history of any of the following are not eligible:

- History of noncompliance with other medical therapy for medical conditions that could be impacted by study drug-related adverse events.
- An illness, condition, or other situation that the treating investigator feels would limit compliance with study requirements or would comprise the subject's safety or study endpoints.

# 4 TREATMENT PLAN

## 4.1 Overview

This study plans to enroll two successive cohorts by a Simon two-stage design of 15 and 10 evaluable patients, for possible total of 25 evaluable patients with progressive mCRPC after treatment with at least one first-line therapy for metastatic castration-resistant prostate cancer including new AR targeted therapy (i.e. abiraterone, enzalutamide or similar agents) or chemotherapy (docetaxel or cabazitaxel or noavel taxane). The second cohort will be recruited if the first cohort shows a signal of activity and no signs of unexpected toxicity; see section 6.1 for definitions.

- Patients will receive sEphB4-HSA 1000mg IV (60 ±15 min infusion) on day 1 in a 14-day cycle along with continuation of androgen deprivation therapy. Assessment of response to therapy with PSA will be every odd cycle (every 28 days). Imaging will occur at baseline and 2 months after starting therapy; thereafter, imaging will be conducted every 3 months. After 1 year, imaging can be done every 4 months for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated. Beginning at Cycle 7, cycle length will be 21 days and patients will return for treatment on Day 1 of each cycle.

  *Note: patients will receive ADT per treating physician's choice (LHRH agonist/antagonist or orchiectomy) as standard therapy.*

- Cell Free DNA (cfDNA) will be collected at baseline and on Day 1 of cycle 5, and at disease progression or end of treatment for molecular analysis. We will collect serum for cytokine panel analysis (TNFa, IL1beta, IL2, 4, 5, 6, 8, 10, 12, 13) at baseline Day 1 of cycle 5 prior to drug infusion and at disease progression or end of treatment.

Patients may continue to receive sEphB4-HSA treatment until no longer clinically benefiting (PCWG3), unacceptable toxicity, treatment delay ≥4 weeks, or prohibitive illness/change in patient's condition, or patient decides to withdraw from study. Response assessment with PSA will occur every odd cycle (28 days). Imaging will occur at baseline and 2 months after starting therapy; thereafter, imaging will be conducted every 3 months. After 1 year, imaging can be done every 4 months for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated. Once off treatment continue follow-up for 4 weeks or resolution/stabilization of AE. Patients will be followed every 6 months, until objective progression, or 1 year.

## 4.2 Treatment Administration

**Table 4-2-1: Premedications ***

| Premedication | Dose | Route | Schedule |
|---|---|---|---|
| Diphenhydramine* | 25 mg | Treating investigator discretion / Institutional standards | Administer at least 30 minutes prior to the sEphB4-HSA infusion |
| Acetaminophen* | 650 mg | PO | Administer at least 30 minutes prior to the sEphB4-HSA infusion |

** Refer to Section 4.4.3 for (1) changes to the pre-medication regimen for patients who experience infusion reactions and (2) management of infusion reactions.*

**Table 4-2-2: Treatment Administration Summary**

| Agent | Dose | Route | Schedule | Cycle Length | Supportive Therapies* |
|---|---|---|---|---|---|
| sEphB4-HSA | 1000mg | IV over 60 minutes (±15 min) | Cycles 1-6: Day 1 of each cycle (14 days)<br><br>Cycle 7+: Day 1 of each cycle (21 days) | Cycle 1-6: 14 days<br><br>Cycle 7+: (21 days) | Antiemetics<br><br>Additional medications for prophylaxis and supportive care can be administered as clinically indicated per Institutional standards. |

** Refer to Section 4.4.3 for the management of infusion reactions.*

sEphB4-HSA will be administered intravenously. The dose of sEphB4-HSA will be 1000mg intravenously over 60 minutes (±15 min) on day 1 of a 14-day cycle. Beginning at Cycle 7, cycle length will be 21 days and patients will return for treatment on Day 1 of each cycle. Premedications include diphenhydramine and acetaminophen as described above in Table 4-2-1 . Additional medications for prophylaxis and supportive care can be administered as clinically indicated per institutional protocol.

### 4.3 Toxicity Management & Dose Delays/Modifications

Any patient who receives at least one dose of study therapy will be evaluable for toxicity endpoints. Each patient will be assessed for the development of toxicity according to the timeframe referenced in the Schedule of Events table (section 5). Toxicity will be assessed according to CTCAE v5.0.

- In order to start a new cycle of therapy a patient must have platelets ≥ 100,000, ANC ≥1,000, and resolution of all treatment-related toxicity (except alopecia) to Grade 2 or less or to baseline when baseline was more than Grade 2.
- A dose can be delayed for up to 7 days for resolution of toxicity. The subsequent doses will be delayed so as not to shorten the dosing interval.
- If the dose cannot be given within 7 days of scheduled time it should be omitted. Patients who miss 3 consecutive doses will be removed from the study.
- Patients will be withdrawn from the study if they fail to recover to CTCAE 5.0 ≤1 or baseline from a drug-related toxicity (except alopecia) ≤4 weeks.
- Patients can have a maximum of 2 dose reductions for drug-related toxicity beyond which they will be removed from the study. Dose reduction should follow the dose levels displayed in the table below. Re-escalation is not permitted.

Dose modifications

| Dose Level | Dose of sEphB4-HSA |
|---|---|
| Starting Dose | 1000mg IV over 60 min every 2 weeks |
| -1 | 750 mg IV over 60 min every 2 weeks |
| -2 | 500 mg IV over 60 min every 2 weeks |

#### 4.3.1 Dose modifications for sEphB4-HSA treatment related toxicity

| Toxicity | Grade | sEphB4-HSA |
|---|---|---|
| Non-hematologic toxicities including nausea, vomiting , and diarrhea | Grade 1/2 | If patients develop intolerable Grade 1 or Grade 2 toxicities (per patient) then they can undergo a dose reduction. For Grade 3/4 toxicities despite optimal medical therapy, hold sEphB4-HSA until equal or under Grade 1. Resume at one dose level reduction.<br><br>No routine prophylactic anti-emetic treatment is required at the start of study treatment, however, patients should receive appropriate anti-emetic treatment at the first onset of nausea or vomiting and as required thereafter, in accordance with local treatment practice guidelines |
| General non-hematologic toxicities excluding nausea, | Grade 3/4 | Any patient who experiences Grade 3 or 4 unmanageable non-hematologic toxicity (other than alopecia) at least possibly related to the study |

| | | |
|---|---|---|
| vomiting, and diarrhea | | drug will require immediate dose interruption. Treatment will be withheld until toxicities have resolved to Grade 1 or baseline. After resolution of the toxicity, a one dose level reduction will be initiated. |
| AST/ALT | Grade 3 | Grade 3 AST and/or ALT that does not represent at least a two grade increase from baseline – hold therapy, recheck weekly. If AST and ALT return to baseline grade within 7 days, retreat at same dose level. If AST and ALT do not return to baseline within 7 days, treatment should be restarted once AST and ALT have returned to baseline and dose should be reduced by one level.<br><br>Grade 3 AST and/or ALT that represents at least a two grade increase from baseline or AST/ALT ≥ 10XULN –hold therapy, recheck weekly, initiate retreatment when resolved to baseline with one dose level reduction. |
| | Grade 4 | Grade 4 AST and/or ALT– hold therapy, recheck weekly, initiate retreatment when resolved to baseline with a one level dose reduction.<br><br>Any grade AST or ALT that represents a doubling from baseline AND that is concurrent with a doubling of the total bilirubin- hold therapy, recheck weekly, initiate retreatment when resolved to baseline grade or grade 1 with one level dose reduction. |
| Hypertension[1] | Grade 2 | Patients with grade 2 hypertension as evidenced by two or more different measurements that are at least one our apart should be initiated on antihypertensive therapy per the treating physician. BP should be rechecked in one week after initiation of antihypertensive therapy; if SBP is still >150 and/or DBP is > 100, physician should increase the dose of the antihypertensive or add a second antihypertensive drug as indicated. |
| | Grade 3 | First occurrence, patients may continue on treatment while their antihypertensive therapy is optimized. A second occurrence of grade 3 hypertension despite antihypertensive therapy would require holding treatment with sEphB4-HSA until hypertension returns to ≤ grade 2 at which point treatment can be resumed with one dose reduction. |
| | Grade 4 | Withhold study drug. Optimal management with intensive IV support in ICU. |
| Platelets | Grade 3/4 | Hold therapy, recheck weekly, initiate retreatment when resolved to Grade 1 or less and restart treatment with a one level dose reduction. |
| Neutropenia | Neutropenic fever or Grade 4 neutropenia | Hold therapy, recheck weekly, initiate retreatment when resolved to Grade 1 or less and restart treatment with a one level dose reduction. |
| Anemia | Grade 1 or Grade 2 (Hemoglobin (Hb) > 8 g/dl) | Investigate and manage as deemed appropriate by the investigator with or without interruption of study drug or change in dose, taking into account previous history of anemia. Common treatable causes of anemia (e.g., iron, vitamin B12 or folate deficiencies and hypothyroidism) should be excluded. In some cases management of anemia |

| | | |
|---|---|---|
| | | may require blood transfusions. |
| | Grade 3 (Hb < 8g/dl) or worse | Study treatment should be interrupted for up to maximum of 4 weeks to allow for bone marrow recovery and the patient should be managed appropriately. Study treatment can be restarted at the reduced dose level -1 if Hb has recovered to > 10 g/dl. Any subsequently required anemia related interruptions, considered likely to be dose related, or coexistent with newly developed neutropenia, and or thrombocytopenia, will require a further study treatment dose reduction to level -2.<br><br>If a patient has been treated for anemia with multiple blood transfusions without study treatment interruptions and becomes blood transfusion dependent as judged by investigator, study treatment should be permanently discontinued. |

1. All patients will be educated about the hypertension side effect and instructed to contact the study team if their blood pressure remains under poor control between visits. The patients may be asked to keep a blood pressure log as per treating physician discretion.

*NOTE: Stopping or reducing the dose of study drug can cause a decrease in BP. The treating physician should monitor the patient for hypotension and adjust the number and dose of antihypertensive medication(s) accordingly.*

If the patient experiences a grade 4 allergic or hypersensitivity reaction the drug should be permanently discontinued.

AR targeted therapy will continue per standard of care regardless of sEphB4-HSA toxicity.

### 4.3.2 Toxicities that require discontinuation from protocol therapy

Protocol therapy should be discontinued if a patient experiences:

- Any CTC Grade 4 toxicity with the exception of:
  - CTC Grade 4 neutropenia of 5 days or fewer duration
  - CTC Grade 4 nausea/vomiting or diarrhea that resolves to Grade 3 or less within 72 hours despite optimal supportive care.
  - CTC Grade 4 laboratory abnormalities that can be readily corrected with 72 hours and do not result in hospitalization.
- CTC Grade 3 thrombocytopenia with hemorrhage
- CTC Grade 3 febrile neutropenia
- Hy's Law (AST or ALT >3x ULN with concomitant serum bilirubin >2x ULN and no alternate etiology)

### 4.3.3 Management of Infusion Reaction

Since sEphB4-HSA contains only human protein sequences, it is unlikely to be immunogenic and to induce infusion or hypersensitivity reactions. Since sEphB4-HSA specifically binds to EphrinB2, this makes it less likely that such a reaction would occur. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgia, hypo- or hypertension, bronchospasm, or other symptoms. Prophylactic premedications will be administered as described in Table 4-2-1. Infusion reactions should be graded according to the CTCAE Version 5.0 guidelines. Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines, as appropriate:

For Grade 1 symptoms: (Transient flushing or rash, drug fever < 38C). Remain at bedside and monitor subject until recovery from symptoms.

For Grade 2 symptoms: (Rash, flushing, urticarial, dyspnea, drug fever > 38C) Stop the sEphB4-HSA infusion, begin an i.v. infusion of normal saline, and treat the subject with 25 mg additional diphenhydramine (for total dose of 50 mg, considering prior premedication with 25 mg) i.v. (or equivalent) and/or acetaminophen; remain at bedside and monitor subject until resolution of symptoms. Corticosteroid therapy (e.g., hydrocortisone, PRN, or other Institutional standard) may also be administered as appropriate. Additional supportive care may be administered per Institutional standard practice. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further sEphB4-HSA will be administered at that visit. The amount of study drug infused must be recorded on the case report form (CRF). The following prophylactic premedications are required for future infusions: diphenhydramine 50 mg (or equivalent) and acetaminophen 650 mg (or equivalent). Corticosteroids (e.g., hydrocortisone or other Institutional standard) are also recommended, if clinically indicated. These premedications should be administered at least 30 minutes before additional sEphB4-HSA administration. Remain at bedside and monitor subject until recovery from symptoms.

For Grade 3 or Grade 4 symptoms: (grade 3: Symptomatic bronchospasm, with or without urticarial, requiring parental medications; allergy related edema/angioedema, hypotension OR grade 4: Anaphylaxis as defined as a life-threatening event characterized by the rapid onset of airway obstruction including bronchospasm, stridor, hoarseness, urticarial and/or hypotension): Immediately discontinue infusion of sEphB4-HSA. Begin an i.v. infusion of normal saline, and treat the subject as follows: Recommend bronchodilators, epinephrine 0.3 mg of a 1:1,000 solution for intramuscular administration, or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for i.v. administration, and/or diphenhydramine 50 mg i.v. with methylprednisolone 100 mg i.v. (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. sEphB4-HSA will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

Severe infusion reactions, including fatal reactions, have been observed in mice (but not in monkeys). All clinical sites must have immediate access to emergency medications and equipment and staff trained in CPR in addition to the ability to rapidly transfer patients to an intensive care setting if a patient experiences a severe infusion reaction.

### 4.4 Concomitant Medications/Treatments

Required premedications are described in Table 4-2-1. There are no other known concomitant medications or treatments associated or recommended with sEphB4-HSA at this time.

In the absence of treatment delays due to adverse event(s), concurrent therapy for prostate cancer with the exception of ongoing androgen deprivation and anti-resorptive agents such as bisphosphonates or denosumab are not permitted. If a patient develops a single symptomatic or imminently dangerous metastasis (such as fracture or vertebral compression) then intervention with radiation therapy and/or surgery is permitted after discussion with the study PI. In this case, study drug would be held during the intervention and recommenced after

completion and recovery from any adverse effects. The hold on study drug should not exceed 4 weeks.

### 4.5 Duration of Therapy

Treatment may continue until one of the following criteria applies:

- No longer clinically benefiting (PCWG3, symptomatic or objective disease progression by RECIST v1.1 (See Appendix IV))
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s) as evaluated by the treating physician (see section 4.3)
- Therapy delay for ≥4 weeks
- Patient decides to withdraw from the study or elects to discontinue investigational therapy
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

### 4.6 Duration of Follow Up

Patients removed from study for progression will return for an End of Treatment (EOT) visit 30 (±3) days of the last dose of study treatment or prior to initiating new therapy. Patients removed from study for unacceptable treatment-related adverse event(s) will be followed until resolution or stabilization of the adverse event. If off treatment for reasons other than disease progression, patients will be followed every 6 months, until objective progression or patient starts alternative therapy for a maximum of 1 year.

### 4.7 Removal of Subjects from Study Treatment and/or Study as a Whole

Patients can be taken off the study treatment and/or study as a whole at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation must be clearly documented on the appropriate eCRF and may include:

- Patient voluntarily withdraws from treatment (follow-up permitted)
- Patient withdraws consent (no follow-up permitted)
- Patient is unable to comply with protocol requirements
- Patient demonstrates disease progression
- Patient experiences unacceptable toxicity
- Treating physician determines that continuation on the study would not be in the patient’s best interest
- Patient develops a second malignancy that requires treatment which would interfere with this study
- Patient is lost to follow-up (LTF)

### 4.8 Patient Replacement

Any patient who signs consent and is registered for the trial but does not receive study treatment may be replaced.

## 5 STUDY PROCEDURES

| Time Period | Screening (≤1 month unless otherwise indicated) [17,3] | On Treatment (1 Cycle = **14 Days**) | | | | | | | Off Treatment | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Cycle 1 | Cycle 2 | Cycle 3 | Cycle 4 | Cycle 5 | Cycle 6 | Cycle 7+[13] | End of Treatment[14] | Follow -up[15] |
| | Baseline | Week 1 Day 1 | Week 3 Day 1 | Week 5 Day 1 | Week 7 Day 1 | Week 9 Day 1 | Week 11 Day 1 | Week 13 Day 1 | | |
| **Assessment or Activity** | | | | | | | | | | |
| Informed Consent | X | | | | | | | See Table Below | | |
| Medical history[16] | X[16] | | | | | | | | X[16] | |
| Physical exam[1] | X | X | X | X | X | X | X | | X | |
| ECOG status | X | X | X | X | X | X | X | | X | |
| Toxicity assessment | | X | X | X | X | X | X | | X | |
| Review Concomitant Medications | X | X | | X | | X | | | X | |
| Tumor Imaging[2] | X | X[2,12] | | | | | | | X | |
| CBC + diff, chemistry, liver enzymes*[,3, 4] | X | X | X | X | X | X | X | | X | |
| PSA*[,5] | X | X | | X | | X | | | X | |
| EKG*[,6] | X | | | X | As clinically indicated | | | | As Clinically Indicated | |
| Research blood[7] | X | | | | | X | | | X | |
| Archival tissue sample[8, 9, 10] | X | | | | | | | | X | |
| sEphB4-HSA administration[11] | | X | X | X | X | X | X | | | |
| Survival Status | | | | | | | | | | X |

*Can be competed ±3 days of drug administration

1. Includes vital signs (pulse, blood pressure) and height (baseline only) and weight.
2. Bone scan, CXR or CT of chest, CT or MRI or abdomen and pelvis; with IV contrast if renal function adequate; the same modality used at baseline should be used throughout as clinically indicated. Imaging will occur at baseline and 2 months (± 7 days) after starting therapy; thereafter, imaging will be conducted every 3 months (± 7 days). After 1 year, imaging can be done every 4 months (± 7 days) for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated.
3. Study labs include: CBC with diff, alkaline phosphatase, albumin, total bilirubin, BUN, creatinine, calcium, glucose, AST, ALT, and electrolytes. These study safety labs are to be conducted within ≤2 weeks prior to registration.
4. Testosterone level only at baseline.
5. Assessment of response to therapy with PSA will occur on day 1 of odd cycles,
6. Required for all patients at baseline and C3D1. Then only as clinically indicated thereafter.
7. Blood will be collected at baseline, cycle 5 day 1, and at end of treatment for additional molecular analysis (see section 9).

8. Permission to obtain archival tissue samples of primary lesion and metastatic lesion sites will be obtained at baseline. The samples themselves will be collected only if available and only when requested by the Principal Investigator or Principal Investigator's designee; they may be collected at any time during the study, per PI request. Refer to the lab manual for details.
9. If there is concern about transformation to small cell cancer histology then a standard of care biopsy may be appropriate given that this is an exclusion criteria for this trial.
10. If tumor tissue is biopsied or resected as part of standard medical care or assessment from the time of study enrollment until progression then archival tissue from this biopsy/resection may be used for assay of the markers defined within the protocol to document molecular pathway characteristics and immune cell infiltration at that point to correlate with whether the cancer is responding, stable or progressing.
11. Patients will receive sEphB4-HSA 1000mg IV (60min infusion) on day 1 of each cycle along with continuation of androgen deprivation therapy. Refer to Table 4-2-1 for required premedication regimens.
12. Imaging must be completed and reviewed within 6 days (whenever feasible) prior to receiving treatment.
13. **Please see Table Below.** Beginning at Cycle 7, cycle length will be 21 days and patients will return for treatment on Day 1 of each cycle.
14. Patients will return for an End of Treatment (EOT) visit 30 (±3) days of the last dose of study treatment or prior to start of new treatment.
15. If off treatment for reasons other than disease progression, patients will be followed every 6 months, until objective progression or patient starts alternative therapy for a maximum of 1 year.
16. If patient has had molecular testing (i.e., NGS testing, genetic testing, genomic testing, etc.) performed as standard of care prior to study entry or at any time during study participation, collect a copy of the results report for correlative analyses. Refer to Section 9 for details.
17. Screening/baseline assessments are to be conducted within 1 month prior to registration unless otherwise indicated.

Study Calendar Beginning at Cycle 7

| Time Period | On Treatment (1 Cycle = **21 Days**) | | | | | | Off Treatment | |
|---|---|---|---|---|---|---|---|---|
| | Cycle 7 | Cycle 8 | Cycle 9 | Cycle 10 | Cycle 11 | Cycle 12+ | End of Treatment[14] | Follow-up[15] |
| | Week 13 Day 1 | Week 16 Day 1 | Week 19 Day 1 | Week 22 Day 1 | Week 25 Day 1 | Week 28 Day 1 | | |
| **Assessment or Activity** | | | | | | | | |
| Informed Consent | | | | | | | | |
| Medical history[16] | | | | | | | X[16] | |
| Physical exam[1] | X | X | X | X | X | X | X | |
| ECOG status | X | X | X | X | X | X | X | |
| Toxicity assessment | X | X | X | X | X | X | X | |
| Review Concomitant Medications | X | X | X | X | X | X | X | |
| Tumor Imaging[2] | | | | X[12, 13] | | | X | |
| CBC + diff, chemistry, liver enzymes*[,3, 4] | X | X | X | X | X | X | X | |
| PSA*[,5] | X | X | X | X | X | X | X | |
| EKG*[,6] | As clinically indicated | | | | | | | |
| Research blood[7] | | | | | | | X | |
| Archival tissue sample[8, 9, 10] | X | | | | | | | |
| sEphB4-HSA administration[11] | X | X | X | X | X | X | | |
| Survival Status | | | | | | | | X |

*Can be competed ±3 days of drug administration

1. Includes vital signs (pulse, blood pressure) and height (baseline only) and weight.
2. Bone scan, CXR or CT of chest, CT or MRI or abdomen and pelvis; with IV contrast if renal function adequate; the same modality used at baseline should be used throughout as clinically indicated.
3. Study labs include: CBC with diff, alkaline phosphatase, albumin, total bilirubin, BUN, creatinine, calcium, glucose, AST, ALT, and electrolytes.
4. Testosterone level only at baseline.
5. Assessment of response to therapy with PSA will occur on day 1 of each cycle,
6. Required for all patients at baseline and C3D1. Then only as clinically indicated thereafter.
7. Blood will be collected at baseline, cycle 5 day 1, and at end of treatment for additional molecular analysis (see section 9).
8. Permission to obtain archival tissue samples of primary lesion and metastatic lesion sites will be obtained at baseline. The samples themselves will be collected only if available and only when requested by the Principal Investigator or Principal Investigator's designee; they may be collected at any time during the study, per PI request. Refer to the lab manual for details.
9. If there is concern about transformation to small cell cancer histology then a standard of care biopsy may be appropriate given that this is an exclusion criteria for this trial.
10. If tumor tissue is biopsied or resected as part of standard medical care or assessment from the time of study enrollment until progression then archival tissue from this biopsy/resection may be used for assay of the markers defined within the protocol to document molecular pathway characteristics and immune cell infiltration at that point to correlate with whether the cancer is responding, stable or progressing.
11. Patients will receive sEphB4-HSA 1000mg IV (60min infusion) on day 1 of each cycle along with continuation of androgen deprivation therapy. Refer to Table 4-2-1 for required premedication regimens.
12. Imaging must be completed and reviewed prior to receiving treatment.

13. Imaging will occur at baseline and 2 months (± 7 days) after starting therapy; thereafter, imaging will be conducted every 3 months (± 7 days). After 1 year, imaging can be done every 4 months (± 7 days) for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated.
14. Patients will return for an End of Treatment (EOT) visit 30 (±3) days of the last dose of study treatment or prior to start of new treatment.
    If off treatment for reasons other than disease progression, patients will be followed every 6 months, until objective progression or patient starts alternative therapy for a maximum of 1 year.
15. If off treatment for reasons other than disease progression, patients will be followed every 6 months, until objective progression or patient starts alternative therapy for a maximum of 1 year.
16. If patient has had molecular testing (i.e., NGS testing, genetic testing, genomic testing, etc.) performed as standard of care prior to study entry or at any time during study participation, collect a copy of the results report for correlative analyses. Refer to Section 9 for details.

# 6 ENDPOINT ASSESSMENT

## 6.1 Definitions

### 6.1.1 Evaluable patients

Evaluable for primary endpoint of PSA response. All patients who received at least 1 dose of the study drug and who also had a followup PSA test conducted prior to initiating subsequent therapy will be evaluable for PSA response.

Evaluable for toxicity. All patients who received at least 1 dose of the study drug will be evaluable for toxicity.

Evaluable for objective response. Only those patients who have measurable disease present at baseline will be considered evaluable for objective response. These patients will have their response classified according to the definitions stated below.

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

### 6.1.2 Disease Parameters by RECIST V1.1

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

*Note: Tumor lesions that are situated in a previously irradiated area should not be considered measurable unless they have demonstrated progression.*

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow- up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis of skin or lung, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

*Note: Cystic lesions that meet the criteria for radiographically defined simple cysts*

*should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.*

*'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.*

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

### 6.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 6 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and 10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same

pulse sequence. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

**6.1.4 Methods of Evaluation of Bone disease:**
Bone disease will be evaluated using technetium Radionuclide bone scan and PCWG3 using PCCTC bone scan forms.

**6.1.5 PSA response criteria:**
These definitions are intended to characterize the PSA changes on study for the purpose of reporting of results.

Complete Response (CR): Undetectable PSA (≤0.2 ng/ml) that is confirmed by another PSA level at no less than 4 weeks interval (+/- 3 days).

Partial Response (PR): Decrease in PSA value by ≥ 50% that is confirmed by another PSA level at no less than 4 weeks interval (+/- 3 days).

Stabilization (SD): Patients who do not meet the criteria for PR or PD for at least 90 days on study will be considered stable

Progression (PD): 25% increase over baseline or nadir whichever is lower and an increase in the absolute value of PSA level by 2 ng/ml that is confirmed by another PSA level at no less than 4 weeks interval.

**6.1.6 Evaluation of target lesions by RECIST v1.1**
Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

**6.1.7 Evaluation of Non-Target Lesions by RECIST v1.1 (excluding bone lesions, which will be assessed as below)**
Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

**6.1.8 Evaluation of bone disease based on Radionuclide bone scans by PCWG3:**
Changes in intensity will not be used as an outcome measure.

Stable or Improved: A stable or improved classification requires that no new lesions appear on radionuclide bone scan or that new pain has not developed in an area that was previously visualized.

Progression (Non-Response): Appearance of two or more new skeletal lesions that is confirmed on a repeat scan. *An increase in the size or intensity of known skeletal lesions will not be considered progression*.

**6.1.9 Evaluation of best overall response by RECIST V1.1**
The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (*i.e.*, Target Disease)

| Target Lesions | Non-Target Lesions | New Lesions | Overall Response | |
|---|---|---|---|---|
| CR | CR | No | CR | |
| CR | Non- CR/Non-PD | No | PR | |
| CR | Not evaluated | No | PR | |
| PR | Non- CR/Non-PD/not evaluated | No | PR | |
| SD | Non- CR/Non-PD/not evaluated | No | SD | |
| PD | Any | | PD | no prior SD, PR or CR |
| Any | PD*** | | PD | |
| Any | Any | Yes | PD | |

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non -Measurable Disease (*i.e.*, Non Target Disease)

| Non-Target Lesions | New Lesions | Overall Response |
|---|---|---|
| CR | No | CR |
| Non-CR/non-PD | No | Non-CR/non-PD* |
| Not all evaluated | No | not evaluated |
| Unequivocal PD | Yes or No | PD |
| Any | Yes | PD |

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

## 6.2 Assessment of primary endpoint

The assessment of the primary endpoint of confirmed PSA response rate is defined as the proportion of subjects who received at least 1 dose of the study drug achieving a post-treatment PSA partial response or complete response as defined by PSA response criteria in section 6.1.5

## 6.3 Assessment of secondary endpoints

6.3.1 The safety and tolerability of sEphB4-HSA in patients with mCRPC will be assessed according to NCI CTCAE v 5.0 and reported by the toxicity, severity, and attribution will be recorded for each cycle.

6.3.2 The time to PSA progression will be assessed by calculating the interval from administration of the first dose of drug on cycle 1 day 1 to PSA progression. PSA progression is defined by the criteria in section 6.1.5 PSA will be assessed every odd cycle.

6.3.3 The overall response rate will be the proportion of patients with measurable disease who received at least 1 dose of the study drug and as their best response achieved a partial or complete response (responder). A subject will be classified as a responder if the RECIST 1.1 criteria for a CR or PR are satisfied (see 10.1.6 and10.1.7) as well as the absence of confirmed progression on bone scan assessed by PCWG3 (see 10.1.8) at any time up to and including the defined analysis cut-off point. Disease evaluation will be assessed by imaging at baseline and 2 months after starting therapy; thereafter, imaging will be conducted every 3 months. After 1 year, imaging can be done every 4 months for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated.

6.3.4 The time to radiologic progression (rPFS) will be assessed by calculating the interval from administration of the first dose of drug on cycle 1 day 1 to the time to radiologic progression by RECIST 1.1 or PCWG3 bone criteria (10.1.6-108) or death from any cause. Imaging will occur at baseline and 2 months after starting therapy; thereafter, imaging will be conducted every 3 months. After 1 year, imaging can be done every 4 months for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated.

### 6.4 Exploratory Endpoints

6.4.1 EphB4 and ephrinB2 expression will be assessed by IHC staining of primary and/or metastatic site (recent archival specimen or new biopsy). EphB4 and other biomarker abnormalities will be assessed by analyzing genomic alterations that are described in the results of molecular analysis reports that are obtained from standard of care tests (i.e., results from standard of care NGS testing/ genetic testing/ genomic testing, etc.)

6.4.2 Analysis of cfDNA for abnormalities in PI3K pathway, MYC or TP53.

6.4.3 IHC for CD3, CD4, CD8, and NK cell markers to characterize the immune infiltrate in tumor specimen.

## 7 ADVERSE EVENTS

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University. The level of risk attributed to this study requires High Risk Monitoring, as outlined in the DSMP. In addition, the study will abide by all safety reporting regulations, as set forth in the Code of Federal Regulations.

### 7.1 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (see Section 5 for time points). In addition, certain adverse events must be reported in an expedited manner to allow for optimal monitoring and patient safety and care.

All patients experiencing an adverse event, regardless of its relationship to study drug, will be followed until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

### 7.2 Definitions & Descriptions

#### 7.2.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

Recording of AEs should be done in a concise manner using standard, acceptable medical terms. In general, AEs are not procedures or measurements, but should reflect the reason for the procedure or the diagnosis based on the abnormal measurement. Preexisting conditions that worsen in severity or frequency during the

study should also be recorded (a preexisting condition that does not worsen is not an AE). Further, a procedure or surgery is not an AE; rather, the event leading to the procedure or surgery is considered an AE.

If a specific medical diagnosis has been made, that diagnosis or syndrome should be recorded as the AE whenever possible. However, a complete description of the signs, symptoms and investigations which led to the diagnosis should be provided. For example, if clinically significant elevations of liver function tests are known to be secondary to hepatitis, "hepatitis" and not "elevated liver function tests" should be recorded. If the cause is not known, the abnormal test or finding should be recorded as an AE, using appropriate medical terminology (e/g/ thrombocytopenia, peripheral edema, QT prolongation).

### 7.2.2 Severity of AEs

All non-hematologic adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The CTCAE v 5.0 is available at https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm

If no CTCAE grading is available, the severity of an AE is graded as follows:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living*.
Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living**.
Grade 4: Life-threatening consequences; urgent intervention indicated.
Grade 5: Death related to AE
*Instrumental activities of daily living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self care activities of daily living refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

### 7.2.3 Serious Adverse Events (SAEs)

All SAEs, regardless of attribution, occurring from time of signed informed consent, through 30 days after the last administration of study drug, must be reported upon discovery or occurrence.
An SAE is defined in regulatory terminology as any untoward medical occurrence that:

- **Results in *death*.**
  If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.
- **Is life-threatening.**
  The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
- **Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.**
- **Results in persistent or significant disability or incapacity.**
- **Is a congenital anomaly/birth defect.**
- **Is an important medical event.**

Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of “Serious Adverse Event“.
For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

#### 7.2.4 Unanticipated Problems Involving Risks to Subject or Others

A UPIRSO is a type of SAE that includes events that meet ALL of the following criteria:

- is unexpected (in terms of nature, severity, or frequency) given the procedures described in the research protocol documents (e.g., the IRB-approved research protocol and informed consent document) and the characteristics of the human subject population being studied
- is related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places human subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized, even if no harm has actually occurred.

## 7.3 Adverse Event Reporting

### 7.3.1 Routine Reporting

All routine adverse events, such as those that are expected, or are unlikely or definitely not related to study participation, are to be reported on the appropriate eCRF. Routine AEs will be reviewed by the Data and Safety Monitoring Committee (DSMC) according to the study’s phase and risk level, as outlined in the DSMP.

### 7.3.2 Determining if Expedited Reporting is Required

This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

1) Identify the type of adverse event using the NCI CTCAE v 5.0.
2) Grade the adverse event using the NCI CTCAE v 5.0.
3) Determine whether the adverse event is related to the protocol therapy.
   Attribution categories are as follows:
   - Definite: AE is clearly related to the study treatment.
   - Probable: AE is likely related to the study treatment.
   - Possible: AE may be related to the study treatment.
   - Unlikely: AE not likely to be related to the study treatment.
   - Unrelated: AE is clearly NOT related to the study treatment.
4) Determine the prior experience of the adverse event.
   Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:
   - the current protocol
   - the drug package insert
   - the current Investigator’s Brochure

### 7.3.3 Expedited Reporting of SAEs/Other Events

#### 7.3.3.1 Reporting to the Northwestern University QAM/DMC

All SAEs must be reported to the assigned QAM within 24 hours of becoming aware of the event. Completion of the NU CTO SAE Form, provided as a separate document, is required.

The completed form should assess whether or not the event qualifies as a UPIRSO. The report should also include:

- Protocol description and number(s)
- The patient's identification number
- A description of the event, severity, treatment, and outcome (if known)
- Supportive laboratory results and diagnostics
- The hospital discharge summary (if available/applicable)
- Country of incidence

All SAEs will be reported to, and reviewed by, the DMC per the DSMP.

#### 7.3.3.2 Reporting to the Northwestern University IRB

*The following information pertains to the responsibilities of the lead site (Northwestern University). Additional participating sites should follow their local IRB guidelines for reporting to their local IRBs.*

- Any death of an NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.
- Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be reported to Northwestern University and to the NU IRB within 5 working days of notification.
- Information pertaining to an NU subject that fits into any of the categories listed on the Reportable New Information page will be reported to the NU IRB within 5 business days of knowledge or notification

#### 7.3.3.3 Reporting to VasGene Therapeutics, Inc

SAEs will be reported within 24 hours/1 business day to VasGene Therapeutics by e-mail (linda@vasgene.com) using the NU CTO SAE form.

#### 7.3.3.4 Reporting to the FDA

The FDA will be notified within 7 calendar days of any SAE that is associated with study treatment, is unexpected, and is fatal or life-threatening.

The FDA will be notified within 15 calendar days of any SAE that is associated with the study treatment, unexpected, and serious but not fatal or life-threatening. This includes any previous SAEs that were not initially deemed reportable, but are later determined to meet the criteria for reporting (i.e. by the DSMC).

All other SAEs will be reported on an annual basis as part of the annual FDA report.

# 8 DRUG INFORMATION

## 8.1 sEphB4-HSA

### 8.1.1 Other names

None

### 8.1.2 Classification - type of agent

sEphB4-HSA is a fully human recombinant protein consisting of the extracellular domain (aa 1 to 522) of EphB4 receptor at the N-terminus and human albumin (aa 1 to 585) at the C-terminus. sEphB4-HSA specifically binds to EphrinB2, a trans-membrane ligand and member of Ephrin ligand family. EphrinB2 is the only ligand for EphB4 receptor.

### 8.1.3 Mode of action

sEphB4-HSA binds to EphrinB2 with high affinity (5-10nM) and blocks interaction with cell surface EphB receptors, thus blocking forward and reverse signaling.

### 8.1.4 Storage and stability

The Drug Product contains sEphB4-HSA at 25 mg/mL in 10 mM L-Histidine, 150 mM Sodium Chloride, and 10% Sucrose, pH 7.0.

sEphB4-HSA vials must be stored at a temperature 2-8°C and should be protected from light. Recommended safety measures for preparation and handling of sEphB4-HSA using standard practice. Once diluted in an IV 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.

### 8.1.5 Protocol dose specifics

sEphB4-HSA will be given as 1000mg IV infusion over 60 minutes (±15 minutes) every 2 weeks on day 1 (± 3) of each cycle during Cycles 1-6 (1 cycle =14 days). Beginning at Cycle 7, patients will receive sEphB4-HSA every 3 weeks on Day 1 of each cycle (1 cycle = 21 days).

Refer to Table 4-2-1 for required premedication regimens.

### 8.1.6 Preparation

- Each vial contains 250 mg of drug in a 10 mL volume (vial concentration 25 mg/mL).
- The product is a clear, colorless to light straw yellow sterile solution in 10 mL glass vials.
- Allow the appropriate number of vials of sEphB4-HSA to stand at room temperature for approximately 15 minutes before preparation.
- Ensure that the sEphB4-HSA solution is a colorless, clear to slightly opalescent solution, essentially free of particles on visual inspection.
- Aseptically withdraw the required volume of sEphB4-HSA solution into a syringe, and dispense into an IV bag. (If multiple vials are needed for a subject, it is important to use a separate sterile syringe and needle for each vial to prevent problems such as dulling of needle tip, stopper coring, repeated friction of plunger against syringe barrel wall and so on).
- The necessary dose should be diluted in appropriate size bag of 0.9% sodium chloride to a total volume of 200 mL. EVA, PVC-free or PVC bags can be used for drug preparation.
- Mix by GENTLY inverting several times. DO NOT shake.
- Visually inspect the final solution. If the infusion is not clear or the contents appear to contain precipitate, the solution should be discarded and documented on the Drug Accountability Log.
- Administer over 60 (±15) minutes via 0.2 µm in-line filter.
- Care must be taken to ensure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent.

#### 8.1.7 Route of administration for this study

Administered IV via peripheral or central line. Administer with 0.2 μm in-line filter.

#### 8.1.8 Incompatibilities

No known incompatibilities, sEphB4-HSA should be given through a dedicated intravenous line. Do not co-administer other medications at the same line.

#### 8.1.9 Availability & Supply

sEphB4-HSA will be shipped to each site from VasGene Therapeutics, Inc. Additional supplies can be ordered using the drug supply form provided VasGene Therapeutics, Inc. The product is a clear, colorless to light straw yellow sterile solution in 10 ml glass vials. Each vial contains 250 mg of drug in a 10 mL volume (vial concentration 25 mg/ml). The vial is closed with an appropriately sized stopper and seal.

#### 8.1.10 Side effects

Please refer to most recent for IB for full list of Reported Adverse Events.

#### 8.1.11 Nursing implications

Attach the IV bag containing the sEphB4-HSA solution to administration set (tubing) with 0.2 μm in-line filter, and infusion pump. The infusion rate of the infusion pump should be adjusted to allow for a total infusion time of 60 minutes (±15 minutes). At the end of the infusion period, flush the line with a sufficient quantity of normal saline. Do not administer study drug as an i.v. push or bolus injection.

#### 8.1.12 Return and Retention of Study Drug

Any partially used and unused drug can be destroyed per the institution’s drug destruction policy

## 9 CORRELATIVES/SPECIAL STUDIES

| Correlative Samples - Details for Lab Manual | | | |
|---|---|---|---|
| Correlative study | Recent archival tumor tissue (primary and metastatic) if available. | Blood | |
| Mandatory or Optional | Optional | Mandatory | |
| Timing (+/- windows) | When requested by the Principal Investigator or Principal Investigator's designee; Samples may be collected at any time during the study per PI request. | Baseline, cycle 5 day 1, EOT | |
| Volume Needed (blood only) | -- | 10ml | 2ml |
| Tube type needed (blood only) | -- | 10ml EDTA tubes | Draw blood in a plain, red-top tube(s), serum gel tube is acceptable. |
| Tissue thickness and/or # slides (tissue only) | Up to 12 slides at 5µm thickness | -- | |
| Processing center (e.g. PCF-CTU) | Each Institution | Each Institution | Spin down within 2 hours and send 1 mL of serum frozen in a plastic vial. |
| Sample handling/processing instructions | Each institution will send samples to NU PCF-CTU | Each institution will send samples to NU PCF-CTU | Each institution will send samples to NU PCF-CTU |
| Contact person | CTUtissuerequests@northwestern.edu | PCF-CTU@northwestern.edu | PCF-CTU@northwestern.edu |
| Shipping/delivery info | PCF-CTU<br><br>Samples must be shipped overnight Monday-Thursday **only**. | PCF-CTU<br><br>Samples must be shipped overnight Monday-Thursday **only**. | PCF-CTU<br><br>Samples must be shipped overnight Monday-Thursday **only**. |
| Storage needs | For long term, sections will be stored at 4°C | For long term, samples will be stored at -80°C at NU PCF-CTU | For long term, samples will be stored at -80°C |
| Analysis center | Abdulkadir Lab | Samples will be stored for future analysis at Northwestern University. | Abdulkadir Lab |
| Assay methodology | IHC<br><br>Note: Tissue for IHC will be second priority. | Sequencing | Cytokine Panel |

### 9.1 Description of Correlative studies:

#### 9.1.1 The role of EpHB4 in driving mCRPC

Abundant evidence from human and transgenic mouse studies indicate that alterations in key cancer genes, including MYC, an oncogene and PTEN, a tumor suppressor and negative regulator of PI3K activity can conspire to lead to aggressive prostate cancer [23]. EphB4 supports cMYC expression at transcriptional level in

colon cancer [25]. Further, kinomic analyses of EphB4 mutations in lung cancer revealed the involvement of PTEN or TP53 loss, and activation of EGFR or IGF1R pathways [26]. EphB4 can promote tumorigenesis by engaging multiple signaling pathways, including the PI3 kinase/AKT pathway [27]. We are particularly interested in determining if sEphB4-HSA treatment modulates PI3K/AKT pathways in Myc driven tumorigenesis. For this, we will evaluate the expression of MYC, PI3K and downstream mediators pS6K.

In addition to PI3K pathways, preliminary studies from Dr. Abdulkadir and Dr. Gill's laboratories show that the expression of EphB4 level is retained after Androgen Deprivation Therapy (ADT), indicating that it can be a target in cancers after ADT. We also want to explore if sEphB4-HSA cooperates with enzalutamide or abiraterone. There is an interesting relationship between the PI3K and AR pathways in castration-resistant prostate cancer with therapeutic implications. Combining AR inhibition with PI3K inhibition may be an effective strategy for treating Pten-null CRPC [28]. As shown in the preliminary results, sEphB4 treatment inhibits that PI3K pathway, thereby providing a rationale or combining sEphB4 with AR pathway inhibition. We will evaluate the expression of AR in primary and metastatic tissue of patient and correlate the status of AR as a predictive biomarker for response to sEphB4-HSA treatment.

Mechanistically, sEphB4-HSA acts by binding to Ephrin B2 and preventing it's binding to EphB4. Given the cooperation between Ephrin B2 expressing endothelial cells and EphB4 expressing tumor cells [29], it is important to investigate the expression of both molecules in this treatment setting. To this end, we will evaluate the expression of EphB4 and Ephrin B2 in the tumor microenvironment of patient tissue and correlate to treatment response.

We also aim to understand the impact of sEphB4-HSA treatment on the tumor microenvironment in general and immune cell infiltration to the metastatic tumor site in particular. Ephrin B2, the binding partner of sEphB4-HSA and EphB4 receptor plays a prominent role in suppression of activated T cells [30]. Our preliminary *in vivo* studies showed the increase of CD3 cells in transgenic mice (Pten-,Myc+ P53-) treated with sEphB4-MSA. Therefore, it is worthwhile to study tumor-infiltrating lymphocytes in tumor tissues from baseline evaluation and response evaluation time points. Specifically, we will investigate the expression of $CD4^+$ Helper T cells and $CD8^+$ Cytotoxic T cells, and $CD31^+$ Endothelial cells.

#### 9.1.2 EphB4 activation and other associated genes as a biomarker for response

EphB4 is known to be upregulated in most cancer tissues. Furthermore, preclinical evidence from mouse model of aggressive prostate cancer with Myc amplification and PTEN showed significant tumor regression following EphB4 targeted therapy. Therefore it is of high significance to identify genomic alterations in EphB4 and other associated genes like Myc, p53, PI3k and AR to predict clinical response. We will analyze standard of care genetic/genomic/NGS testing reports to identify genetic alterations including MYC, AR, P53 and other most commonly mutated cancer driver genes. We will also analyze through immunohistochemistry the baseline level of EphB4 and Ephrin B2 and the molecular drivers of mCRPC mentioned in 9.1.1, their co activation with EphB4 and contribution to development of mCRPC [18].

#### 9.1.3 The landscape of genomic alterations in the course of sEphB4 treatment

CfDNA has emerged as an efficient non-invasive biomarker that not only correlates to cancer stage and response to therapy but also identifies somatic mutations acquired with therapy predicting pathways involved in drug resistance [31]. cfDNA will be

isolated from the blood samples and stored for future analysis and analyzed by NGS testing

### 9.2 Sample Collection Guidelines

From each patient, specimens will be collected as below:

a. If available, archival tissue of primary tumor and metastic tumor will be obtained.
b. Blood for cfDNA (pre-treatment, Cycle 3 Day 1 and at clinical progression or when off study, whichever is first)

Please refer to Lab Manual for instructions on sample collection.

### 9.3 Sample Processing, Storage, and Shipment

See the Lab Manual for instructions on sample processing, storage and shipment.

### 9.4 Assay Methodology

9.4.1 Immunohistochemistry:

For metastatic and primary tumor samples we will follow the standard protocol for immunohistochemistry to perform qualitative and quantitative measurement of EphB4, EphrinB2, PI3K-AKT pathway, MYC, PTEN pathway, AR, and for markers of Tumor Infiltrating lymphocytes and endothelial cells For immune cell staining, the pathologist will assign scores of 0, 1+, 2+ or 3+ based on staining intensity and percent positive cells per unit area of section

#### 9.4.2 Next Generation Sequencing

cfDNA samples will be analyzed by NGS assays at Northwestern University.

### 9.5 Specimen Banking

Research samples will be stored at the Abdulkadir Lab or Northwestern University Pathology Core Facility until the material has been exhausted or the repository is discontinued after the trial is completed. If the repository is permanently closed, all stored sample material will be destroyed. Samples may be maintained beyond the death of the participant.

## 10 STATISTICAL CONSIDERATIONS

This is a single-arm study evaluating the efficacy, safety, and tolerability of sEphB4-HSA in patients with mCRPC. Analysis populations are defined below.

### 10.1 Sample size and Statistical and data analysis plan for the primary objective.

To estimate **preliminary efficacy** we will assess PSA response rate (PR and CR) with a **Simon two stage minimax design**. We assume the undesirable overall response rate (null hypothesis) to be approximately 10% or less, and the alternate hypothesis suggesting success to be approximately 30% or more. Fifteen patients will be added in the first stage. If 2 or more respond, then an additional 10 patients will be added for a total of 25 evaluable patients. If not, we will stop the study after the first stage. The study will be assessed by the DSMC for excess toxicity at this point in time and stopped if DSMC finds excessive toxicity. If the second stage is completed, five or fewer successes will suggest failure and six or more responses a success of the entire study. This design has a Type I error rate of less than 5% and 80% or greater power. It has a 55% chance of stopping early after the first stage if the true response rate is 10% or less, and a total expected long run average sample size of n=19.5. See below, using Pass 11 software.

**Two-Stage Clinical Trials Sample Size**
**Possible Designs For P0=0.100, P1=0.300, Alpha=0.050, Beta=0.200**

| N1 | R1 | PET | N | R | Ave N | Alpha | Beta | Constraints Satisfied |
|---|---|---|---|---|---|---|---|---|
| 25 | 5 | 0.000 | 25 | 5 | 25.00 | 0.033 | 0.193 | Single Stage |
| **15** | **1** | **0.549** | **25** | **5** | **19.51** | **0.033** | **0.198** | **Minimax** |
| 10 | 1 | 0.736 | 29 | 5 | 15.01 | 0.047 | 0.195 | Optimum |

**References**

Simon, Richard. 'Optimal Two-Stage Designs for Phase II Clinical Trials', Controlled Clinical Trials, 1989, Volume 10, pages 1-10.

**Report Definitions**

N1 is the sample size in the first stage.
R1 is the drug rejection number in the first stage.
PET is the probability of early termination of the study.
N is the combined sample size of both stages.
R is the combined drug rejection number after both stages.
Ave N is the average sample size if this design is repeated many times.
Alpha is the probability of rejecting that P<=P0 when this is true.
Beta is the probability of rejecting that P>=P1 when this is true.
P0 is the response proportion of a poor drug.
P1 is the response proportion of a good drug.

PSA response rate will be reported with two-sided 80% exact binomial confidence interval (corresponding to the one-sided α=0.10).

### 10.2 Analysis of secondary and exploratory objectives

To estimate the distributions of time to PSA progression and rPFS (defined in Section 6.3), we will use Kaplan Meier methods, and estimate the median with two-sided 90% CI.

The estimated overall response rate in patients with measurable disease (Section 6.1.1) will be reported with two-sided 90% exact binomial CI.

For each patient within the population evaluable for toxicity (Section 6.1.1), recorded AE data over time will consolidated as the maximum grade observed of each AE type. All reported AE types will be tabulated by maximum grade using frequencies and percentages. Data on type, timing, frequency and attribution of AEs will also be summarized.

To explore if PSA response is associated with expression of EphB4 and ephrinB2 in archival metastatic and primary tumor CRPC specimens, and/or with aberrations in the PI3K pathway, P53, MYC. Marker values will be summarized according to PSA response status. Summaries will be descriptive and graphical; hypothesis testing is not planned.

### 10.3 Accrual

This multi-site trial will accrue patients at University of Chicago, University of Southern California and Northwestern University. Four-eight potentially eligible patients are seen per month at participating sites. Accordingly, we plan to enroll 1-2 patients per month. We expect it will take 18 months to accrue our study population.

## 11 STUDY MANAGEMENT

### 11.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

### 11.2 Amendments

The Principal Investigator will formally initiate all amendments to the protocol and/or informed consent. All amendments will be subject to the review and approval of the appropriate local, institutional, and governmental regulatory bodies, as well as by VasGene Therapeutics Inc. Amendments will be distributed by the lead institution (Northwestern) to all affiliate sites upon approval by the Northwestern University IRB.

### 11.3 Registration Procedures

For potential patients for this study, study teams are asked to inform the QAM of the date and time that the patient will need to be registered (croqualityassurance@northwestern.edu).

BEFORE a patient can be treated on study, please complete and submit the following items to confirm eligibility and receive an identification number:

- Patient's signed and dated informed consent form (upload to NOTIS and keep original hard copy in a secure location/study chart)
- Eligibility checklist (signed and dated by the treating physician – upload to NOTIS)
- Eligibility eCRF (complete in NOTIS)
- Copy of the pathology report (upload to NOTIS)

The QAM will review all source documentation required to confirm eligibility that is readily available in the patient's electronic medical record (EMR). Any information that is not available in the EMR must be de-identified and emailed to the QAM. Once the QAM confirms the patient is eligible, he or she will register the patient, assign a subject identification number, provide a cohort assignment, and send a confirmation of registration to involved personnel. Registration will then be complete and the patient may begin study treatment.

### 11.4 Data Submission

Once a patient is confirmed and registered to the study, eCRFs should be submitted according to the study procedures table. Generally, for all phase II patients, data are due within 10 days of a visit. A set amount of data may also be requested for any screen failures, as is defined by the study. In most instances, this will include collection of adverse events and baseline data from the time of registration to the date of screen failure.

### 11.5 Data Management and Monitoring/Auditing

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (please refer

to NOTIS for additional information). The level of risk attributed to this study requires *High Risk* as outlined in the DSMP. The assigned QAM, with oversight from the Data Monitoring Committee, will monitor this study in accordance with the study phase and risk level. Please refer to the NOTIS for additional data submission instructions.

## 11.6 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

### 11.6.1 Emergency Modifications

For any such emergency modification implemented, an IRB modification form must be completed within 5 business days of making the change, and the QAM must be notified within 24 hours of such change.

### 11.6.2 Other Protocol Deviations

All other deviations from the protocol must be reported to the assigned QAM using the appropriate form.

A protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that is under the investigator's control and that has not been approved by the Institutional Review Board (IRB). Protocol deviations must be reported according to the policies and procedures of the IRB of record.

A protocol deviation may be considered an instance of Promptly Reportable Non-Compliance (PRNC) if it:

- Has harmed or increased the risk of harm to one or more research participants.
- Has compromised the rights and welfare of the research subject
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

## 11.7 Investigator Obligations

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The PI is responsible for personally overseeing the treatment of all study patients. The PI must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected, entered onto the appropriate eCRFs, and submitted within the study-specific timeframes. Periodically, monitoring visits may be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. The study may also be subject to routine audits by the Audit Committee, as outlined in the DSMP.

## 11.8 Publication Policy

All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in accordance with the DSMC Data Release Policies and Processes. The assigned QAM will prepare a preliminary data set for DSMC approval no later than 3 months after the study reaches its primary completion date, as defined by ClinicalTrials.gov. This is the date that the final patient was examined or received an intervention for the purposes of final collection of data for the primary outcome, whether the clinical trial concluded according to the pre-specified protocol or was

terminated. If the investigator would like data release to be approved by the DSMC prior to when study design specifies, and/or prior to three months after a study's primary completion date, the PI must send a written request for data approval to the QAM which includes justification. Requests must be made a minimum of six to eight weeks in advance of the expected deadline. The request will be presented to the DSMC at their next available meeting. Any DSMC decisions regarding data release will be provided to the PI. If the request is approved, the QAM will present the data set to the DSMC for approval. A final, DSMC-approved dataset, as applicable, will then be released 6-8 weeks after the request was made. The investigators are expected to use only DSMC-approved data and statistical analyses any time they are disseminating trial data. The investigators must send a copy of the draft abstract/poster/manuscript to the study's biostatistician and assigned QAM to confirm that the DSMC-approved data and statistical analyses are used appropriately. Once the biostatistician and QAM gives final approval, the publication may be submitted to external publisher.

## References

1. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer Statistics, 2017.* CA Cancer J Clin, 2017. **67**(1): p. 7-30.
2. Harris, W.P., et al., *Androgen deprivation therapy: progress in understanding mechanisms of resistance and optimizing androgen depletion.* Nat Clin Pract Urol, 2009. **6**(2): p. 76-85.
3. Fizazi, K., et al., *Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study.* Lancet Oncol, 2012. **13**(10): p. 983-92.
4. Rathkopf, D.E., et al., *Updated interim efficacy analysis and long-term safety of abiraterone acetate in metastatic castration-resistant prostate cancer patients without prior chemotherapy (COU-AA-302).* Eur Urol, 2014. **66**(5): p. 815-25.
5. Scher, H.I., et al., *Increased survival with enzalutamide in prostate cancer after chemotherapy.* N Engl J Med, 2012. **367**(13): p. 1187-97.
6. Berthold, D.R., et al., *Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer: updated survival in the TAX 327 study.* J Clin Oncol, 2008. **26**(2): p. 242-5.
7. de Bono, J.S., et al., *Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial.* Lancet, 2010. **376**(9747): p. 1147-54.
8. Kantoff, P.W., et al., *Sipuleucel-T immunotherapy for castration-resistant prostate cancer.* N Engl J Med, 2010. **363**(5): p. 411-22.
9. West, T.A., B.E. Kiely, and M.R. Stockler, *Estimating scenarios for survival time in men starting systemic therapies for castration-resistant prostate cancer: a systematic review of randomised trials.* Eur J Cancer, 2014. **50**(11): p. 1916-24.
10. Bianchini, D., et al., *Antitumour activity of enzalutamide (MDV3100) in patients with metastatic castration-resistant prostate cancer (CRPC) pre-treated with docetaxel and abiraterone.* Eur J Cancer, 2014. **50**(1): p. 78-84.
11. Caffo, O., et al., *Clinical Outcomes of Castration-resistant Prostate Cancer Treatments Administered as Third or Fourth Line Following Failure of Docetaxel and Other Second-line Treatment: Results of an Italian Multicentre Study.* Eur Urol, 2015. **68**(1): p. 147-53.
12. Boyd, A.W., P.F. Bartlett, and M. Lackmann, *Therapeutic targeting of EPH receptors and their ligands.* Nat Rev Drug Discov, 2014. **13**(1): p. 39-62.
13. Karantanos, T., P.G. Corn, and T.C. Thompson, *Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches.* Oncogene, 2013. **32**(49): p. 5501-5511.
14. Kawano, H., et al., *A novel feedback mechanism by Ephrin-B1/B2 in T-cell activation involves a concentration-dependent switch from costimulation to inhibition.* Eur J Immunol, 2012. **42**(6): p. 1562-72.
15. Shiuan, E. and J. Chen, *Eph Receptor Tyrosine Kinases in Tumor Immunity.* Cancer Res, 2016.
16. Wang, B., *Cancer cells exploit the Eph-ephrin system to promote invasion and metastasis: tales of unwitting partners.* Sci Signal, 2011. **4**(175): p. pe28.
17. Xia, G., et al., *EphB4 expression and biological significance in prostate cancer.* Cancer Res, 2005. **65**(11): p. 4623-32.
18. Lee, Y.C., et al., *Investigation of the expression of the EphB4 receptor tyrosine kinase in prostate carcinoma.* BMC Cancer, 2005. **5**: p. 119.
19. Grasso, C.S., et al., *The mutational landscape of lethal castration-resistant prostate cancer.* Nature, 2012. **487**(7406): p. 239-43.
20. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer statistics, 2017.* CA: A Cancer Journal for Clinicians, 2017. **67**(1): p. 7-30.
21. Sweeney, C.J., et al., *Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer.* New England Journal of Medicine, 2015. **373**(8): p. 737-746.
22. James, N.D., et al., *Abiraterone for Prostate Cancer Not Previously Treated with Hormone Therapy.* New England Journal of Medicine, 2017. **377**(4): p. 338-351.

23. Markert, E.K., et al., *Molecular classification of prostate cancer using curated expression signatures.* Proc Natl Acad Sci U S A, 2011. **108**(52): p. 21276-81.
24. El-Khoueiry, A., et al., *A first-in-human phase I study of sEphB4-HSA in patients with advanced solid tumors with expansion at the maximum tolerated dose (MTD) or recommended phase II dose (RP2D).* European Journal of Cancer. **69**: p. S11.
25. McCall, J.L., et al., *KSR1 and EPHB4 Regulate Myc and PGC1beta To Promote Survival of Human Colon Tumors.* Mol Cell Biol, 2016. **36**(17): p. 2246-61.
26. Ferguson, B.D., et al., *Novel EPHB4 Receptor Tyrosine Kinase Mutations and Kinomic Pathway Analysis in Lung Cancer.* Sci Rep, 2015. **5**: p. 10641.
27. Li, X., et al., *The differential expression of EphB2 and EphB4 receptor kinases in normal bladder and in transitional cell carcinoma of the bladder.* PLoS One, 2014. **9**(8): p. e105326.
28. Mulholland, D.J., et al., *Cell autonomous role of PTEN in regulating castration-resistant prostate cancer growth.* Cancer Cell, 2011. **19**(6): p. 792-804.
29. Noren, N.K., et al., *Interplay between EphB4 on tumor cells and vascular ephrin-B2 regulates tumor growth.* Proc Natl Acad Sci U S A, 2004. **101**(15): p. 5583-8.
30. Nguyen, T.M., et al., *EphB and Ephrin-B interactions mediate human mesenchymal stem cell suppression of activated T-cells.* Stem Cells Dev, 2013. **22**(20): p. 2751-64.
31. Bettegowda, C., et al., *Detection of circulating tumor DNA in early- and late-stage human malignancies.* Sci Transl Med, 2014. **6**(224): p. 224ra24.

# 12 APPENDICES

## 12.1 Appendix I: ECOG Performance Status

| Grade | Description |
|---|---|
| 0 | Normal activity. Fully active, able to carry on all pre-disease performance without restriction. |
| 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). |
| 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. |
| 3 | In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours. |
| 4 | 100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair. |
| 5 | Dead. |

* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

### 12.2 Appendix II: Common Terminology Criteria for Adverse Events V5.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm)

### 12.3 Appendix III: Highly Effective Methods of Contraception

| Barrier Methods | Intrauterine Device Methods | Hormonal Methods |
|---|---|---|
| • Male condom plus spermicide<br>• Cap plus spermicide<br>• Diaphragm plus spermicide | • Copper T<br>• Progesterone T<br>• Levonorgestrel-relasing | • Implants<br>• Hormone shot or injection<br>• Combined pill<br>• Minipill<br>• Patch |

NOTE: choice of contraception should be discussed with primary treating oncologist to discuss the risks and benefits of different modalities of contraception. Abstinence is not an acceptable method

### 12.4 Appendix IV: Response Evaluation Criteria in Solid Tumors (RECIST) 1.1

Criteria for Evaluating Response in Solid Tumors

RECIST version 1.1* will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

* As published in the European Journal of Cancer:

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

## 12.5 Appendix V: Summary of Changes

| ***Amendment 1 – October 22, 2018*** SRC Approved - 10/26/18 | | | |
|---|---|---|---|
| ***Section(s) affected*** | ***Prior version*** | ***Amendment 1 changes*** | ***Rationale*** |
| Cover page; Section 7.3.3.3 (Reporting to Ephos Biosciences Inc); Section 8.1.9 (Availability & Supply); Section 11.2 (Amendments) | Drug sponsor "Vasgene Therapeudics Inc" | Drug Sponsor "Ephos Biosciences Inc" | Administrative. Drug company changed names. |
| ***Amendment 2 – March 4, 2019*** SRC Approved - 3/5/19 | | | |
| ***Section(s) affected*** | ***Prior version*** | ***Amendment 2 changes*** | ***Rationale*** |
| Study Summary; Section 2.3.1 (Exploratory Objectives); Section 6.4.1 (Exploratory Endpoints) | Exploratory endpoint "molecular changes associated with EphB4 and ephrinB2 expression in **metastatic** tumor specimens" | Exploratory endpoint: "molecular changes associated with EphB4 and ephrinB2 expression in tumor specimens **(primary and/or metastatic tissue)**" | Updated the language to include use of primary and/or metastatic tumor tissue for analysis. |
| Section 1.7 (Rationale for exploratory studies) | "sEphB4-**HAS"** | "sEphB4-**HSA**" | Corrected error |
| Section 1.7.2 (Rationale for exploratory studies); Section 9.1.2 (EphB4 activation and other associated genes as a biomarker for response) | Use of the TEMPUS xO panel for analysis | Use of the TEMPUS xF panel for analysis | After discussion with TEMPUS and study PIs, xF panel is more appropriate for analysis. |
| Section 9 (Correlatives/Special Studies) | IHC analysis performed at **Northwestern PCF** and Abdulkadir Lab | IHC analysis performed at Abdulkadir Lab | Updated to indicate that all IHC analysis will be performed in Abdulkadir Lab |
| ***Amendment 3 – July 24, 2019*** SRC Approved - | | | |
| ***Section(s) affected*** | ***Prior version*** | ***Amendment 3 changes*** | ***Rationale*** |
| Cover Page | n/a | David VanderWeele listed as Co-investigator | Administrative update |
| | n/a | IND number has been added | Administrative update |
| Cover page; Section 8.1.9 (Availability and Supply); Section 11.2 (Amendments) | Funding source "Ephos Biosciences Inc" | Funding Source "Vasgene Therapeutics Inc" | Funding sponsor has changed their name and cover page was updated to match the study contract |
| List of Abbreviations; Study Schema; Study Summary; Section 1.7.2 (Rationale for Exploratory Endpoints); Section 4.1 (Treatment Overview); Section 6.4.2 (Exploratory Endpoints); Section 9.1.3 (Description of Correlative studies); Section 9.2 (Sample Collection) | "circulating tumor DNA (ctDNA)" | "cell free DNA (cfDNA)" | Cell free DNA is a more accurate term than circulating tumor DNA since not all the circulating DNA comes from tumor cells. Some is from normal cells. |

| | | | |
|---|---|---|---|
| Study Summary; Section 1.5 (Summary of clinical trial findings) | Frequency of toxicities from the phase 1 study | Updated frequency of toxicities from the phase 1 study | New IB has been released with updated information on toxicities. |
| Section 1.7.2 (Rationale for Exploratory Endpoints); Section 9 (Correlatives/Special Studies); Section 9.1.2 (EphB4 activation and other associated genes as a biomarker for response); Section 9.1.3 (The landscape of genomic alterations in the course of sEphB4 treatment); 9.4.2 (Next Generation Sequencing) | Reference to exploratory studies using Tempus NGS | Removed mention of exploratory studies using Tempus NGS. Samples will be stored until final analysis center is determined. | Contract with Tempus is pending and this will be updated in a later amendment. |
| Section 3.1.4 (Inclusion Criteria) | "Patients must have received at least one first-line therapy for mCRPC and have progressive disease (as defined below). No more than 3 prior treatment therapies (life prolonging) are permitted." | "Patients must have received **and progressed on** at least one **second generation AR targeted therapy for castration resistant disease irrespective of prior chemotherapy**. No more than 3 prior treatment therapies **for castration resistant disease** (life prolonging) are permitted." | **FDA Requested Change**<br><br>Clarification of inclusion criteria |
| Section 4.1 (Overview) | Blood samples for cytokine collected at every cycle. | Blood samples for cytokine collected at baseline, day 1 of cycle 5 and disease progression or end of treatment. | Blood collection was incorrected. |
| Section 4.3.3 (Management of Infusion Reaction) | n/a | Added instruction for how to manage infusion reactions | **FDA Requested Change**<br><br>Additional instruction for how to manage infusion reactions has been included. |
| Section 7.3.3.3 (Reporting to VasGene Therapeutics) | SAE reporting to Ephos Biosciences | SAE reporting to VasGene Therapeutics | Contact information for SAE reporting has been updated |
| Section 8.1.6 (Preparation) | n/a | Bullet point moved under section 8.1.6 | Corrected formatting error |
| Section 9 (Correlatives/Special Studies) | Blood collection on cycle **3** day 1 | Blood collection on cycle **5** day 1 | Corrected error in timing of collection |
| | Blood collected in "2 pink top EDTA tubes" | Blood collected in "2 10ml EDTA tubes" | Corrected the type of blood tubes used for collection |
| | 8 slides needed for IHC analysis | Up to 12 slides for IHC analysis | Allowed for the possibility of additional slides to be used for IHC. |
| | Destination of all samples was either to Abdulkadir Lab or Tempus | All samples will be sent to NU PCF-CTU | Updated destination of all samples. |

| | | | |
|---|---|---|---|
| | Sarki Abdulkadir listed as contact | NU PCF-CTU Listed as contact | Administrative update. PCF-CTU will handle all samples. |
| | Instructions for shipping of samples | All samples will be shipped to NU PCF-CTU | Administrative update. PCF-CTU will handle all samples. |
| | Instructions for storage needs | Included instructions for long term storage for blood samples | Updated instructions for sample storage. |
| Section 9 (Correlatives/Special Studies); Section 9.2 (Sample Collection Guidelines) | N/a | Analysis of tissue samples will be prioritized first for sequencing and then for IHC. | Priority for tissue samples has been included due to the likelihood of low tissue volumes. |
| Section 9.5 (Specimen Banking) | Research samples will be stored at the Abdulkadir Lab | Research samples will be stored at the Abdulkadir Lab **and Northwestern University Pathology Core Facility** | Tissue samples for IHC and blood serum will be stored in the Abdulkadir Lab and the tissue and blood for sequencing will be stored at NU PCF-CTU. |
| ***Amendment 4 – January 29, 2020*** | | | |
| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 4 Changes*** | ***Rationale*** |
| Cover Page and Throughout | Protocol version dated July 24, 2019 | Updates protocol version date to January 29, 2020 (Amendment 4) | Administrative update |
| Cover Page and Section 9 (Correlative/Special Studies Table) | N/A | Adds hyperlinks to email addresses | Administrative update |
| Table of Contents | N/A | Updates page numbers | Administrative update |
| Study Summary and Section 10 (Statistical Considerations) | Referenced Section 4.9 for continuous monitoring plan using Pocock-type boundary | Removes reference to Section 4.9 | Correction of typographical error.<br><br>The reference to Section 4.9 and the Pocock-type boundary continuous monitoring plan was included in error and has now been removed. |
| Study Summary | Exploratory objective: IHC staining for expression of EphB4 and ephrinB2 **in metastatic tumor samples** and correlate the level of alteration with the MYC, PTEN/PI3K, AR, and p53 pathways. | Exploratory objective: IHC staining for expression of EphB4 and ephrinB2 **in tumor samples** and correlate the level of alteration with the MYC, PTEN/PI3K, AR, and p53 pathways. | Corrects error. Either primary or metastatic archival tissue may be collected for this objective. |
| Study Schema; 1.7 (Rationale for Exploratory Studies); | Patients are to undergo optional biopsy and/or provide archival tissue for | No longer permits fresh biopsy for research. | Updates tissue collection procedures and |

| | | | |
|---|---|---|---|
| Exploratory Objective 2.3.3;<br>5.0 (Study Procedures);<br>6.4 (Exploratory Endpoints);<br>9 (Correlatives/Special Studies) | next generation sequencing (NGS) and immunohistochemistry (IHC). | Instead of using tissue from fresh biopsy, IHC data are to be obtained from primary and metastatic archival tissue, if available.<br><br>NGS of tumor tissue will no longer be performed for research; instead, if patients have undergone molecular testing as a standard of care procedure, the study will analyze genomic alterations that are described in the results of these reports (i.e., results from standard of care NGS testing/ genetic testing/ genomic testing, etc.) | analysis for feasibility and due to budgetary constraints |
| Section 3.1 (Inclusion Criteria);<br>Section 5 (Study Procedures) | Eligibility criteria allowed a 28 day window prior to registration for select screening labs; this was not referenced in the Study Procedures Table. | Updates the Study Procedures table to clarify that screening/baseline assessments are to be conducted within 1 month prior to registration, unless otherwise indicated; safety labs are to be conducted within 2 weeks prior to registration; updates eligibility criteria to align with this by specifying a 2 week window prior to registration for screening safety labs. | Clarification requested by the QAM and PI. |
| Section 3.2 (Exclusion Criteria) | N/A | Adds Exclusion Criterion 3.2.14: Patients with a history of significant thromboembolic events, (including recurrent DVT, CVAs, or recurrent pulmonary embolism) are not eligible. | Exclusion 3.2.14 has been added as a cautionary measure for safety. sEphB4-HSA is an angiogenesis inhibitor. Other angiogenesis inhibitors can cause thromboembolitic events. |
| Section 3.2 (Exclusion Criteria) | N/A | Adds Exclusion Criterion 3.2.15: Patients with a history of any of the following are not eligible:<br>· History of noncompliance with other medical therapy for medical conditions that could be impacted by study drug-related adverse events.<br>· An illness, condition, or other situation that the | Exclusion 3.2.15 has been added to ensure compliance for patient safety and study endpoints. |

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| | | treating investigator feels would limit compliance with study requirements or would comprise the subject's safety or study endpoints. | |
| Section 5 (Study Procedures);<br>Section 9 (Correlative Samples Table) | Permission to obtain archival tissue samples of primary lesion and metastatic lesion sites will be obtained at baseline, and samples will be collected at baseline. | Permission to obtain archival tissue samples of primary lesion and metastatic lesion sites will be obtained at baseline. The samples themselves will be collected only if available and only when requested by the Principal Investigator or Principal Investigator's designee; they may be collected at any time during the study, per PI request. Refer to the lab manual for details.<br><br>Also adds an X to the Study Procedures table to indicate this collection. | Clarifies the timing of collection of archival tissue |
| Section 5 (Study Procedures) | Footnote 15 was included in the first study procedures table but was missing from the second study procedures table | Adds footnote 15 to the second study procedures table to align with the first study procedures table. | Corrects error. Adds missing footnote. |
| Section 5 (Study Procedures) | N/A | Adds footnote 16:<br>[16] If patient has had molecular testing (i.e., NGS testing, genetic testing, genomic testing, etc.) performed as standard of care prior to study entry or at any time during study participation, collect a copy of the results report for correlative analyses. Refer to Section 9 for details. | Updates for feasibility and due to budgetary constraints. NGS of tumor tissue will no longer be performed in for research; instead, if patients have undergone molecular testing as a standard of care procedure, the study will analyze genomic alterations that are described in the results of these reports (i.e., results from standard of care NGS testing/ genetic testing/ genomic testing, etc.) |
| Section 7.2.4 (UPIRSOs) | Previously stated:<br>A UPIRSO is a type of SAE that includes events that meet ALL of the following criteria:<br>• Is unanticipated in terms of nature, severity, or frequency<br>• Places the research | Now states:<br>A UPIRSO is a type of SAE that includes events that meet ALL of the following criteria:<br>•is unexpected (in terms of nature, severity, or frequency) given the procedures described in | Updates the definition of UPIRSO. This is an administrative change to align with Northwestern University's new protocol template language. |

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| | subject or others at a different or greater risk of harm<br>• Is deemed to be at least possibly related to participation in the study. | the research protocol documents (e.g., the IRB-approved research protocol and informed consent document) and the characteristics of the human subject population being studied<br>•is related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and<br>•suggests that the research places human subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized, even if no harm has actually occurred. | |
| Section 7.3.3.1 (Expedited Reporting of SAEs/Other Events) | N/A | Now requires completed SAE forms to include country of incidence | Updates the requirements for SAE forms. This is an administrative change to align with Northwestern University's new protocol template language. |
| Section 7.3.3.1 (Expedited Reporting of SAEs/Other Events) | All SAEs will be reported to, and reviewed by, the DSMC at their next meeting. | All SAEs will be reported to, and reviewed by, the DSMC per the DSMP. | Updates language regarding reviewing SAEs by the DSMC. This is an administrative change to align with Northwestern University's new protocol template language. |
| Section 7.3.3.2 (Reporting to the Northwestern IRB) | •Any death of an NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.<br>•Any death of an NU subject that is actively on study treatment (regardless of whether or not the event is possibly | •Any death of an NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.<br>•Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be | Updates language regarding the reporting of SAEs to the NU IRB. This is an administrative change to align with Northwestern University's new protocol template language. |

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| | related to study treatment)<br>•Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be reported to the NU IRB within 5 working days of notification. | reported to Northwestern University and to the NU IRB within 5 working days of notification.<br>•Information pertaining to an NU subject that fits into any of the categories listed on the Reportable New Information page will be reported to the NU IRB within 5 business days of knowledge or notification | |
| Section 7.3.3.4 (Reporting to the FDA) | N/A | Adds Section 7.3.3.4 (Reporting to the FDA)<br><br>The FDA will be notified within 7 calendar days of any SAE that is associated with study treatment, is unexpected, and is fatal or life-threatening.<br><br>The FDA will be notified within 15 calendar days of any SAE that is associated with the study treatment, unexpected, and serious but not fatal or life-threatening. This includes any previous SAEs that were not initially deemed reportable, but are later determined to meet the criteria for reporting (i.e. by the DSMC).<br><br>All other SAEs will be reported on an annual basis as part of the annual FDA report. | Adds standard protocol template languge for reporting to the FDA. |
| Section 9.1 (Description of Correlative Studies) | cfDNA will be isolated from the blood samples and stored for future analysis. | cfDNA will be isolated from the blood samples and stored for future analysis and analyzed by NGS testing. | Updates language to specify that cfDNA samples will be used for NGS. |
| Section 9 (Correlative Samples Table) | cfDNA blood samples for sequencing listed an analysis center of TBD | Updates analysis center to Northwestern University | PI decision to perform sample analysis at Northwestern University |
| Section 9 (Correlative Samples Table) | Blood samples for cytokine analysis listed an analysis center of Mayo | Updates analysis center to the Abdulkadir Lab | Corrects error in analysis center. Mayo was listed incorrectly as a typographical error. |
| Section 9.4.2 (Next Generation Sequencing) | We will employ TEMPUS xO gene panel for primary tumor samplesequencing. | cfDNA samples will be analyzed by NGS assays at Northwestern University. | Updates to specify that NGS of cfDNA samples will be performed at Northwestern |

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| Section 11.4 (Data Submission) | Once a subject is confirmed and registered to the study, eCRFs should be submitted according to the detailed data submission guidelines (provided in a separate document). For all patients, data are due at the end of every cycle. | Once a patient is confirmed and registered to the study, eCRFs should be submitted according to the study procedures table. Generally, for all phase II patients, data are due within 10 days of a visit. A set amount of data may also be requested for any screen failures, as is defined by the study. In most instances, this will include collection of adverse events and baseline data from the time of registration to the date of screen failure. | Updates language regarding data submission. This is an administrative change to align with Northwestern University's new protocol template language. |
| Section 11.6.2 (Other Protocol Deviations) | A protocol deviation is any unplanned variance from an IRB approved protocol that:<br>•Is generally noted or recognized after it occurs.<br>•Has no substantive effect on the risks to research participants.<br>•Has no substantive effect on the scientific integrity of the research plan or the value of the data collected.<br>•Did not result from willful or knowing misconduct on the part of the investigator(s). | A protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that is under the investigator's control and that has not been approved by the Institutional Review Board (IRB). Protocol deviations must be reported according to the policies and procedures of the IRB of record. | Updates language regarding protocol deviations. This is an administrative change to align with Northwestern University's new protocol template language. |
| Section 11.8 (Publication Policy) | All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in accordance with the policies and processes set forth in the Lurie Cancer Center DSMP. For trials that require high intensity monitoring, the assigned QAM will prepare a preliminary data summary (to be approved by the DSMC) no later than 3 months after the study reaches its primary completion date (the date that the final subject is examined or receives an intervention for the purposes of final data | All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in accordance with the DSMC Data Release Policies and Processes. The assigned QAM will prepare a preliminary data set for DSMC approval no later than 3 months after the study reaches its primary completion date, as defined by ClinicalTrials.gov. This is the date that the final patient was examined or received an intervention for the purposes of final collection of data for the primary outcome, whether | Updates language regarding the publication policy. This is an administrative change to align with Northwestern University's new protocol template language. |

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| | collection for the primary endpoint). If the investigator's wish to obtain DSMC-approved data prior to this point (or prior to the point dictated by study design), the PI must send a written request for data to the QAM which includes justification. If the request is approved, data will be provided no later than 4 weeks after this request approval. The data will be presented to the DSMC at their next available meeting, and a final, DSMC-approved dataset will be released along with any DSMC decisions regarding publication. The investigators are expected to use only DSMC-approved data in future publications. The investigators should submit a copy of the manuscript to the biostatistician to confirm that the DSMC-approved data are used appropriately. Once the biostatistician gives final approval, the manuscript may be submitted to external publishers. | the clinical trial concluded according to the pre-specified protocol or was terminated. If the investigator would like data release to be approved by the DSMC prior to when study design specifies, and/or prior to three months after a study's primary completion date, the PI must send a written request for data approval to the QAM which includes justification. Requests must be made a minimum of six to eight weeks in advance of the expected deadline. The request will be presented to the DSMC at their next available meeting. Any DSMC decisions regarding data release will be provided to the PI. If the request is approved, the QAM will present the data set to the DSMC for approval. A final, DSMC-approved dataset, as applicable, will then be released 6-8 weeks after the request was made. The investigators are expected to use only DSMC-approved data and statistical analyses any time they are disseminating trial data. The investigators must send a copy of the draft abstract/poster/manuscript to the study's biostatistician and assigned QAM to confirm that the DSMC-approved data and statistical analyses are used appropriately. Once the biostatistician and QAM gives final approval, the publication may be submitted to external publisher. | |
| Section 12.4 (Appendix IV: Response Evaluation Criteria in Solid Tumors (RECIST) 1.1) | In addition, volumetric analysis will be explored by central review for response assessment. | Deletes strikethough text: ~~In addition, volumetric analysis will be explored by central review for response assessment.~~ | Correction of typographical error.<br><br>Reference to central review was included in error and has now been removed. |

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| Throughout | N/A | Minor corrections to typographical errors, style, and formatting | Administrative update |

| ***Amendment 5 – August 19, 2020*** | | | |
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| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 5 Changes*** | ***Rationale*** |
| Cover Page and Throughout | Protocol version dated January 29, 2020 (Amendment 5) | Updates protocol version and date to August 19, 2020 (Amendment 5) | Administrative update |
| Table of Contents | N/A | Updates page numbers | Administrative update |
| Table 4-2-1 (Premedications Table) | Premedications were not required. | Adds Table 4-2-1 to require the below premedication schedule:<br>Administer the two below pre-medications at least 30 minutes before the infusion:<br>Diphenhydramine, 25 mg<br>Acetaminophen, 650 mg,<br>** Refer to Section 4.4.3 for (1) changes to the pre-medication regimen for patients who experience infusion reactions and (2) management of infusion reactions.* | Safety update. Adds a required premedication regimen as a safety precaution in response to an enrolled subject having experienced an infusion reaction. |
| Table 4-2-2 (Treatment Administration Summary Table) | N/A | Names the Table "Table 4-2-2"<br>Adds the following text: Additional medications for prophylaxis and supportive care can be administered as clinically indicated per Institutional standards.<br>Adds the following footnote: * Refer to Section 4.4.3 for the management of infusion reactions. | Administrative updates.<br>Adds a name to the table.<br>Reiterates what is stated in Section 4.2.<br>Provides a reference to Section 4.4.3 for ease of use of protocol end-users. |
| Section 4.2 (Treatment Plan) | There are no specifically required or recommended supportive care medications. However, | Premedications include diphenhydramine and acetaminophen as described above in Table 4-2-1 . Additional | Safety update. Adds a required premedication regimen as a safety precaution in response to an enrolled subject |

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| | medications for supportive care can be administered as clinically indicated per institutional protocol. | medications for prophylaxis and supportive care can be administered as clinically indicated per institutional protocol. | having experienced an infusion reaction. |
| Section 4.3.3 (Management of Infusion Reaction) | | | Safety updates. |
| | Prophylactic premedication may be given any time after the first dose of Cycle 1. However, if any subject experiences a Grade 3 infusion reaction, all subsequently enrolled subjects will be required to receive prophylactic premedication before all infusions of sEphB4-HAS... | Prophylactic premedications will be administered as described in Table 4-2-1… | Adds a required premedication regimen as a safety precaution in response to an enrolled subject having experienced an infusion reaction. |
| | For Grade 2 symptoms: (Rash, flushing, urticarial, dyspnea, drug fever > 38C) Stop the sEphB4-HSA infusion, begin an i.v. infusion of normal saline, and treat the subject with diphenhydramine 50 mg i.v. (or equivalent) and/or acetaminophen; remain at bedside and monitor subject until resolution of symptoms. Corticosteroid therapy may also be administered as appropriate… | For Grade 2 symptoms: (Rash, flushing, urticarial, dyspnea, drug fever > 38C) Stop the sEphB4-HSA infusion, begin an i.v. infusion of normal saline, and treat the subject with 25 mg additional diphenhydramine (for total dose of 50 mg, considering prior premedication with 25 mg) i.v. (or equivalent) and/or acetaminophen; remain at bedside and monitor subject until resolution of symptoms. Corticosteroid therapy (e.g., hydrocortisone, PRN, or other Institutional standard) may also be administered as appropriate. Additional supportive care may be administered per Institutional standard practice… | Provides additional guidance for the management of infusion reactions. |
| | …The following prophylactic premedications are recommended for future infusions: | … The following prophylactic premedications are required for future infusions: | Adds a required premedication regimen as a safety precaution in response to an enrolled subject having |

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| | diphenhydramine 50 mg (or equivalent), acetaminophen, and/or corticosteroids should be administered at least 30 minutes before additional sEphB4-HSA administration… | diphenhydramine 50 mg (or equivalent) and acetaminophen 650 mg (or equivalent). Corticosteroids (e.g., hydrocortisone or other Institutional standard) are also recommended, if clinically indicated. These premedications should be administered at least 30 minutes before additional sEphB4-HSA administration. | experienced an infusion reaction. |
| Section 4.4. (Concomitant Medications / Treatments) | There are no known concomitant medications or treatments associated or recommended with sEphB4-HSA at this time. | Required premedications are described in Table 4-2-1. There are no other known concomitant medications or treatments associated or recommended with sEphB4-HSA at this time. | Safety update. Adds a required premedication regimen as a safety precaution in response to an enrolled subject having experienced an infusion reaction. |
| Section 5.0 (Study Procedures Table) | Footnote 11:<br>Patients will receive sEphB4-HSA 1000mg IV (60min infusion) on day 1 of each cycle along with continuation of androgen deprivation therapy. | Footnote 11:<br>Patients will receive sEphB4-HSA 1000mg IV (60min infusion) on day 1 of each cycle along with continuation of androgen deprivation therapy. Refer to Table 4-2-1 for required premedication regimens. | Safety update. Adds a required premedication regimen as a safety precaution in response to an enrolled subject having experienced an infusion reaction. |
| Section 8.1.5 (Protocol Dose Specifics) | sEphB4-HSA will be given as 1000mg IV infusion… | sEphB4-HSA will be given as 1000mg IV infusion…Refer to Table 4-2-1 for required premedication regimens. | Safety update. Adds a required premedication regimen as a safety precaution in response to an enrolled subject having experienced an infusion reaction. |
| Appendix V (Summary of Changes) | N/A | Describes revisions that are made in protocol Amendment 5 | Administrative update |
| Throughout | N/A | Minor adjustments to formatting and style throughout to correct errors and accommodate revisions. | Administrative update |

| ***Amendment 6 – September 18, 2020*** | | | |
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| ***Section(s) Affected*** | ***Prior Version*** | ***Amendment 6 Changes*** | ***Rationale*** |
| Cover Page and Throughout | Protocol version dated August 19, 2020 (Amendment 5) | Updates protocol version and date to September 18, 2020 (Amendment 6) | Administrative update |
| Study Schema | Response assessment: Day 1 of odd cycles (PSA) and every 8 weeks (imaging) | Response assessment:<br>• PSA: Day 1 of odd cycles<br>• Imaging: After 2 months of therapy and every 3 months thereafter (after 1 year, patients with stable disease or better may have imaging every 4 months) | Clarifies imaging schedule. |
| Study Summary; Treatment Plan<br><br>Section 4.1; Treatment Plan Overview | …imaging will occur every 8 weeks. Imaging for response assessment can be increased to every 12 weeks if disease is stable for ≥6 months and can be increased to 16 weeks if disease is stable for ≥12 months. | Deletion of strikethrough text and addition of bolded text.<br>~~…imaging will occur every 8 weeks. Imaging for response assessment can be increased to every 12 weeks if disease is stable for ≥6 months and can be increased to 16 weeks if disease is stable for ≥12 months.~~ **Imaging will occur at baseline and 2 months after starting therapy; thereafter, imaging will be conducted every 3 months. After 1 year, imaging can be done every 4 months for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated.** | Clarifies imaging schedule. |
| Section 4.1; Treatment Plan Overview | CT scan and/or MRI, bone scan will occur every 8 weeks. Imaging for response assessment can be increased to every 12 weeks if disease is stable for ≥6 months. If disease is stable for ≥12 months, imaging will occur every 16 weeks for the remainder of the study. | Deletion of strikethrough text and addition of bolded text.<br>~~CT scan and/or MRI, bone scan will occur every 8 weeks. Imaging for response assessment can be increased to every 12 weeks if disease is stable for ≥6 months. If disease is stable for ≥12 months, imaging will occur every 16 weeks for the remainder of the study.~~ **Imaging will occur at baseline and 2 months after starting therapy;** | Clarifies imaging schedule. |

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| | | **thereafter, imaging will be conducted every 3 months. After 1 year, imaging can be done every 4 months for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated.** | |
| Section 5.0 (Study Procedures Table) | Includes reference to footnote * for tumor imaging: * Can be competed ±3 days of drug administration<br><br>The Study Procedures table included multiple columns and "X's" to indicate the timing of imaging assessments. | Deletes reference to footnote * for tumor imaging.<br><br>Formatting adjustment to the Study Procedures Table. Merges imaging assessments into a single column and single "X" for clarity. | Clarifies imaging schedule and corrects discrepancy. |
| Section 5.0 (Study Procedures Table) | Footnote 2: Imaging is to be repeated after 4 cycles (8 weeks). See table below for subsequent imaging. | Deletion of strikethrough text and addition of bolded text.<br><br>Footnote 2: ~~Imaging is to be repeated after 4 cycles (8 weeks). See table below for subsequent imaging.~~ **Imaging will occur at baseline and 2 months (± 7 days) after starting therapy; thereafter, imaging will be conducted every 3 months (± 7 days). After 1 year, imaging can be done every 4 months (± 7 days) for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated.** | Clarifies imaging schedule. |
| Section 5.0 (Study Procedures Table) | Footnote 12: Imaging for response assessment can be increased to every 12 weeks if disease is stable for ≥ 6 months. If disease is stable for ≥12 months, imaging can be increased to every 16 weeks. | Deletion of strikethrough text and addition of bolded text.<br><br>Footnote 12: ~~Imaging for response assessment can be increased to every 12 weeks if disease is stable for ≥ 6 months. If disease is stable for ≥12 months, imaging can be increased to every 16 weeks.~~ **Imaging will occur at baseline and 2 months (± 7 days) after** | Clarifies imaging schedule. |

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| | | **starting therapy; thereafter, imaging will be conducted every 3 months (± 7 days). After 1 year, imaging can be done every 4 months (± 7 days) for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated.** | |
| Section 6.1.1 (Evaluable Patients) | Evaluable for primary endpoint of PSA response. All patients who received at least 1 dose of the study drug will be evaluable for PSA response. | Addition of bolded text:<br>Evaluable for primary endpoint of PSA response. All patients who received at least 1 dose of the study drug **and who also had a followup PSA test conducted prior to initiating subsequent therapy** will be evaluable for PSA response. | The definition of evaluability for the primary endpoint has been revised, as requested by the Data and Safety Monitoring Committee (DSMC). |
| Section 6.3 (Assessment of Secondary Endpoints) | Disease evaluation will be assessed every 8 weeks. | Deletion of strikethrough text and addition of bolded text:<br>Disease evaluation will be assessed ~~every 8 weeks~~ **by imaging at baseline and 2 months after starting therapy; thereafter, imaging will be conducted every 3 months. After 1 year, imaging can be done every 4 months for patients with stable disease or better. Scans may also be conducted at any time, if clinically indicated.** | Clarifies imaging schedule. |
| Section 6.3 (Assessment of Secondary Endpoints) | Radiologic assessment will be every 8 weeks. Imaging for response assessment can be increased to every 12 weeks if disease is stable for ≥6 months. | Deletion of strikethrough text and addition of bolded text:<br>~~Radiologic assessment will be every 8 weeks. Imaging for response assessment can be increased to every 12 weeks if disease is stable for ≥6 months.~~ **Imaging will occur at baseline and 2 months after starting therapy; thereafter, imaging will be conducted every 3 months. After 1 year, imaging can be done every 4 months for patients with stable disease or better. Scans may also be conducted at any time, if clinically** | Clarifies imaging schedule. |

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| | | **indicated.** | |
| Appendix V (Summary of Changes) | N/A | Describes revisions that are made in protocol Amendment 6 | Administrative update |
| Throughout | N/A | Minor adjustments to formatting, style, and grammar throughout to correct typographical errors and accommodate revisions. | Administrative update |