USC Norris Comprehensive Cancer Center
Part of the Keck School of Medicine of USC

**Local Protocol #:** 9L-16-6

**Version:** May 01, 2018

**Protocol Title:** A pilot/safety study of sEphB4-HSA in combination with a hypomethylating agent (HMA) for patients with relapsed or refractory myelodysplastic syndrome (MDS) and AML previously treated with a hypomethylating agent (IND 133675).

**ClinicalTrialsgov #:** NCT03146871

**PROTOCOL**

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**MODALITY:** Chemotherapy

**TYPE:** Pilot

**PARTICIPATING SITES:** USC/Norris Comprehensive Cancer Center

LAC+USC Medical Center

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|---|---|
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| **DRUG SPONSOR:** | VasGene Therapeutics, Inc. |
| **STUDY DRUG:** | sEphB4-HSA (IND #112629) |

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# LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

| | |
|---|---|
| AE | Adverse Event |
| ALT | Alanine Aminotransferase |
| ALC | Absolute Lymphocyte Count |
| AST | Aspartate Aminotransferase |
| ALT | Alanine transferase |
| AML | Acute myeloid leukemia |
| ANC | Absolute Neutrophil count |
| b-HCG | Beta-human chorionic gonadotropin |
| BUN | Blood Urea Nitrogen |
| CBC | Complete Blood Count |
| CISO | Clinical Investigation Support Office (at University of Southern California) |
| CMP | Comprehensive Metabolic Panel |
| CMML | Chronic Myelomonocytic Leukemia |
| CR | Complete Response |
| CRF | Case Report Form |
| CT | Computed Tomography |
| CTCAE | Common Terminology Criteria for Adverse Events |
| DLT | Dose Limiting Toxicity |
| DSMB | Data and Safety Monitoring Board |
| ECOG | Eastern Cooperative Oncology Group |
| FDA | Food & Drug Administration |
| G-CSF | Granulocyte Colony Stimulating Factor |
| H&P | History & Physical Exam |
| HRPP | Human Research Protections Program |
| ICF | Informed Consent Form |
| IRB | Institutional Review Board |
| IV (or iv) | Intravenously |
| IWG | International Working Group |
| LLN | Lower Limit of Normal |
| MDS | Myelodysplastic Syndrome |
| MTD | Maximum Tolerated Dose |
| NCI | National Cancer Institute |
| ORR | Overall Response Rate |

| | |
|---|---|
| OS | Overall Survival |
| PBMCs | Peripheral Blood Mononuclear Cells |
| PD | Progressive Disease |
| PFS | Progression Free Survival |
| PO | Per os/by mouth/orally |
| PI | Principal Investigator |
| PR | Partial Response |
| RBC | Red Blood Cell |
| RP2D | Recommended Phase 2 Dose |
| SAE | Serious Adverse Event |
| SD | Stable Disease |
| SGOT | Serum Glutamic Oxaloacetic Transaminase |
| SGPT | Serum Glutamic Pyruvic Transaminase |
| SU2C | Stand Up to Cancer |
| SUSAR | Serious, Unlisted/Unexpected and At least possibly Related to the drug |
| TSH | Thyroid Stimulating Hormone |
| ULN | Upper Limit of Normal |
| UC-RH | University of Copenhagen-Rigshospitalet |
| USC | University of Southern California, Norris Comprehensive Cancer Center |
| VARI-SU2C | Van Andel Research Institute-Stand Up to Cancer Epigenetics Dream Team |
| WBC | White Blood Cells |
| WHO | World Health Organization |
| IIT | Investigator Initiated Trial |

| | |
|---|---|
| Title | A pilot/safety study of sEphB4-HSA in combination with a hypomethylating agent (HMA) for patients with relapsed or refractory myelodysplastic syndrome (MDS) and AML previously treated with a hypomethylating agent |
| Short Title | Pilot study of sEphB4-HSA and a hypomethylating agent (HMA) in relapsed/refractory MDS and AML |
| Protocol Number | 9L-16-6 |
| Phase | Pilot/Safety |
| Methodology | Clinical Trial |
| Study Duration | 3 years |
| Study Center(s) | United States Sites:<br>• University of Southern California (USC), Norris Comprehensive Cancer Center<br>  ○ Los Angeles, California |
| Objectives | To describe the toxicities and assess the tolerability of sEphB4-HSA in combination with a HMA among patients with relapsed/refractory MDS and AML.<br>To assess efficacy of sEphB4-HSA in combination with an HMA as manifest by IWG response criteria (Appendices 1 and 2), as well as time to development of acute myeloid leukemia (AML) in patients with MDS, time to progression in patients. |
| Number of Subjects | 12 |
| Diagnosis and Main Inclusion Criteria | Six patients with advanced MDS requiring treatment with HMA and either refractory to at least 4 cycles or progressing after previously documented response to HMA.<br><br>Six patients with AML who were previously treated with HMA who are unfit for intensive chemotherapy. |
| Study Product(s), Dose, Route, Regimen | sEphB4-HSA 15mg/kg IV q 2 weeks<br>plus either:<br>Decitabine 20mg/m2 IV/1hr D1-5 q28days<br>-or-<br>Azacitidine 75mg/m2 SC or IV D1-7 q28days |
| Duration of administration | Minimum of 2 cycles unless there is an SAE or progression of disease based on >50% increase in blast count in the blood or marrow; maximum 30 cycles with the combination for patients with ongoing clinical benefit. |
| Reference therapy | 5-azacytidine, decitabine monotherapy |
| Statistical Methodology | We will treat 12 patients in this pilot study and describe the results. |

## 1.0 Introduction

### 1.1 Disease Background

Myelodysplastic syndrome (MDS) is a clonal hematopoietic neoplasm that results in bone marrow failure and frequently leads to acute myeloid leukemia (AML). Patients with MDS can have variable cytopenias, and as many as 20% of the marrow cells may be leukemic blasts. Cytogenetic abnormalities are present in 50% of patients and more subtle molecular abnormalities are also found but do not necessarily distinguish MDS from other pre-leukemic myeloid malignancies.

#### 1.1.1 Current Management

Current, FDA-approved agents for MDS include the hypomethylating agents (HMAs), decitabine and 5-azacytidine. These drugs can improve peripheral blood cytopenias in as many as 50% of patients and improve median overall survival from 15 months to 24 months, but they lead to complete remission (CR) in only 10-20% of patients (Fenaux 2009). Furthermore, these agents are never curative and the average duration of remission is 1-2 years. Allogeneic stem cell transplant (allo-SCT) is the only curative intervention but is not an option for the majority of affected patients due to comorbid conditions, lack of a donor, and poor performance status.

There are no FDA-approved treatment options for patients who fail standard available HMA therapy for MDS, and the median survival in such patients is only 5.6 months. Investigators have attempted to optimize the efficacy of HMA therapy by pursuing combinations with other DNA-active agents such as histone deacetylase inhibitors (HDACs) or with immunomodulatory agents such as lenalidomide. Phase III clinical trials have failed to show convincing evidence thus far to support a change in the treatment paradigm (Sekeres 2014); therefore, patients with MDS who are refractory to treatment with HMAs or who relapse after initially responding and who are not candidates for alllo-SCT are generally offered either low-dose chemotherapy with cytarabine, supportive care, or hospice.

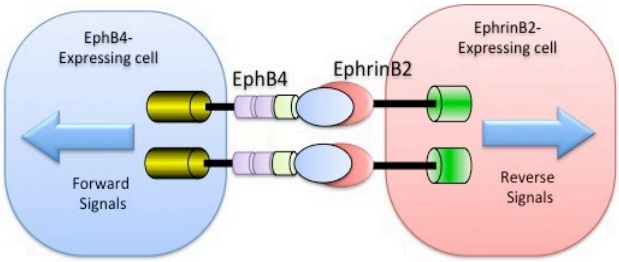
The mechanism of resistance or loss of sensitivity to HMAs is not known. One possible explanation that is being explored in clinical trials at several centers is that treatment with HMAs leads to immunologic changes in the host T cell repertoire that silences their endogenous anti-tumor responses. HMAs induce expression of tumor antigens such as cancer testis antigens (CTAs) and patients with both MDS and AML whose blasts express such antigen after HMA therapy demonstrate upregulation of target specific CD8+ T–cells (Goodyear 2010). More recently, upregulation of PD-L1, PD-L2, PD-1, and CTLA-4 has been demonstrated in marrow samples of patients receiving treatment with HMAs for MDS (Yang 2013). Our collaborators have recently shown that almost

half of patients receiving HMA therapy for MDS demonstrate demethylation of the PD-1 promotor which correlates with increased expression of PD-1 in T cells (Orskov 2015). Patients who failed to demethylate the PD-1 promotor (therefore with silenced PD-1 expression) had significantly better overall responses (60% vs. 8%, p = 0.014) to azacitidine than patients who demonstrated PD-1 demethylation. There are several novel immune checkpoint inhibitors that are being explored for their potential role in hematologic malignancies. The primary drawback of these agents is the development of autoimmune complications, most of which respond to steroid treatment. However, in patients with MDS autoimmune complications and steroid treatment are both potentially more harmful because of the frequent baseline cytopenias.

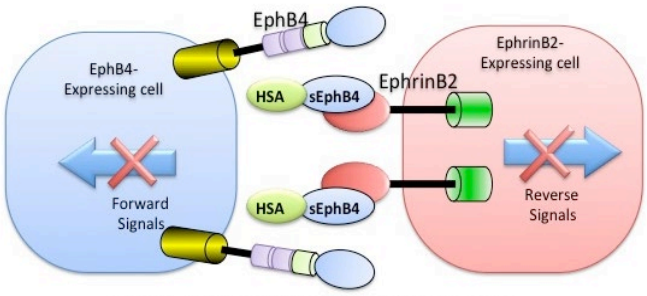
Our group has identified a novel receptor-ligand interaction, comprised of receptor EphB4 and membrane localized ligand EphrinB2, which appears to be a target unique to many cancers. We have created a human fusion protein, sEphB4-HSA, to block this interaction, the effect of which appears to be both anti-proliferative as well as T-cell engagement. In phase I clinical trials, there were no myelosuppressive effects from the agent. Given its saftey in phase I, its pre-clinical efficacy against EphB4-expressing myeloid blasts, and the potential to engage T cells to overcome HMA resistant, we propose a pilot trial to evaluate its safety in combination with HMAs among patients with MDS and AML who have progressed on HMA treatments. If safety is confirmed, we plan a larger randomized trial in the setting of earlier stage disease to assess its role as an adjunct to HMA therapy in MDS and AML.

### 1.2 Study Agent Background: sEphB4-HSA

Our group has identified a novel receptor-ligand interaction, comprised of receptor EphB4 and membrane localized ligand EphrinB2, which appears to be a target unique to many cancer types. In normal tissue, EphB4 is expressed on venous endothelial cells and its ligand EphrinB2 is expressed on arterial endothelium and pericytes. Their interaction is essentially for maturation of new blood vessels and EphrinB2 is mediates VEGF signaling. In tumor cells, the EphB4-EphrinB2 interaction induces bidirectional signaling that results in both enhanced survival and resistance to apoptosis. Knock down



EphB4/ephrinB2 binding causes bi-directional signaling



sEphB4-HSA Blocks EphB4/ephrinB2 Signaling

of EphB4 results in tumor cell death and increased sensitivity to TRAIL-induced apoptosis. These findings led to the development of sEphB4-HSA, a fully human fusion protein composed of soluble EphB4 extracellular domain fused at the C-terminus with albumin. sEphB4-HSA is a 123.3 kDa protein that bind specifically to EphrinB2, functioning as a decoy protein that blocks bidirectional signaling. The protein has anti-tumor efficacy in numerous cell lines and has both direct anti-proliferative effects (e.g. reduction in PI3K signaling) as well as anti-angiogenesis effects.
In addition to its direct anti-proliferative effects in several tumor types, targeting of the EphrinB2-EphB4 interaction appears to increase T cell infiltration in tumors. Moreover, depletion of Tcells appears to abrogate the effect of sEphB4-HSA on tumor cell survival. We have also demonstrated that PD1 expression by T cells decreases with sEphB4-HSA implicating the role of immune checkpoint inhibition in the mechanism of action of this agent.

A high rate of tumor inhibition was noted in multiple human tumors using a xenograft model at a dose of 20mg/kg prompting the initiation of a phase I clinical trial in various solid tumors which has now been completed at the Norris Comprehensive Cancer Center. sEphB4-HSA has demonstrated minimal toxicity (Table 2), without demonstrable myelosuppression in 61 treated patients with solid tumors. We therefore propose to utilize the agent in combination with an HMA among previously-treated MDS patients to determine safety and feasibility of combining sEphB4-HSA with HMA.

### 1.3 Evidence of anti-leukemic activity of sEphB4-HSA

Previous published studies from our group and others, have established critical roles for both the receptor EphB4 and its ligand ephrinB2 in the growth of solid tumors. The role of EphB4/ephrinB2 signaling in AML has not been reported. We have profiled leukemia cell lines (Fig1. A) and blast cells (Fig1. B) from patients with AML and MDS/AML and that the majority of leukemia cells express EphB4 at levels greater than normal CD34+ progenitor cells, suggesting that EphB4 could be a viable therapeutic target in myeloid malignancies.(Fig1. C)

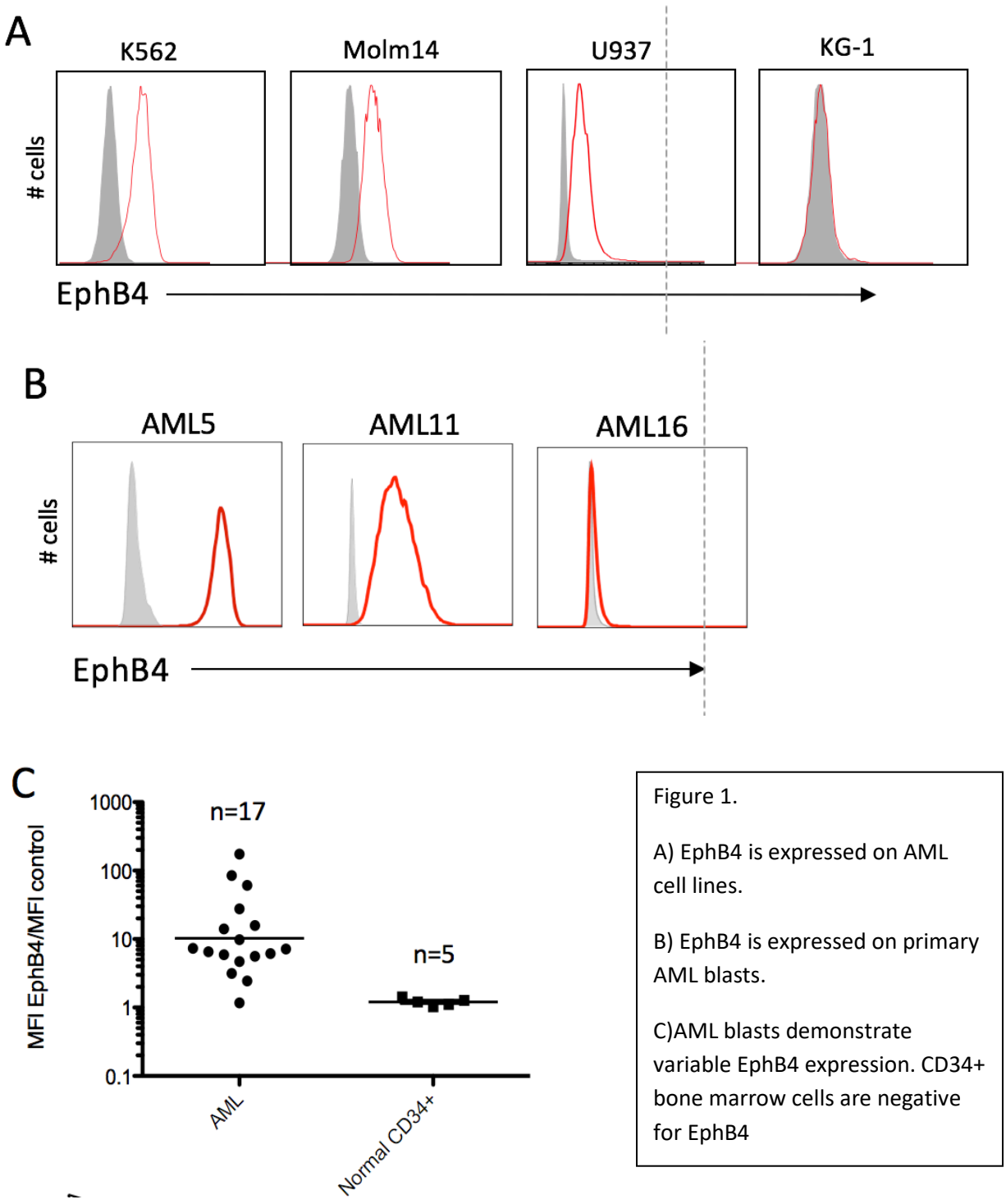



Figure 1.

A) EphB4 is expressed on AML cell lines.

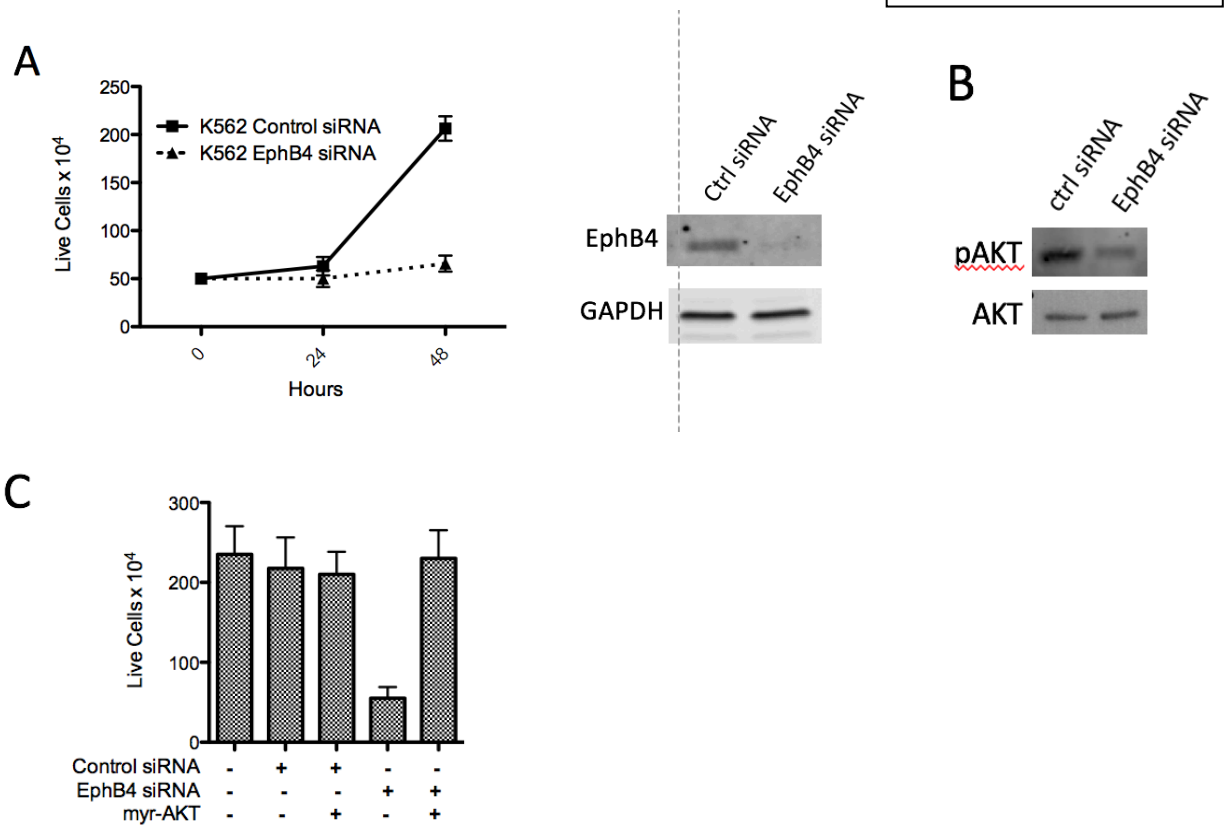
B) EphB4 is expressed on primary AML blasts.

C)AML blasts demonstrate variable EphB4 expression. CD34+ bone marrow cells are negative for EphB4

To understand the role of EphB4 in leukemia cells, we performed siRNA knock down experiments of EphB4 in the erythroleukemia cell line, K562. Knock down of EphB4 significantly impaired the growth of leukemia cells at 48 hours and was associated with

a decrease in pro-survival signaling protein pAKT.(Fig. 2A) Co-transfection of K562 with myr-AKT (a constitutively active form of AKT), in EphB4 knock-down cells, restored normal growth demonstrating that the lost of cell growth in EphB4 knock down cells was due to a loss of AKT activity. (Fig 2B)

Figure 2. A) siRNA knock down of EphB4 leads to reduced proliferation and survival of leukemia cells B) EphB4 knock down leads to decreased p-AKT C) Cell growth can be rescued after EphB4 knockdown by expression of constitutively active AKT (myr-AKT)



This further suggests that measuring pAKT levels can serve as a biomarker for EphB4 pathway inhibition in leukemia cells. To demonstrate the activity of sEphB4-HSA on leukemia cell growth, K562 cells were treated with sEphB4-HSA alone and in combination with cytarabine, a drug commonly used for the treatment of leukemia. While sEphB4-HSA alone demonstrated modest anti-leukemia activity in this *in vitro* experiment, it demonstrated highly synergistic leukemia cell killing when combined with cytarabine. The combinatorial index of the two drugs is plotted in Fig. 3, which levels les than 1 indicating synergy.

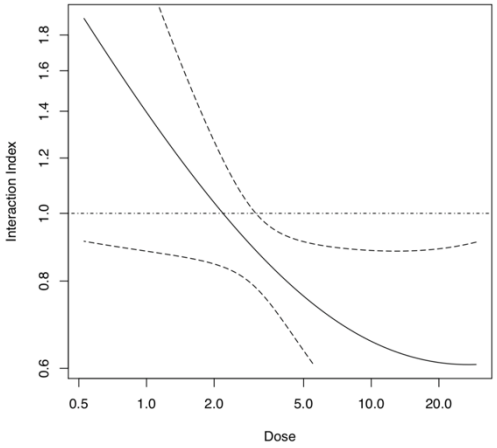
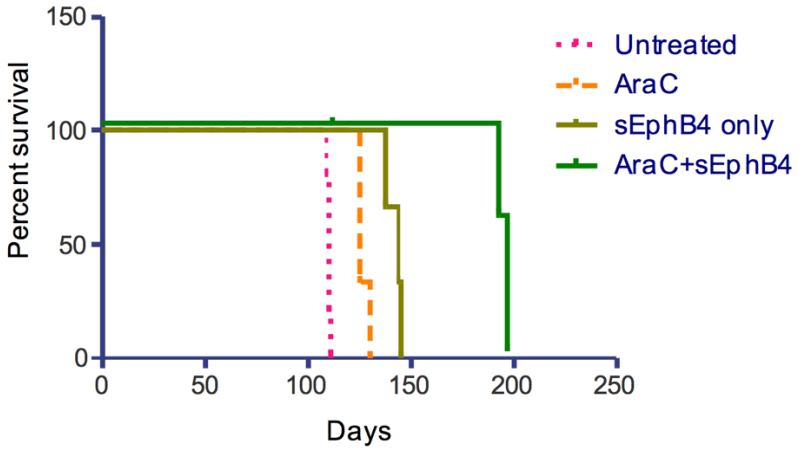



Figure. 3. Synergy plot demonstrating *in vitro* synergy of AraC with sEphB4-HSA in K562 cell line. Interaction index less than 1 indicates synergy. Dotted lines represent confidence intervals.

To further explore the anti-leukemia activity of sEphB4-HSA, we tested the drug alone and in combination with chemotherapy in a primary human xenograft mouse model of leukemia. Immuno-deficient mice were injected with leukemia cells collected from patients and once the disease was established, mice were treated with cytarabine, sEphB4-HSA or the combination. sEphB4-HSA alone demonstrated activity that is moderately more effective than chemotherapy alone. (Fig. 4) Most striking, however, is the highly synergistic activity

Figure 4. AML PDX model demonstrating single agent and combined activity of sEphB4-HSA. $p<0.05$ for sEphB4-HSA vs untreated and $p<0.01$ for AraC + sEphB-HSA vs AraC alone

of the drugs in combination.




Finally, we tested the anti-leukemia activity of sEphB4-HSA in combination with the hypomethylator decitabine (DAC). Mice were injected with K562 cells and treated with DAC, sEphB4-HSA, and the combination and observed for survival. The combination of DAC+sEphB4 extended median survival from 46 to 77 days.(Fig. 5)

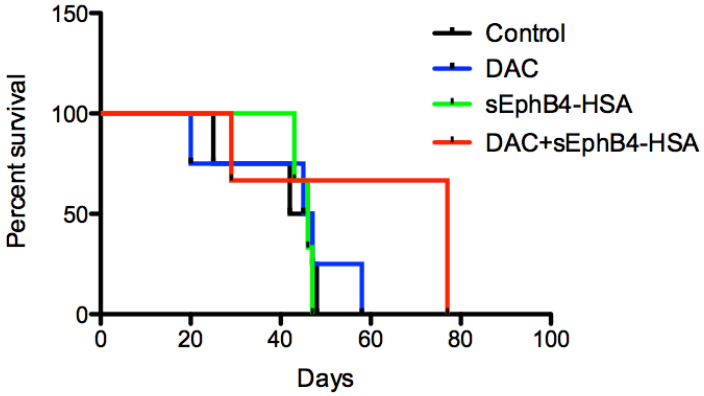



Figure 5. K562 mouse xenografts were treated with DAC, sEphB4-HSA, and DAC+sEphB4-HSA and observed for survival.

It is important to note that while we do believe sEphB4-HSA can potently activate anti-tumor immune response (as discussed below), these data in *immune-deficient* mice suggest that in myeloid leukemia, sEphB4-HSA does have direct anti-tumor activity that is mediated through AKT.

## 1.4 Role of EphrinB2 and EphB4 in the anti-tumor immune response

It is well established that cancer induces an immune-deficient state which allows tumors to escape immune surveillance. The precise mechanism of how treatment with sEphB4-HSA causes immune cell activation, however is unknown, although the literature suggest several possible mechanisms.
Recent work has revealed that there are suppressive counterparts to all of the effector cells of the immune system that normally act to inhibit autoimmunity and prevent runaway inflammatory responses. Three immunosuppressive cell types, in particular, are thought to be maliciously up-regulated by cancer via cytokines and re-purposed cell surface proteins in order to create a climate of immune tolerance toward the cancer.

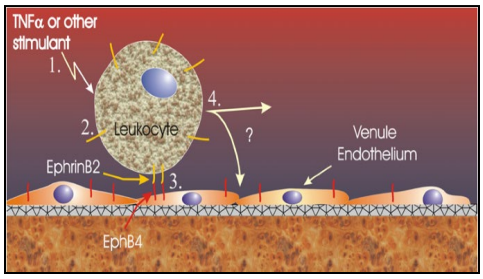
Regulatory T-cells are a subset of CD4+ T cells that suppress immune responses and are believed to play a role in autoimmunity and cancer immune-evasion (Antony 2005)(Whiteside 2014). Induced T regulatory cells (iTregs) expand in response to tumor antigens and are presumably responsible for the suppression of anti-tumor immune responses (Shenghui 2011)(Iversen 2013).

T-cell exhaustion is a state of T-cell dysfunction that develops during chronic exposure to antigen, including cancer neoantigens. Exhausted CD8+ T-cells lose capacity to produce activating cytokines, proliferate, and kill, and eventually undergo apoptosis and deletion. Signaling via inhibitory surface receptors including PD-1, 2B4, TIM-3 and CD160 play key roles in T cell exhaustion (Crespo 2013)(Wherry 2011). Drugs which target the inhibitory receptors responsible for maintaining the exhausted state restore T-cell cytotoxic potency and have shown remarkable clinical efficacy ('immune checkpoint inhibitors'; ipilimumab, nivolumab, pembrolizumab).

Myeloid-derived suppressor cells (MDSC) are abnormal myeloid cells that inhibit lymphocyte proliferation, and cytotoxic T lymphocyte induction and activity. These cells lack membrane markers for mature T cells, B cells, NK cells, or macrophages. Studies have revealed that circulating MDSC numbers correlate with a poor prognosis and tumor evasion of immune responses (Talmadge 2013)(Lechner 2011)(Messmer 2015).

Eph/Ephrin receptors regulate adhesion and activation of immune cells

The functions of Ephrin/Eph molecules in immune cells are just beginning to be appreciated. Little is known about the expression profiles of ephrinB2 and EphB4 in differentiated leukocytes of humans. EphrinB2 has been shown to be expressed on human monocytes and in bone marrow stromal cells, whereas EphB4 is expressed on human hematopoietic progenitor cells (Zamora 2006). Since ephrinB2 and EphB4 can modulate the adhesive and repulsive activities of migrating cells, molecules may also function to regulate these aspects of leukocyte function. In support of this notion, it has been demonstrated that some Ephrin ligands of A and B classes can modify the migratory properties of T-cells in vitro (Zamora 2006).



Proposed model of ephrinB2-EphB4 interaction in leukocyte recruitment (from

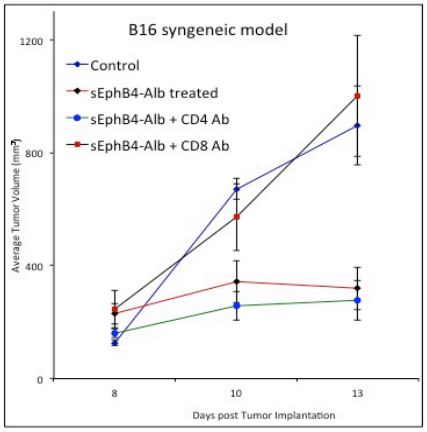
## Eph/Ephrin Interaction Inhibits and Repels T lymphocytes

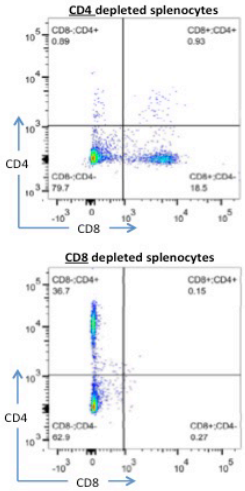
In T lymphocytes, the activation of Eph receptors is usually repulsive, therefore ephrin-Eph-mediated interactions between leukocytes and endothelial cells are thought to act to limit leukocyte adhesion to the vascular endothelium. Therefore, increased expression of Eph receptors in tumor vasculature and/or in leukocytes could act to increase such inhibition and attenuate leukocyte adhesion, extravasation, and tissue transmigration.

T-cell receptor (TCR)-mediated primary T-cell activation has been demonstrated to be highly governed by EphB/ephrinB axis. Wu and colleagues have demonstrated TCR costimulatory effects of all ephrinB using ephrinB-Fc chimeric proteins that act by blocking EphB4 activation (Wu 2005)(Kawano 2012). Nguyen et al has demonstrated that EphB/ephrinB interactions play an important role in mediating human mesenchymal stem cell inhibition of activated T-cells (Nguyen 2013). These studies suggest that, in mature T-cells, EphB4 may act to decrease T-cell cytotoxicity. Overexpression of EphB4 and ephrinB2 by the tumor and tumor-associated vasculature may be an adaptive tumor response to T-cell surveillance that serves to repel T-cells and attenuate the cytotoxicity of T-cells penetrating into the vicinity of the tumor (in this fashion, EphB4 may function in a manner analogous to other known immune checkpoint signaling proteins).

## Eph/Ephrin Interaction Attracts Myeloid Cells

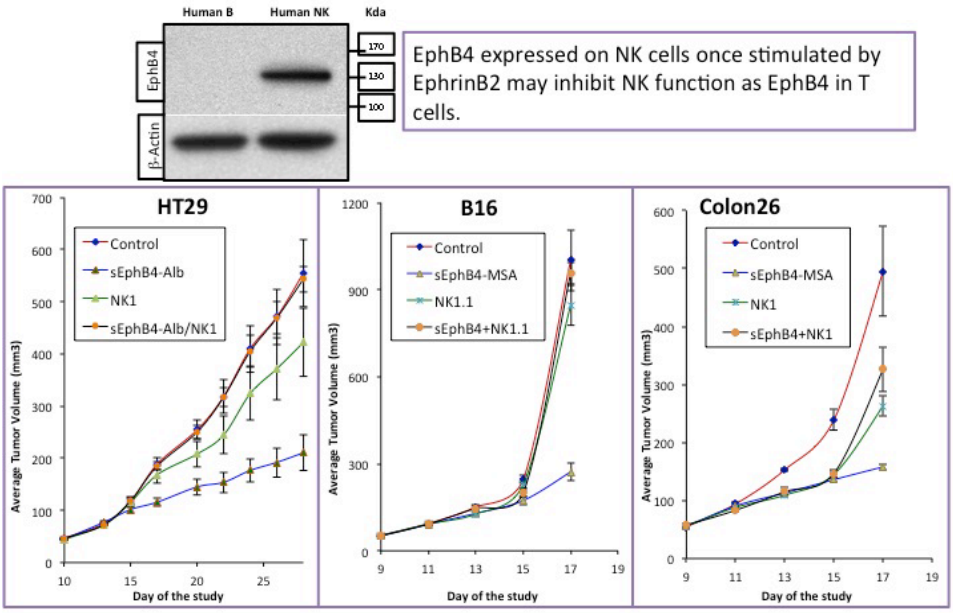
EphB4-ephrinB2 interaction has been shown to contribute to monocyte adhesion and diapedesis across arterial endothelium in a manner dependent on both EphB4 forward signaling and ephrinB2 reverse signaling (Korff 2008)(Pfaff 2008)(Kamei 2010). In contrast to the repulsive function of EphB4 signaling in T lymphocytes, in monocytes (which abundantly express EphB4) (Pfaff 2008), ephrinB2 (expressed on vascular endothelial cells) appears to have a pro-adhesive function. When the monocyte encounters a high density of ephrinB2 receptors (as is found in abnormal tumor vasculature), the monocyte is recruited to migrate through the vessel wall and into the extravascular space. In tumor vasculature, mechanism for normal monocyte recruitment may be co-opted to bring in highly immunosuppressive MDSCs. In our preliminary analysis of patients treated with sEphB4-HSA, the most dramatic responses to therapy were seen in patients that had the highest numbers of MDSC pre-treatment. If sEphB4-HSA works by blocking MDSC infiltration at tumor sites, this release T cells from the suppressive effects of MDSC would unleash the nascent T cell anti-tumor immune response. There are currently no immune-check point type therapies that directly target MDSC making this approach highly novel and potentially synergistic with other checkpoint inhibitors.




CD8 but not CD4 cells are required for sEphB4-HSA activity

Depleting NK cells leads to loss of sEphB4-HSA activity




Using syngeneic animal and cell line xenograft models, we have demonstrated that certain immune subsets are required for sEphB4-HSA activity. Mice implanted with syngeneic tumors in the B16 murine melanoma cancer model were treated with antibodies against CD4 and CD8. Analysis of spleens demonstrated 98-99% depletion of corresponding cell type. Depletion of CD8+ cells, but not CD4+ cells, abolishes the anti-tumor activity of sEphB4-HSA. Similarly, treatment with the antibody NK1.1, which depletes murine NK cells, was also to abolish clinic activity of sEphB4 in the same model. Additionally, treatment of humanized mice xenografted with HT29 and colon26 cell lines, with antibody NK, which depletes human NK cells, also lead to decreased activity of sEphB4-HSA.

Preclinical data utilizing EphrinB2 knockout models demonstrate that the anti-tumor effect of EphrinB2 blockade is reliant on the presence of T cells (Figure 6.). Furthermore, treatment with sEphB4-HSA initiates an influx of T cells into various tumor types in a mouse model (Figures 7a-7b). We have confirmed that this occurs in the tumors of treated patients on the phase I clinical trial (Figure 8), indicating a potent impact of sEphB4-HSA on T cell mobilization in humans.

## EphrinB2 Modulates Immune Response (Genetic Study)

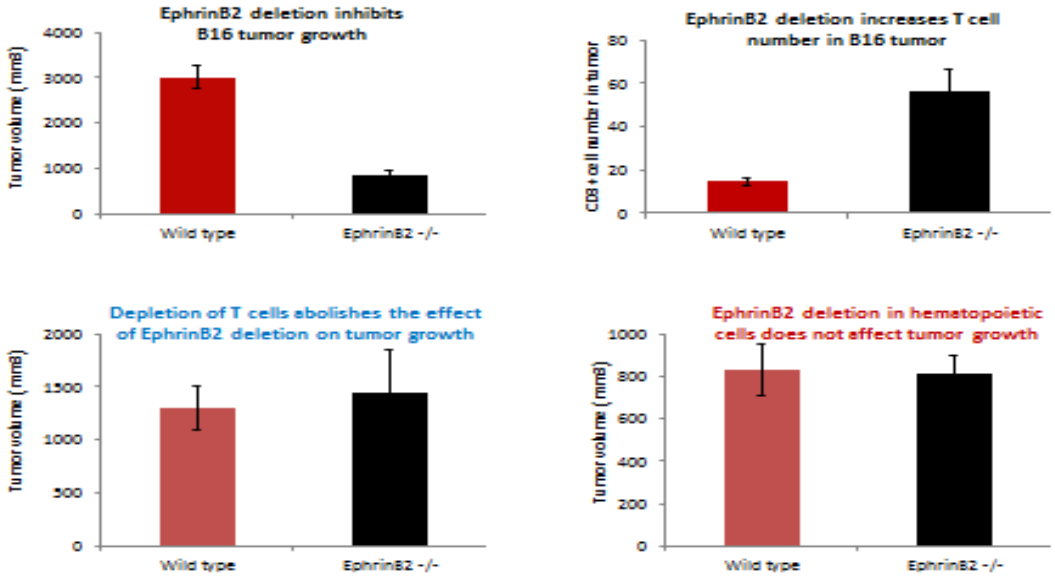



**Figure 6. The anti-tumor effect of EphrinB2 deletion relies on the presence of T cells.**

**Figure 7a-7b. T cell influx into tumor tissue occurs upon treatment with sEphB4-HSA.**

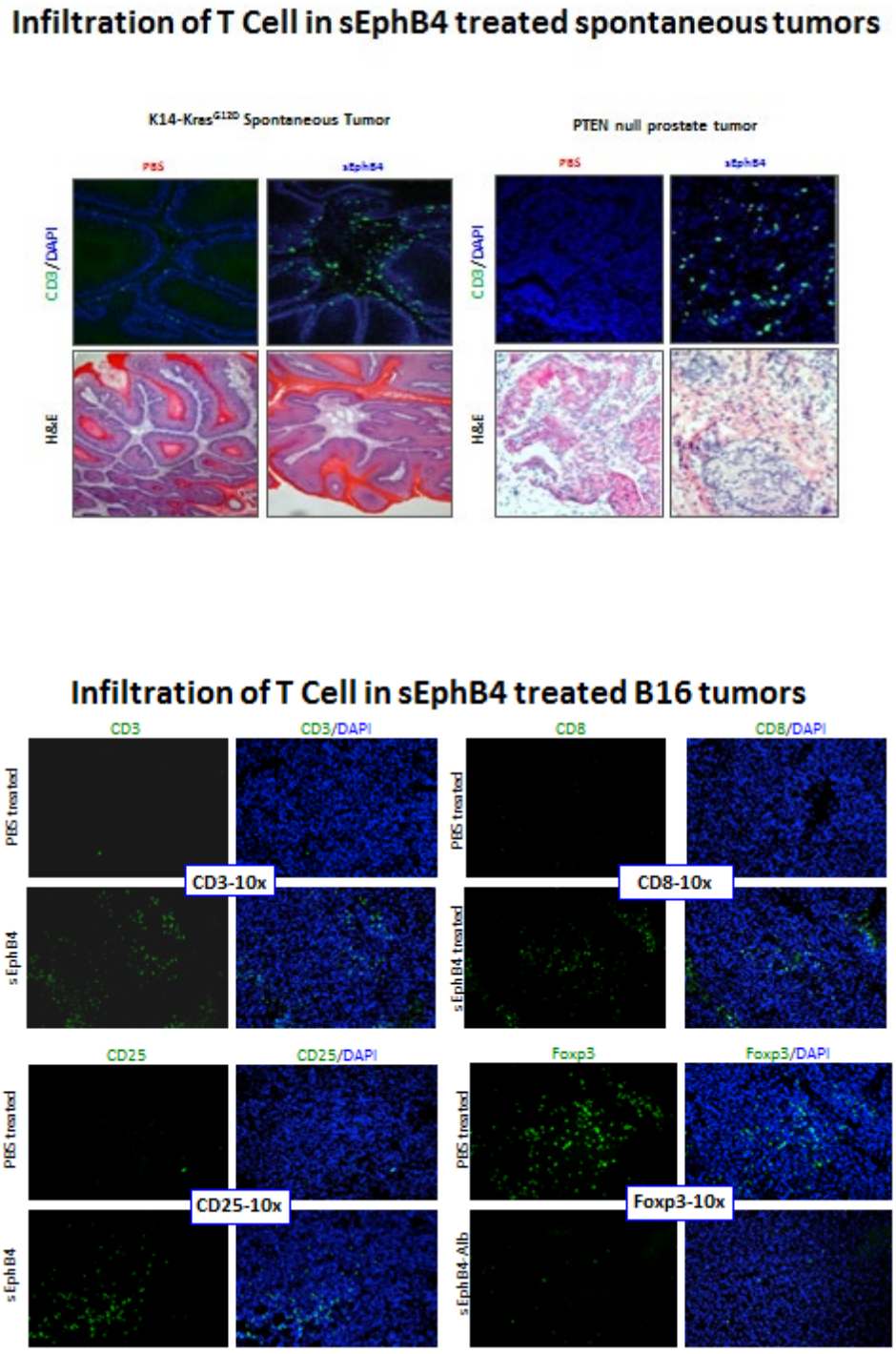
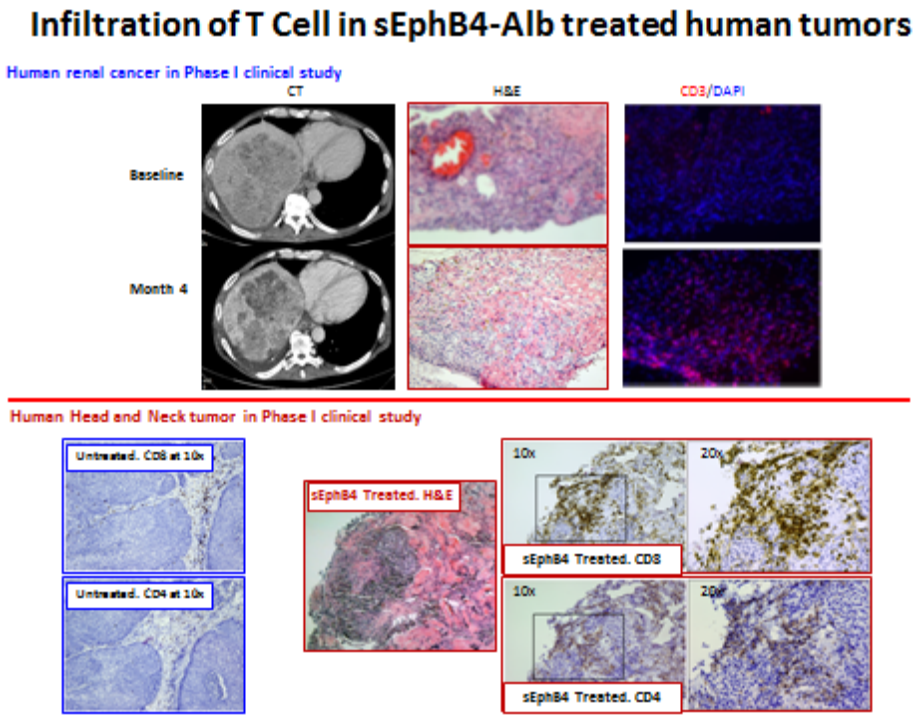


**Figure 8. Tumoral tissue before and after sEphB4-HSA treatment in humans enrolled on Phase I clinical**




## 1.5 Preclinical Toxicology and Immunogenicity

In cynomolgus monkeys, once a week I.V. infusion of 0/mg/kg (control) and 3, 10, 30 mg/kg for a total of 5 doses was well tolerated. No apparent toxicity was observed and all animals survived until their scheduled necropsy intervals. Full necropsy was performed on days 35 and 49 for selected monkeys and tissues were examined microscopically. No pathologic changes were observed in any organs examined including heart, lung, liver, kidney, spleen, adrenal glands, eyes, etc. sEph-B4-HSA was immunogenic in monkeys following i.v. administration (anti-drug antibody response ranged from 0-164 ug of anti-sEphB4-HSA antibodies per ml of serum). In the 5-weeks toxicity study, the immunogenicity appeared to impact the pharmacokinetics as the majority of the animals had lower serum concentrations of sEph-B4-HSA after drug administration on Day 29 ($5^{th}$ dose) compared to Day 1 (First dose). None of the monkey sin the 5-weeks toxicity study developed reactions consistent with anaphylaxis after sEphB4-HSA. Anti-sEphB4-HSA antibodies were detected in 75% of animals exposed to low drug dose (3mg/kg), 100% of animals exposed to middle drug dose (10mg/kg) and 50% of animals exposed to high drug dose (30mg/kg). Immune response in monkeys was associated with decreased drug levels.

## 1.6 Preclinical Pharmacokinetics (PK)

Pharmacokinetic studies were performed in two species. PK studies in mice showed a half-life of 20-25 hours. PK studies in cynomolgus monkeys showed a half-life of 5-6

days. Weekly infusion of the drug led to accumulation of the drug, with increase in peak and trough levels.

### 1.7 Preclinical Rationale for sEphB4-HSA Dosage Selection

Dose selection was based on the anticipated half-life (5 days) of the molecule, previous toxicology studies, and in vivo activity in tumor xenograft models with sEphB4-HSA. In xenograft tumor models in mice, activity was seen at doses of 5 mg/kg with maximal activity of 20 mg/kg. In a 1-month toxicity study of sEphB4HSA in cynomolgus monkeys showed that doses of 3, 10, and 30 mg/kg given weekly for a total of 5 doses were generally well tolerated. Repeat-dose toxicity of sEphB4-HSA in cynomolgus monkeys, a relevant animal model, have been conducted. The longest of these studies involved the administration of 5 weekly doses over a 4 week period at dose levels up to 30 mg/kg.

### 1.8 Safety and Efficacy in Humans

A phase I first-in-human clinical trial of sEphB4-HSA in various solid tumors was initiated in 2013. The study was IRB-approved and included a dose escalation followed by a dose expansion phase. The first three dosing cohorts were evaluated using the weekly administration schedule; 3 patients at 2.5mg/kg, 3 patients at 5mg/kg, 3 patients at 10 mg/kg and 1 patient at 15mg/kg. At that point, no DLTs were noted. Based on the PK profile, biweekly (every other week) dosing of sEphB4-HSA was then evaluated. Three patients were treated 10mg/kg, 6 patients were treated at 15mg/kg and 6 patients at 20mg/kg, every 2 weeks. There was one DLT of hypertension at the 20mg/kg dose. Based on PK analysis it was decided to return to the weekly dosing schedule and expanded the 10mg/kg weekly dose with 3 additional patients to bring the total number treated at 10mg/kg weekly to 6. There was 1 DLT of QTc prolongation (asymptomatic). Based on this, the 10mg/kg weekly dose was determined to be the MTD and RP2D.

**Table 1: Summary of Phase I Cohorts, Schedule and DLTs**

| Dose Level | Schedule | # of pts treated | # of pts evaluable for DLT | DLT |
|---|---|---|---|---|
| 2.5 mg/Kg | Q week | 3 | 3 | No |
| 5 mg/Kg | Q week | 3 | 3 | No |
| 10 mg/Kg | Q week | 6 | 6 | 1 QTc prolongation |
| 15 mg/kg | Q week | 1 | 1 | No |
| 10 mg/Kg | Q 2 weeks | 3 | 3 | No |
| 15 mg/Kg | Q 2 weeks | 7 | 6 | No |
| 20 mg/Kg | Q 2 weeks | 6 | 6 | 1 Grade 3 hypertension |

**Table 2. Grade 3 or 4 or Dose-limiting Toxicities, at least possibly related (N=31)**

| CTCAE AE | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|
| Hypertension | 13 (42%) | 9 (29%) | 0 |
| Fatigue | 4 (13%) | 3 (10%) | 0 |
| Weight loss | 3 (10%) | 0 | 0 |
| ALT | 0 | 1 (3%) | 0 |
| AST | 1 (3%) | 0 | 0 |
| Vomiting | 2 (6%) | 1 (3%) | 0 |
| Nausea | 1 (3%) | 1 (3%) | 0 |
| Mucositis | 1 (3%) | 0 | 0 |
| Hoarseness | 1 (3%) | 0 | 0 |
| Anorexia | 1 (3%) | 0 | 0 |
| Hyponatremia | 0 | 1 (3%) | 0 |
| Headache | 0 | 1 (3%) | 0 |
| Allergic Reaction | 1(3%) | 1 (3%) | 0 |

### 1.9 Pharmacokinetics in Humans

Murine in vivo efficacy, PK and human PK were examined to guide selection of dose and schedule. In vivo efficacy studies in mice showed dose response at 5, 10, and 20 mg/kg given IP three times a week. In addition, 50 mg/kg IP three times a week has shown potency in tumor growth inhibition. PK studies in mice at 5, 10, 20 and 50 mg/kg (iv) show peak level of 71, 178, 272, 743 ug/mL and half-life of 12-18 hours. Trough drug level in mice at a dose of 50mg/kg at 48 hours was 112 +/- 9. We thus estimated that the desired peak drug level in humans is around 200-300 ug/mL and trough level around 60-100 ug/mL. Human PK studies at 2.5, 5, 10mg/kg IV infusion over 60 minutes once a week show peak drug levels of 46.4, 86.6, and 188 ug/ml respectively.

Once a week dosing leads to accumulation of drug following repeated infusion. Repeat dosing leads to higher Cmax and trough levels. Specifically at dose level of 10mg/kg given weekly repeat dosing shows accumulation followed by homeostasis after 3-4 doses. Peak dose level after dose 1 190±35 (Cmax), rose to 282±52 (Cmax on week 3) and trough after the first dose of 66+6 (Pre 2nd dose, Day 8) rose to 116+21 after 3 doses followed by homeostasis. PK studies were also done at 10, 15, and 20mg/kg given twice a week. While there is accumulation over time, the drug levels during the second week remain low (Table 3).

**Table 3. PK parameters (means) of sEphB4-HSA following a 1 hour intravenous infusion (weekly)**

| Dose (mg/kg) | $C_{max}$ (ug/mL) | $AUC_{0\text{-}96h}$ (h*ug/mL) | $AUC_{0\text{-}tlast}$ (h*ug/mL) | $AUC_{0\text{-}\infty}$ (h*ug/mL) | $t_{1/2}$ (h) | CL (mL/h/kg) |
|---|---|---|---|---|---|---|
| 2.5 | 46.4 | 2400 | 3270 | 4580 | 130 | 0.315 |
| 5.0 | 86.6 | 4980 | 7330 | 10600 | 157 | 0.283 |
| 10 | 188 | 11700 | 17300 | 30400 | 302 | 0.133 |
| 15 | 345 | 23500 | 23600 | 49000 | 226 | 0.132 |

**Table 4. Summary of PK data in humans on Q2 weekly schedule**

| Dose | Cmax (ug/mL) | Day 7 post infusion (ug/mL) | Day 14 post infusion (ug/mL) |
|---|---|---|---|
| 10 mg/kg | 190 | 66 | ND |
| 15 mg/kg | 345 | 72 | 23 |
| 20 mg/kg | 422 | 107 | 54 |

These data indicate that serum profile from each dose can be fitted to a 2-compartment model since the PK of sEphB4-HSA is non-linear in the tested dose range.

Mean serum half-life from all 4 doses ranged from 130-302 hours.

In order to achieve peak drug level around 200 ug/ml and trough around 60-100 ug/ml, 10 mg/kg weekly was determined to be a suitable dose and utilized for the dose expansion phase of the first-in-human clinical trial.

## 2.0 OBJECTIVES

### 2.1 PRIMARY

2.1.1 To describe the toxicities and assess the tolerability of sEphB4-HSA in combination with an approved HMA among patients with MDS who are refractory to or have lost their response to one or more HMAs and among patients with relapsed/refractory AML previously treated with a HMA.

### 2.2 SECONDARY

2.2.1 To measure the expression of EphB4 among marrow and peripheral blood blasts in patients with MDS & AML at baseline and over the course of treatment

2.2.2 To measure the expression of immune check-point activating ligands (such as PD-L1, PD-L2) on marrow and peripheral blood blasts in patients treated with HMA and sEphB4-HSA in combination.

2.2.3 To profile immune subsets (activated and exhausted T cells, NK cells, T regulatory cells, and myeloid derived suppressor cells) in the peripheral blood and marrow in patients treated with HMA and sEphB4-HSA in combination.

2.2.4 To assess efficacy of sEphB4-HSA in combination with an HMA as manifest by IWG response criteria (Appendices 1 and 2), as well as time to development of acute myeloid leukemia (AML) in patients with MDS and time to progression.

## 3. Study Design

### 3.1 Description of the study

This is a pilot study designed to include 6 patients with relapsed refractory MDS and 6 patients with AML who were refractory to or relapsed after HMA treatment and who are deemed unfit for chemotherapy. Treatment will include the FDA-approved HMA that the patient was last treated with, administered per standard of care, in combination with the study agent.

#### 3.1.1 Pilot

The dose of sEphB4-HSA will be 15mg/kg. The study drug will be administered every 14 days.

### 3.2 End of Study

Patients who are responding to the combination therapy may continue them as per the study schema for as long as they are receiving clinical benefit up to 12 months, after which they can continue to receive the FDA-approved hypomethylating agent per standard of care.

The end of this study is defined as the date when the last patient, last visit (LPLV) occurs on the date of progression (or death if this occurs first)) or safety follow-up is received from the last patient, whichever occurs later. LPLV is expected to occur no more than 24 months after the last patient is enrolled.

### 3.3 Rationale for study design

Treatment options outside of allogeneic stem cell transplant are limited for patients with higher risk MDS who fail or lose their response to the 2 available FDA-approved HMAs. The same is true for patients with AML who are unfit for chemotherapy and may respond for a short duration to HMA treatment. Combination strategies that aim to increase the ORR and CR rates of HMAs have failed thus far and thus relapsed/refractory MDS and AML remain an unmet need in oncology.

This clinical trial aims to investigate the toxicities and obtain preliminary estimates of the efficacy of adding sEphB4-HSA to an HMA (azacitabine or decitabine). sEphB4-HSA has been shown to demonstrate an antileukemic effect and acts synergistically in combination with chemotherapy both in vitro and in mouse models. The agent also acts by causing immune cell activation, most likely via T cell mobilization. The primary objective of this phase of the study will be to describe the toxicities and establish that the proposed dose is well tolerated. In addition, data will be collected regarding the efficacy of sEphB4-HSA in combination with an approved HMA among patients with MDS and AML who are refractory to or have lost their response to one or more HMAs.

Eligibility for this trial will include relapsed and refractory MDS and AML patients who are unfit for intensive chemotherapy. Please refer to inclusion/exclusion criteria.

The primary endpoints will be toxicity and tolerability. Secondary endpoints will include efficacy endpoints as described below. This brief pilot study will establish that sEphB4-HSA is well tolerated when given with an approved HMA among patients MDS and AML who are refractory to or have lost their response to one or more HMAs. In addition, this trial will collect clinical and biological data that can be used to design follow-up investigations.

## 3.4 Endpoints

### 3.4.1 Primary endpoints

- Toxicity and tolerability: Toxicity will be assessed and graded according to the CTCAE v4, after each cycle. Tolerability will be defined as able to complete two cycles of treatment without the occurrence of dose limiting toxicity (DLT) as defined in Section 4.3.5 and are able to begin Cycle 3 within 4 weeks (unless for reasons unrelated to treatment toxicities).

### 3.4.2 Secondary endpoints

#### 3.4.2.1 Efficacy Endpoints

- Overall response – occurrence of CR, mCR, PR, or HI; anytime during the first two cycles of treatment based on the IWG Working Group Criteria for MDS and AML (Appendices 1 and 2)
- Time to disease progression – time from the start of treatment to the first disease progression or recurrence. Patients who are progression free at the time of last follow-up will be censored; death prior to progression will be counted as an event.
- Time to development of AML for patients with MDS - time from the start of treatment to the first evidence of development of AML. Patients who have not developed AML at the time of last follow-up will be censored; death prior to AML development will be counted as an event.
- Time to death from any cause - time from start of treatment to death from any cause. Patients who are alive at the time of last follow-up will be censored.
- Whether a patient who was transfusion-dependent on study entry, became transfusion-independent for at least 4 weeks.

#### 3.4.2.2 Correlative/Biological Endpoints

- Percent of bone marrow and peripheral blood blasts expressing EphB4 at baseline and during treatment
- Degree of PD-L1 and PD-L2 expression by IHC or flow cytometry in the bone marrow and peripheral blood blasts before and at different time points during treatment
- T-cell subset profile at baseline and during treatment: (activated and exhausted T cells, NK cells, T regulatory cells, and myeloid derived suppressor cells) in the peripheral blood and marrow

## 4. MATERIALS AND METHODS

### 4.1 Study Population

Patients with MDS or CMML, Intermediate-1 risk or higher, relapsed after or refractory to one or more standard therapies including at least 1 HMA given for at least 4 cycles. Patient with AML with 20-30% bone marrow blasts without WBC>25 x $10^3$ transformed from MDS that was previously treated with HMA.

### 4.2 Patient Eligibility

Eligibility waivers are not permitted. Subjects must meet all of the inclusion and exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered.

#### 4.2.1 Inclusion Criteria

- Adult subjects (18 years of age or older) with advanced MDS requiring treatment with HMA and either refractory to at least 4 cycles or progressing after previously documented response.
    - Patient must be treated within 6 months of the last HMA treatment and **must be willing to be treated with the same agent they last received on this study**
    - Prior treatment with novel HMA analog of decitabine on clinical trial is allowed; in such cases, decitabine will be used as the standard of care agent
- MDS classified as Intermediate 1-risk or high risk according to the international prognostic scoring system (IPSS) or revised-IPSS
- Chronic myelomonocytic leukemia (CMML)
- Acute myeloblastic leukemia (AML) that was previously treated with HMA and is unfit for intensive chemotherapy
    - Patient must be within 6 months of prior treatment with HMA and must be willing to be treated with the same agent on this study
- During the 8 weeks prior to inclusion in study, subjects must have a baseline bone marrow examination including all of the following:

    - Cytomorphology to confirm bone marrow blasts
    - Cytogenetics
- ECOG Status 0-2.
- Subject is able to understand and willing to comply with protocol requirements and instructions.
- Subject has signed and dated informed consent.
- Adequate baseline organ function defined by the criteria below
- Total bilirubin (except for Gilbert's Syndrome) ≤ 2.5xULN
- ALT and AST ≤ 3xULN
- Creatinine ≤ 2.5 x ULN
- Women of childbearing potential (WOCBP) and male patients with WOCBP as partners must be using an adequate method of contraception to avoid pregnancy throughout the study and for up to 12 weeks after the last dose of the investigational agent. Subject is practicing an acceptable method of contraception (documented in CRF). WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal. Post menopause is defined as:
    - Amenorrhea ≥ 12 consecutive months without another cause or
- For women with irregular menstrual periods and on hormone replacement therapy (HRT), a documented serum follicle stimulating hormone (FSH) level >35mIU/mL
- Women who are using oral contraceptives, other hormonal contraceptives (vagina products, skin patches, or implanted or injectable products), or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy, or are practicing abstinence or where their partner is sterile (e.g., vasectomy) should be considered to be of childbearing potential.
- WOCBP must have a negative serum test (minimum sensitivity 25 IU/L or equivalent units of human chorionic gonadotropin [HCG]) within 72 hours prior to the start of investigational product.

### 4.2.2 Exclusion Criteria

- Patients with AML whose white blood cell count exceeds 25,000/mcL.
- Patients with uncontrolled hypertension
- QTc (Fridericia Correction Formula) > 480 ms on ECG
- Patients whose electrolytes (sodium, potassium, calcium, magnesium) are abnormal or cannot be normalized with standard intervention on the day of treatment with study drug
- Patients who are actively receiving any other anticancer therapy.

- Patients with a history of allergic reactions attributed to compounds of similar chemical or biologic composition to HMAs
- Patients with a diagnosis of acute promyelocytic leukemia.
- Patients with short life expectancy (less than 3 months) due to comorbidity other than MDS
- Female subjects who are nursing or pregnant (positive serum or urine Beta-human chorionic gonadotropin [B-hCG] pregnancy test)
- Patients with current alcohol or drug abuse.
- Patients who have received treatment with an investigational drug within 30 days preceding the first dose of study medication.
- Patients with uncontrolled inter-current illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- Patients known to be infected with Hepatitis B, C or Human Immunodeficiency Virus (HIV). If known, then must be on stable and effective antiviral treatment.
- Patients with a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalent) or other immunosuppressive medications within 14 days of randomization. Inhaled or topical steroids and adrenal replacement steroid doses >10mg daily prednisone equivalent, are permitted in the absence of uncontrolled autoimmune disease.
- Patients must not have uncontrolled hypertension as defined by SBP ≥ 160mmHg or DBP ≥ 90mmHg; patients whose blood pressure can be controlled medically are allowed to be rescreened once BP is under control.

### 4.2.3 Medication-Related Exclusion Criteria

- Hypertension was a DLT in Phase I. Therefore, patients with uncontrolled HTN (>160/90) will not be admitted onto the study.

## 4.3 Study Treatment Plan

Treatment with HMA azacitidine or decitabine (patients should be given the same drug they were last treated with and failed for their MDS) will proceed per standard of care as indicated on the FDA labeling. Azacitidine can be given as an intravenous or subcutaneous infusion at a dose of 75mg/$m^2$ on days 1-7 or days 1-5 and 8-9 on a 28-day cycle. Patients who required dose reductions of the HMA should be treated with the last tolerated dose of the same HMA. Dose adjustments during subsequent cycles can be made if indicated in the opinion of the treating physician and with approval of the PI if they are in keeping with the FDA labeling. Decitabine is given days 1-5 at a dose of 20 mg/$m^2$ intravenously on a 28-day cycle. sEphB4-HSA is given intravenously at a dose of 15mg/kg days 1and 15 of a 28 day cycle. Dose adjustments during subsequent cycles can be made if indicated in the opinion of the treating physician and with approval of the PI if they are in keeping with the FDA labeling. Administration of sEphB4-HSA can be before or after the HMA but it should not be administered concurrently. Details of administration are described in Section 4.3.4.

### 4.3.1 Study Drug

sEphB4-HSA is a fusion protein composed of the full length extra-cellular domain of EphB4 receptor tyrosine kinase and seamless fusion at its C-terminus with full length human albumin. This monomeric protein binds EphrinB2 with high affinity (5-10nM) and blocks interaction with cell surface EphB receptors, thus blocking forward and reverse signaling. sEphB4-HSA is given intravenously at a dose of 15mg/kg days 1 and15 of a 28 day cycle.

### 4.3.2 Return and Retention of Study Drug

When a study is opened at two or more institutions, the institutional policy will be followed. Such policies will be provided to the USC Clinical Investigation Support Office (CISO) QA prior to enrolling 1st patient.

The study pharmacist at each participating site will be responsible for maintaining a record of shipment, receipt and dispensation of study medication(s). The study pharmacist will utilize an NCI drug accountability template for documenting dates, and amounts/doses received from the sponsor and dates, patient initials and doses dispensed to the patient.

The study Research Coordinator(s) and Data Manager(s) will be responsible for drug accountability of dispensed and returned drug in accordance with the CISO SOP 8.0.

This study will consist of a phase II study in which 2 cohorts of patients will be treated with the combination at the recommended phase II dose of the two drugs.

The drug is being provided free of charge by VasGene Therapeutics Inc. We will apply under the existing IND #112629.

### 4.3.3 Drug Formulation and Storage

sEphB4-HSA is a fusion protein composed of the full length extra-cellular domain of EphB4 receptor tyrosine kinase and seamless fusion at its C-terminus with full length human albumin. This monomeric protein binds EphrinB2 with high affinity (5-10nM) and blocks interaction with cell surface EphB receptors, thus blocking forward and reverse signaling.

The Drug product contains sEphB4-HSA at 25 mg/ml in 10 mM L-Histidine, 150 mM Sodium Chloride, and 10% Surose, pH 7.0. The Final Container product is a clear, colorless to light straw yellow sterile solution in 10 cc glass vials. Each vial contains 250 mg of drug in a 10 mL volume. The vial is closed with an appropriately sized stopper and seal.

sEphB4-HSA is very stable at -800C and -200C for a period of 6-12 months. Freeze and thaw in general can be detrimental to protein stability, although sEPhB4-HSA can

undergo freeze and thaw at least three times without any adverse effects. sEphB4-HSA vials must be stored at a temperature of -80 0C or -200C and should be protected from light. Any excursion from recommended storage conditions must be brought to the attention of the Sponsor's representative to seek advice on future use of the product. can be stored for up to 12 hours at room temperature/under room light and 24 hours at 20 to 80C 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.

### 4.3.4 Drug Preparation and Administration

sEphB4-HSA will be on the basis of actual body weight at the start of each cycle. The drug will be prepared as follows:

1. Allow the appropriate number of vials of sEphB4-HSA to stand at room temperature for approximately 15 minutes before preparation.
2. Ensure that the sEphB4-HSA solution is a colorless, clear to slightly opalescent solution, essentially free of particles on visual inspection.
3. Aseptically withdraw the required volume of sEphB4-HSA solution into a syringe, and dispense into an i.v. 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).
4. The total dose to be administered will be diluted to a total volume of 200 mL with sterile normal saline and placed in EVA mixing container (B.Braun Cat #2112389).
5. Prepare the sEphB4-HSA solution for infusion per the example provided below: Total dose should be calculated as follows: Subject body weight in kg x 10 mg= total dose in mg; a subject with a body weight of 70 kg would be administered 1050 mg of sEphB4-HSA (70 kg x 15 mg/kg = 1050 mg).
   Forty-two mL of sEphB4-HSA and 158 mL of normal saline would be mixed in the i.v. bag and the solution would be infused over 60 minutes.
6. Mix by GENTLY inverting several times. DO NOT shake.
7. Visually inspect the final solution. If the infusion is not clear or the contents appear to contain precipitate, the solution should be discarded (according to the instructions in Section 7.7) and documented on the Drug Accountability Log.
8. Record the time sEphB4-HSA was prepared on the i.v. bag label.
9. Attach the i.v. bag containing the sEphB4-HSA solution to the Non- PVC tubing with infusion set, 0.2 µm in-line filter, and infusion pump.
10. The infusion rate of the infusion pump should be adjusted to allow for a total infusion time of 60 minutes.
11. At the end of the infusion period, flush the line with a sufficient quantity of

normal saline. Do not enter into each vial more than once. Do not prepare sEphB4-HSA for infusion in glass syringes. Do not administer study drug as an i.v. push or bolus injection.

### 4.3.5 Dose-Limiting Toxicity (DLT)

The following toxicities considered to be at least possibly related to the study therapy are considered DLTs:

- Any Grade 5 toxicity
- Any Grade 4 non-hematologic toxicity
- All Grade 3 non-hematologic toxicity excluding:
    - Nausea, vomiting, and diarrhea that does not require hospitalization or total parenteral nutrition support and can be managed with supportive care to ≤ Grade 2 within 48 hours
    - Constipation that does not resolve to ≤ Grade 2 within one week
    - Alopecia;
    - Grade 3 fatigue that returns to grade 2 or less within 28 days; and
    - First occurrence of grade 3 hypertension that is not accompanied by any hypertension related symptoms and that is manageable with medical therapy to ≤ grade 2 within 2 weeks of initiation of antihypertensive therapy. A second occurrence of grade 3 hypertension despite medical antihypertensive therapy will be considered a DLT
- Related febrile neutropenia that was not present prior to dosing, does not resolve within 14 days and is not related to underlying disease.
- Related Grade 4 neutropenia that represents a 2 grade increase over baseline, does not resolve to Grade 3 or less within 14 days, and is not related to underlying disease
- Related Grade 4 thrombocytopenia that represents a 2 grade increase over baseline, dose not resolve within 14 days and is not related to underlying disease
- Any toxicity at least possibly related to sEphB4 or the HMA that results in a delay in treatment of 4 or more weeks or leads to the termination of treatment

#### 4.3.5.1 Definition of Evaluable for DLT

To be evaluable for DLT, a patient must receive at least the majority of each of the 1st 2 cycles of treatment (i.e. at least 3 doses of the HMA and 1 dose of the sEphB4 in each cycle) or have experienced a DLT during the 1st 2 cycles or been unable to begin Cycle 3 within 4 weeks due to treatment toxicities. All patients who are not evaluable for dose limiting toxicity will be replaced.

#### 4.3.5.2 DLT and Tolerability Monitoring Rules

Up to 6 evaluable subjects will be enrolled in each cohort and results of the trial will be formally reviewed by the Phase I Committee after every 3 patients have completed Cycle 1 within each cohort.

Toxicity will be reviewed on an ongoing basis and all DLT's and treatment discontinuations due to adverse events will be discussed in real time. As soon as 2+ patients experience DLT in the 1st 2 cycles, within a cohort, or if 4+/6 patients cannot tolerate this regimen (as defined below) within a cohort, then enrollment will pause and all data in all cycles will be reviewed and consideration will be given to reducing a dose or modifying the regimen – perhaps for one of the HMA agents or for both. If this occurs, the study will be formally amended.

#### 4.3.5.3 Definition of Tolerable

A patient will be defined as having tolerated the regimen if he/she completes 2 cycles without experiencing DLT and is able to begin the 3rd Cycle within 4 weeks (or has gone off therapy due to disease progression or reasons unrelated to toxicities due to the treatment).

### 4.3.6 Maximum Tolerated Dose (MTD)/Recommended Phase II Dose (RP2D)

We anticipate that the doses selected for this regimen will be well tolerated. Unless it is observed that 2+ patients experience Cycle 1 DLT (within a cohort) or that fewer than 3/6 patients are able tolerate 2 cycles, the proposed dose of sEphB4-HSA will be recommended for further study when given in combination with standard of care HMAs. All toxicities and all DLTs over all courses will be reported.

### 4.3.7 Toxicities and Dosing Delays/Dose Modifications

Any patient who receives treatment on this protocol will be evaluated for toxicity. Each patient will be assessed for the development of toxicity according to the Study Calendar (Appendix 8). Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Dose adjustments should be made according to the system showing the greatest degree of toxicity. For the HMAs dose adjustments should be made according to the package insert for toxicities thought to be related to the HMA.

(1) https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021790s006lbl.pdf
(2) https://www.accessdata.fda.gov/drugsatfda_docs/label/2008/050794s011lbl.pdf

Toxicities at least possibly related to sEphB4 should result in holding the next dose of the drug if the toxicity is grade 2 or 3 and represents at least a 1 grade increase over baseline. If the toxicity resolves back to 1 grade lower or baseline, the next administration of sEphB4 can proceed at the same dose.

**sEphB4-HSA dose modifications:**

Dose reductions for sEphB4-HSA

Table 5

| | **sEphB4-HSA** |
|---|---|
| Starting dose | 15 mg/Kg days 1 and 15 |
| Dose reduction -1 | 10 mg/Kg days 1 and 15 |
| Dose reduction -2 | 5 mg/Kg days 1 and 15 |

Normal electrolytes and QTc <480 ms should be confirmed prior to dosing with sEphB4-HSA during cycles 1 and 2. Abnormal electrolytes (potassium, calcium, magnesium) should be corrected prior to dosing and laboratory evaluation should be repeated in order to document that an intervention results in achievement of normal range. If electrolytes cannot be corrected, dosing with sEphB4 should be delayed until they are corrected, but no longer than 48 hours. If dosing is held longer than 48 hours, that dose should be skipped completely. ECG should be performed before and after dosing with sEphB4-HSA throughout cycles 1 and 2. Grade 3 QTc prolongation considered related to the study drug should result in permanent discontinuation of the study drug.

Tumor lysis is unlikely to occur in this population but a sudden increase in the creatinine, phosphorus or potassium should prompt further evaluation including uric acid and lactate dehydrogenase level. Identification of tumor lysis should prompt cessation of therapy and initiation of measures to reduce uric acid and normalize electrolytes as per standard of care.

Hypertension: this is an expected class effect adverse event. All blood pressure measurements should be made after the patient has been in a supine position for 10 minutes or longer. Patients with grade 2 hypertension as evidenced by two or more different measurements that are at least one hour 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 >160 and/or DBP is > 90, physician should increase the dose of the antihypertensive or add a second antihypertensive drug as indicated. In the case of a first occurrence of grade 3 asymptomatic hypertension, 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. 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

### 4.4 Concomitant Medications/Treatments

Other active treatment for MDS (including steroids), other than low permanent doses such as inhaled or topical steroids and adrenal replacement steroid doses >10mg daily prednisone equivalent (in the absence of active autoimmune toxicity requiring treatment), should not be given during the study.

G-CSF can be used with doses according to investigators judgement, in case of neutropenia with infection, or if the patient develops a grade 4 neutropenia (ANC $<0.5x10^9$/l). Subjects who enter the study on G-CSF should, if clinically motivated, continue at the same dose schedule until the optimal dose of study medication has been established.

It is strongly recommended that:

Traditional herbal medicines not be administered because the ingredients of many herbal medicines are not fully studied and their use may result in unanticipated drug-drug interactions that may cause, or confound assessment of, toxicity

Prophylactic medication according to local standard of care. All concomitant medication will be monitored during the study in subjects CRF.

**4.4.1 Prohibited Medications:** Other cancer-directed therapy.

#### 4.4.2 Duration of Therapy

In the absence of treatment delays due to adverse events with both cycles, treatment may continue for up to 12 cycles with the combination therapy and then per standard of care for the HMA as monotherapy thereafter per treating physician, OR until:

- Disease progression;
- Inter-current illness that prevents further administration of treatment;
- Unacceptable adverse event(s);
- Patient decides to withdraw from the study; OR
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

### 4.5 General Plan to Manage Safety Concerns

Measures will be taken to ensure the safety of patients participating in this trial, including the use of stringent inclusion and exclusion criteria (see *Section 4.2.1 and 4.2.2*) and close monitoring (as indicated below and in *Section 4.5.2*). See *Section 7.3.1* for complete details regarding safety reporting for this study.

### 4.5.1 Eligibility Criteria

Eligibility criteria were selected to guard the safety of patients in this trial. Results from the nonclinical toxicology studies with sEphB4-HSA (see Section 4.2).

### 4.5.2 Patient Safety Monitoring

Safety will be evaluated in this study through the monitoring of all serious and non-serious AEs, defined and graded according to NCI CTCAE v4.0. Patients will be assessed for safety (including laboratory values) according to the schedule in *Appendix 9*. Patients will be followed for safety for 90 days following the last dose of study treatment or until receipt of another anticancer therapy, whichever comes first.

General safety assessments will include serial interval histories, physical examinations, and specific laboratory studies, including serum chemistries and blood counts (see *Section 5.1.7* for the list and timing of study assessments). All serious adverse events (SAEs) and protocol-defined events of special interest (see *Section 7.2.2*) will be reported in an expedited fashion (see *Section 7.3*). In addition, the investigators will review and evaluate observed AEs on a regular basis.

Patients who have an ongoing study treatment–related AE upon study completion or at discontinuation from the study will be followed until the event has resolved to baseline grade, the event is assessed by the investigator as stable, new anticancer treatment is initiated, the patient is lost to follow-up, the patient withdraws consent, or until it has been determined that study treatment or participation is not the cause of the AE.

### 4.5.3 Management of Specific Safety Concerns with sEphB4-HSA

Nursing to record blood pressure every 15minutes during the infusion and hold infusion and contact the treating physician or PI if BP>160/90.

### 4.5.4 Guidelines for Dosage Modification & Treatment Interruption or Discontinuation

Treatment modification of either hypomethylating agent will be per the respective prescribing information for decitabine and azacitidine. For the study drug-related HTN, if the blood pressure cannot be reduced after two hours or three medical interventions, study drug will be held and patient will be evaluated for secondary endpoints only. For other toxicities measured as grade 2 or 3 and representing at least a 1 grade increase over baseline at least possibly related to sEphB4, the next dose of the study drug

should be held. If the toxicity resolves back to 1 grade lower or baseline, the next administration of sEphB4 can proceed at the same dose. For grade 3 or 4 toxicities thought to be at least possibly related to study drug see DLT guidelines.

### 4.6 Removal of Patients from Protocol Therapy

Patients will be removed from therapy when any of the criteria listed below apply. Notify the Principal Investigator, and document the reason for study removal and the date the patient was removed from treatment in the Case Report Form. The patient should be followed-up per protocol.

Patients can be taken off the study treatment and/or study 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 of treatment will be documented and may include:

- Patient withdraws consent (follow-up);
- Patient is unable to comply with protocol requirements;
- Patient demonstrates disease progression (unless continued treatment with study drug is deemed appropriate at the discretion of the investigator);
- Patient experiences toxicity that makes continuation in the protocol unsafe;
- Treating physician determines continuation on the study would not be in the patient's best interest;
- Patient becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event);
- Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study; or
- Lost to follow-up.

#### 4.6.1 Duration of Follow up

Patients will be followed as indicated by the study calendar for the first 24 months of therapy. Patients who survive beyond the study period will be contacted every 6 months until progression or death, whichever comes first. This may be by telephone or during standard of care follow-up visits.

Patient replacement: Patients will be considered evaluable for response if they complete one full cycle of combination therapy. Patients who withdraw consent, are removed or are not evaluable for any reason prior to completing one cycle will be replaced.

# 5. STUDY PROCEDURES

## 5.1 Screening/Baseline Procedures

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining written informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

All screening procedures must be performed within 14 days prior to registration unless otherwise stated. The screening procedures include:

### 5.1.1 Study Assessments

Patients will be closely monitored for safety and tolerability throughout the study. All assessments must be performed and documented for each patient.

Patients should be assessed for toxicity prior to each dose; dosing will occur only if the clinical assessment and local laboratory test values do not suggest a DLT or new toxicity of grade 2 or higher (see 4.3.5 and 4.3.7 and 4.5.4). Electrolytes (potassium, calcium, magnesium) should be corrected prior to administration of sEphB4 and ECG should confirm QTc < 480ms.

If the timing of a protocol-mandated study visit coincides with a holiday and/or weekend that precludes the visit, the visit should be scheduled on the nearest following feasible date, with subsequent visits rescheduled accordingly.

### 5.1.2 Medical History

Complete medical and surgical history, history of infections, prior cancer history and treatment, transfusion requirements

Medical history includes clinically significant diseases within the previous 5 years, smoking history, cancer history (including tumor characteristics such as hormone receptor status), prior cancer therapies and procedures, and all medications used by the patient within 7 days before the screening visit (including prescription, over-the-counter, and herbal/homeopathic remedies and therapies).

### 5.1.3 Demographics

Age, gender, race, and ethnicity.
Review subject eligibility criteria.
Review previous and concomitant medications.

### 5.1.4 Vital Signs

Physical exam including Vital signs (temperature, pulse, respiratory rate, pulse oximetry on room air, blood pressure), height, weight, and Performance status (Performance status evaluated prior to study entry according to ECOG score).

For the first infusion of sEphB4-HSA, the patient's vital signs (heart rate, respiratory rate, blood pressure, and temperature) should be determined within 60 minutes before, during (every 15 [± 5] minutes), and 30 (± 10) minutes after the infusion. For subsequent infusions, vital signs will be collected within 60 minutes before and within 30 minutes after the infusion. Vital signs should be collected during the infusion only if clinically indicated. Patients will be informed about the possibility of delayed post-infusion symptoms and instructed to contact their study physician if they develop such symptoms.

### 5.1.5 Physical Examination

A complete physical examination will be performed at screening and at the treatment discontinuation visit and should include the evaluation of head, eye, ear, nose, and throat and cardiovascular, dermatologic, musculoskeletal, respiratory, gastrointestinal, and neurologic systems.

A limited physical examination will be performed at other visits to assess changes from baseline abnormalities and any new abnormalities and to evaluate patient-reported symptoms. New or worsened abnormalities should be recorded as AEs if appropriate.

### 5.1.6 Response Evaluation

Bone marrow biopsy and aspirate results will be used to document baseline IPSS risk score and other indicators of disease severity and will be repeated after the two cycles and in the event of apparent disease progression, unless there is clear evidence of progression to AML in the peripheral blood (blasts confirmed >20% by flow cytometry of the peripheral blood). Bone marrow biopsy must be performed to confirm CR.

### 5.1.7 Laboratory Assessments

#### 5.1.7.1 Hematology

Number and frequency of transfusion over 8 weeks prior to enrollment will be recorded; e.g. 2 units PRBCs q4 weeks, one unit platelets every two weeks, etc.

#### 5.1.7.2 Blood draw for correlative studies

See *Section 9* on Correlatives studies.

#### 5.1.7.3 Serum Chemistries

Comprehensive metabolic panel (CMP) to include: albumin, alkaline phosphatase, ALT/SGPT, AST/SGOT, BUN, creatinine, electrolytes (sodium, potassium, calcium, chloride, bicarbonate), glucose, and total bilirubin.

#### 5.1.7.4 Pregnancy test (for females of child bearing potential)

#### 5.1.7.5 Risk assessment

Revised-IPSS and bone marrow biopsy blast count and cytogenetics.

#### 5.1.7.6 Other

Baseline: labs pertaining to organs that could be susceptible to autoimmune conditions:

- TSH, amylase, lipase;
- Baseline labs pertaining to coagulation status; and
- PT, aPTT.
- Procedures During Treatment

Prior to Each Treatment Cycle:

- Physical exam, vital signs;
- Hematology;
- Serum chemistries; and
- Adverse event evaluation.

Day 1:

- History and Physical exam and continue medications;
- Physical exam, vital signs;
- Hematology;
- Serum chemistries;
- Samples for hematology, serum chemistries, coagulation, urinalysis, and the pregnancy test will be analyzed at the study site's local laboratory.
- Local laboratory assessments will include the following:
    - Hematology (CBC, including RBC count, hemoglobin, hematocrit, WBC count with differential [neutrophils, eosinophils, lymphocytes, monocytes, basophils, and other cells], and platelet count)
    - Serum chemistries (glucose, BUN, creatinine, sodium, potassium, magnesium, chloride, bicarbonate, calcium, phosphorus, total bilirubin, ALT, AST, alkaline phosphatase, LDH, total protein, and albumin)
    - Coagulation (aPTT and INR)

- Pregnancy test (for women of childbearing potential, including women who have had a tubal ligation)
- Urinalysis (specific gravity, pH, glucose, protein, ketones, and blood)
- Thyroid function testing (TSH, free T3 and free T4 if indicated based on abnormal TSH), hepatitis B virus (HBV) serology (HBsAg, antibodies against HBsAg, hepatitis B core antigen), and HCV serology (anti-HCV) as clinically indicated
  - HBV DNA test is required for patients who have known positive serology for anti-HBc
  - HCV RNA test is required for patients who have known positive serology for anti-HCV
- Laboratory assessments will be performed at the participating sites' laboratories (no central labs)

- Biomarker assays

  See *Section 9* for details.

Refer to the laboratory manual for additional details on laboratory assessments and sample handling.

### 5.1.8 Electrocardiograms

Baseline 12 lead electrocardiograms will be performed and reported as per institutional protocol.

## 5.2 Treatment Discontinuation Visit

Patients who discontinue from treatment will be asked to return to the clinic no more than 30 days after the last treatment for a treatment discontinuation visit. The visit at which a response assessment shows progressive disease may be used as the treatment discontinuation visit.

## 5.3 Follow-up Procedures

Patients will be followed every 6 months after completion of (or early withdrawal from) study treatment until death or progression of disease.

## 5.4 Measurement of Effect

Efficacy endpoints are defined in Section 3.4. The criteria for response are included in Appendix**.**

## 5.5 Definitions

**Evaluable for toxicity.** All patients will be included in the toxicity summaries, from the time of their first treatment with study drug.

**Primary Efficacy/ Response Assessment.** Clinical response is assessed following every cycle of treatment. Confirmation of CR by bone marrow biopsy is required. All patients who begin their first treatment with study drug will be accounted for in the listing of patients and their responses.

**Final Response Assessment.** Will occur two months following completion of treatment with sEphB4-HSA and HMA.

### 5.5.1 Post-Treatment Evaluations

Patients will be followed as indicated by the study calendar for the first 24 months of therapy. Patients who survive beyond the study period will be contacted every 6 months for progression and survival data. This may be by telephone or during standard of care follow-up visits. Documentation of disease status should be attempted at time of discontinuation of study treatments. Subsequent bone marrow biopsies and laboratory evaluations will not be performed as part of the study after patients discontinue study treatments but per routine by the treating physician.

Female patients of reproductive potential who are not surgically sterile must practice adequate birth control for a minimum of twelve months post-treatment; male patients who are not surgically sterile must practice adequate birth control for a minimum of three months post-treatment.

## 6. Statistical Considerations

### 6.1 Pilot Study

The study drug, sEphB4-HSA, will be administered at the pre-determined dose of 15mg/kg every 2 weeks, in combination with an FDA-approved hypomethylating agent (either azacitadine or decitabine). We will use the hypomethylating agent with which the patient was last treated. No dose escalation is planned, as the primary end-point of this study is to assess for toxicities, DLTs and tolerability of the combination therapy at the proposed doses (these are defined in Section 4.3.5).

Toxicity will be reviewed on an ongoing basis and all DLT's and treatment discontinuations due to adverse events will be discussed in real time. As soon as 2+ patients experience DLT in Cycle 1 or 2, within a cohort (AML or MDS), or if 4+/6 patients cannot tolerate this regimen within a cohort, then enrollment will pause and all data in all cycles will be reviewed and consideration will be given to reducing a dose or modifying the regimen – perhaps for one of the HMA agents or for both. If this occurs, the study will be formally amended.

### 6.2 Total Sample Size

We will enroll 6 patients with relapsed refractory MDS and 6 patients with relapsed refractory AML who were previously treated with a HMA and are deemed unfit for chemotherapy.

The goal is to have 12 patients treated at the proposed dose. With 12 patients, there is an 86% chance that toxicities that occur in at least 15% of patients are observed at least once; and there is a 93% chance that toxicities that occur in at least 20% of patients are observed at least once. Therefore most common toxicities will be observed in this series of patients.

Although no differences in terms of toxicities and tolerability are expected, a decision was made to require that half of the patients have MDS and half have AML. This will allow a preliminary examination of whether the toxicities are, indeed, similar between the two cohorts. In addition, the two cohorts will be initially analyzed separately in terms of the biological and correlative outcomes.

### 6.3 Analysis of Results

All patients who begin treatment will be accounted for and included in the summaries of the results. Listings of data and standard descriptive methods will be used to display and summarize the results. Toxicities will be tabulated and reported according to grade, type, cycle, and attribution. Cumulative incidence curves will be used to estimate the proportion of patients who will discontinue therapy for reasons of toxicity or general inability to tolerate the regimen. Response rates will be calculated based on all patients who began treatment; exact 95% confidence intervals will be constructed. Time to progression and overall survival will be displayed with Kaplan-Meier plots.

## 7. Safety/Adverse Event Assessment

Baseline adverse events will be assessed. See *Section 7.2* for Adverse Event monitoring and reporting.

Analyses of safety/ toxicity will be performed for all patients having received at least one dose of study drug. The study will use the CTCAE version 4 for reporting of non-hematologic adverse events (http://ctep.cancer.gov/reporting/ctc.html) and modified criteria for hematologic adverse events *(see Appendix 5).*

Safety assessments will consist of monitoring and reporting AEs and SAEs that are considered related to sEphB4-HSA, decitabine, and azacitidine; all events of death; and any study-specific issue of concern.

## 7.1 Risks Associated with sEphB4-HSA and hypomethylating agent

AEs associated with the study drug, sEphB4-HSA, included hypertension and QTc prolongation.

The most commonly occurring adverse reactions to decitabine include neutropenia, thrombocytopenia, anemia, fatigue, pyrexia, nausea, cough, petechiae, constipation, diarrhea, and hyperglycemia. In azacitidine, the most commonly occurring adverse reactions were nausea, anemia, thrombocytopenia, neutropenia, vomiting, pyrexia, diarrhea, injection site erythema (for the subcutaneous route), and constipation.
The two study drugs do not appear to have overlapping toxicity. Toxicities related to myelosuppression will be attributed to hypomethylating agents and toxicities related to hypertension will be attributed to sEphB4-HSA.

A more detailed safety profile for azacitidine and decitabine is provided in the Investigator's Brochures.

## 7.2 Safety Parameters and Definitions

### 7.2.1 Adverse Events

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.

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the patient that emerge during the protocol-specified AE reporting period, including signs or symptoms that were not present prior to the AE reporting period
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations)
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention
- Pre-existing medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period

These non-serious advents should be reported immediately to the USC DSMC for consideration.

Conditions that may be suggestive of an autoimmune disorder, including the following:

- Pneumonitis;
- Hypoxia or dyspnea of Grade ≥3;
- Colitis;
- Endocrinopathies: diabetes mellitus, pancreatitis, or adrenal insufficiency;
- Vasculitis;
- Hepatitis;
- Transaminitis consisting of ALT or AST>3×ULN combined with total bilirubin >2 ×ULN or clinical jaundice, or ALT, or AST>10×ULN;
- Systemic lupus erythematosus;
- Guillain-Barré syndrome;
- Myasthenia gravis;
- Pericardial effusion; and/or
- Skin reactions (e.g., vitiligo, pemphigoid).

Events suggestive of hypersensitivity, cytokine release, influenza−like illness, systemic inflammatory response syndrome, or infusion-reaction syndromes.

### 7.2.2 Serious Adverse Events

A “serious” adverse event 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; or
- 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).

### 7.3 Steps to determine if an adverse event requires expedited reporting

Step 1: Identify the type of adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE v4).

Step 2: Grade the adverse event using the NCI CTCAE v4.

Step 3: Determine whether the adverse event is related to the protocol therapy

Attribution categories are as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely – The AE is unlikely related to the study treatment.
- Unrelated – The AE is clearly NOT related to the study treatment.

Note: 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.

Step 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 known adverse events listed in the agent information section of this protocol;
- the drug package insert;
- the current Investigator's Brochure

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study are collected and reported to the U.S. Food and Drug Administration (FDA), appropriate Institutional Review Boards (IRBs), and VasGene, Inc., in accordance with CFR 312.32 (Investigational New Drug [IND] Safety Reports).

#### 7.3.1 Reporting Requirements for Adverse Events

Expedited Reporting

- The Principal Investigator must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug.
- SAE occurring after consent but before first dose will not require expedited reporting
- Please submit SAE to Vasgene Therapeutics, Inc:
    - Email: Linda@vasgene.com
- The Institutional IRB (US) or videnskabsetisk komite (VEK)/lægemiddelstyrelsen (DK) must be notified of "any unanticipated problems involving risk to subjects or others" in accordance with the Institutional policy. Such policies will be provided to the USC Clinical Investigation Support Office (CISO) QA prior to enrolling 1st patient. (for USC refer to HSPP Policies and Procedures chapter 14 available at http://www.usc.edu/admin/oprs/policies/hspp.htmlUPR/UPIRSO).

SAE notification to the FDA:

- USC: study team to work with CISO QA to submit to the FDA using MedWatch 3500A form in accordance within the FDA required timelines

The following events meet the definition of UPR:

- Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures.
- Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
- Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
- Any new information (e.g., publication, safety monitoring report, updated sponsor safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
- Any breach in confidentiality that may involve risk to the subject or others.
- Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.
- The USC NCCC Data and Safety Monitoring Committee (DSMC) must be notified within 24 hours of submission of such reportable event to the IRB. The patient ID and the study number as well as identifier of the SAE report should be submitted to the DSMC Coordinator via email or Fax to the attention of the DSMC Coordinator at 323-865-0089.
- For IND/IDE trials: The FDA should be notified within 7 business days of any unexpected fatal or life-threatening adverse event with possible relationship to study drug, and 15 business days of any event that is considered: 1) serious, 2) unexpected, and 3) at least possibly related to study participation.

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and initiation of study treatment and ends 60 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

### 7.3.2 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. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care.

All patients experiencing an adverse event, regardless of its relationship to study drug, will be monitored 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.

All AEs and SAEs, whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means, will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the study drug (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

- **Yes**

  There is a plausible temporal relationship between the onset of the AE and administration of sEphB4-HSA and the chosen HMA, and the AE cannot be readily explained by the patient's clinical state, inter-current illness, or concomitant therapies; and/or the AE follows a known pattern of response to sEphB4-HSA/HMA; and/or the AE abates or resolves upon discontinuation of sEphB4-HSA/HMA or dose reduction and, if applicable, reappears upon re-challenge.
- **No**

  Evidence exists that the AE has an etiology other than sEphB4-HSA and the chosen HMA (e.g., pre-existing medical condition, underlying disease, inter-current illness, or concomitant medication); and/or the AE has no plausible

temporal relationship to sEphB4-HSA and the chosen HMA administration (e.g., cancer diagnosed 2 days after first dose of study drug).

Expected AEs are those AEs that are listed or characterized in the Package Insert (PI) or current Investigator's Brochure.

Unexpected AEs are those not listed in the PI or current Investigator's Brochure or not identified. This includes AEs for which the specificity or severity is not consistent with the description in the PI or Investigator's Brochure. For example, under this definition, hepatic necrosis would be unexpected if the PI or Investigator's Brochure only referred to elevated hepatic enzymes or hepatitis.

## 7.4 Procedures for Eliciting, Recording, and Reporting Adverse Events

### 7.4.1 Eliciting Adverse Events

A consistent methodology for eliciting AEs at all patient evaluation time-points should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

### 7.4.2 Routine Reporting

All other adverse events- such as those that are expected, or are unlikely or definitely not related to the study participation- are to be reported annually as part of regular data submission. This report will be forwarded to the USC DSMC Coordinator. All toxicities will be included in the IND annual report.

### 7.4.3 Monitoring Rules for Safety

A USC Data Safety Monitoring Board will be notified of all SAEs reported during the course of this trial.

### 7.4.4 Diagnosis versus Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is acceptable to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

### 7.4.5 Deaths

All deaths that occur during the protocol-specified AE reporting period (see *Section 0*), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death." Deaths that occur during the protocol-specified adverse event reporting period (see *Section 0*) that are attributed by the investigator solely to progression of disease should be recorded only in the study CRF.

### 7.4.6 Pre-existing Medical Conditions

A pre-existing medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A pre-existing medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the pre-existing condition has changed by including applicable descriptors (e.g., "more frequent headaches").

### 7.4.7 Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a patient is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a patient is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for pre-existing conditions;
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study; or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

### 7.4.8 Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a patient has completed or discontinued study participation if attributed to prior sEphB4-HSA exposure.

### 7.4.9 Safety Reconciliation

The Sponsor-investigator agrees to conduct reconciliation for the product. Sponsor-investigator will agree to the reconciliation periodicity and format, but agree at minimum to exchange monthly line listings of cases received by the other party. If discrepancies are identified, the Sponsor-investigator a will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

## 7.5 Adverse Event Reporting

Investigators must report all SAEs to USC IRB and the study's DSMB.

### 7.5.1 MedWatch 3500A Reporting Guidelines

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (item 5) of the MedWatch 3500A form:

- Protocol description (and number, if assigned);
- Description of event, severity, treatment, and outcome if known;
- Supportive laboratory results and diagnostics; and
- Investigator's assessment of the relationship of the AE to each investigational product and suspect medication.

### 7.5.2 Follow-Up Information

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up;
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form; and
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e., date of birth, initial, patient number), protocol description and number, if assigned, brief AE description, and notation that additional or follow-up information is being submitted. (The patient identifiers are important so that the new information is added to the correct initial report.)

MedWatch 3500A (Mandatory Reporting) form is available at FDA website (http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/default.htm).

### 7.6 Additional Reporting Requirements for IND

For investigator-sponsored IND studies, some additional reporting requirements for the FDA apply in accordance with the guidance set forth in 21 CFR § 600.80.

Events meeting the following criteria need to be submitted to the FDA as expedited IND Safety Reports according to the following guidance and timelines:

#### 7.6.1 Seven (7) Calendar Day Telephone or Fax Report

The investigator is required to notify the FDA of any fatal or life-threatening AE that is unexpected and assessed by the investigator to be possibly related to the use of sEphB4-HSA. An unexpected AE is one that is not already described in the sEphB4-HSA Investigator's Brochure. Such reports are to be telephoned or faxed to the FDA and VasGene within 7 calendar days of first learning of the event.

#### 7.6.2 Fifteen (15) Calendar Day Written Report

The Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of sEphB4-HSA/HMA.

Written IND Safety reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the investigator with the IND concerning similar events should be analyzed and the significance of the new report in light of the previous, similar reports commented on.

Written IND safety reports with analysis of similar events are to be submitted to the FDA, VasGene, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500 form, but alternative formats are acceptable (e.g., summary letter).

#### 7.6.3 Contact Information for IND Safety Reports

FDA fax number for IND safety reports:

Fax: (800) FDA-0178

**Site's IRB**: USC Health Sciences Institutional Review Board (HSC IRB)

Phone: (323) 223-2340

Fax: (323) 224-8389

Local IRB at each participating site

### 7.6.4 IND Annual Reports

Copies of all IND annual reports submitted to the FDA by the Sponsor-investigator should be sent to VasGene Drug Safety via fax

### 7.7 Study Close-Out

Any study report submitted to the FDA by the Sponsor-investigator should be copied to VasGene. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to VasGene. Copies of such reports should be mailed to the assigned Clinical Operations contact for the study.

### 7.7.1 sEphB4-HSA Protocols

Notify the Norris Cancer CIC.

### 7.7.2 Post-marketing fifteen (15)-Day "Alert Report"

The Sponsor-investigator is required to notify the FDA of any fatal or life-threatening AE that is unexpected and assessed by the investigator to be possibly related to the use of sEphB4-HSA. An unexpected AE is one that is not already described in the Investigator's Brochure. Such reports are to be submitted to the FDA (2 copies) at the following address:

Central Document Room
12229 Wilkins Avenue
Rockville, MD 20852

## 8. STUDY MANAGEMENT

### 8.1 Conflict of interest

All investigators will follow the University conflict of interest policy. Any USC investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must complete a "Statement of Outside Interests Related to Research" Form. The application is reviewed and approved by the Conflict of Interest Review Committee (CIRC) USC conflict of interest policy is available at http://ooc.usc.edu/conflict-interest-research.

Patients who comply with the requirements of the protocol, are tolerating study treatment, and may be receiving benefit will be offered dosing beyond Cycle 1 at the investigator's discretion after a careful assessment and thorough discussion of the potential risks and benefits of continued treatment with the patient. Such patients may have the option to receive sEphB4-HSA + HMA treatment as long as they continue to experience clinical benefit in the opinion of the investigator until the earlier of

unacceptable toxicity, symptomatic deterioration attributed to disease progression, or any of the other reasons for treatment discontinuation listed in *Section 4.6.*

## 8.2 Patient Informed Consent Process

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 a dated IRB approved consent form.

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. Patients enrolling at USC will receive the *California Experimental Research Subject's Bill of Rights*.

At the time of registration, signed and dated copies of the patient Informed Consent document with the *California Experimental Research Subject's Bill of Rights (California patients only)* and the HIPAA authorization must be given to the patient. Institutional policy regarding distribution and location of original consent documents should be followed. When a study is opened at two or more institutions, a copy of the signed consent and HIPAA should be sent to USC Clinical Investigation Support Office (CISO) QA team as soon as possible, and not later than within 5 business days of obtaining consent. For patients consented at USC/LAC, institutional policy should be followed: a copy of ICF and HIPAA should be uploaded through True to USC CRO and to CISO QA Team. The original will be kept in the patient research chart maintained by the study assigned Data Manager.

The informed consent document must be signed by the subject or the subject's legally authorized representative before his or her participation in the study. The case history for each subject shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent document must be provided to the subject or the subject's legally authorized representative. If applicable, it will be provided in a certified translation of the local language.

Signed consent forms must remain in each subject's study file and must be available for verification by study monitors at any time.

### 8.2.1 Registration Eligibility Worksheet

At the time of registration, the completed Eligibility Worksheet will be submitted to the QA Monitor at CISO for review of eligibility compliance.

## 8.3 Institutional Review Board (IRB) Approval

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB for review and must be approved before the study is initiated. The study will be conducted in accordance with FDA, applicable national and local health authorities, and IRB requirements.

The Principal Investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case, the IRB must be updated at least once a year. The Principal Investigator must also keep the IRB informed of any significant AEs.

Investigators are required to promptly notify their respective IRB of all adverse drug reactions that are both serious and unexpected. This generally refers to SAEs that are not already identified in the Investigator's Brochure and that are considered possibly or probably related to the molecule or study drug by the investigator. Some IRBs may have other specific AE requirements to which investigators are expected to adhere. Investigators must immediately forward to their IRB any written safety report or update provided by Vasgene (e.g., IND safety report, Investigator's Brochure, safety amendments and updates, etc.).

## 8.4 Registration Procedures

### 8.4.1 USC Registration

For patients enrolled at USC, the Research Coordinator must complete the protocol eligibility form to ensure that the patient is eligible. The PI will review the patient eligibility (with assistance from the Research Coordinator- who will assemble the required source documents, and do an initial review) prior to registering the patient on study.

The Research Coordinator or data manager will then register the patient into the Cancer Center database, Café, by accessing the Registration forms. Likewise, after the patient has completed the study, the Off Study forms in cafe will need to be completed, for Off Treatment and Off Study.

## 8.5 Records and data submission

### 8.5.1 Confidentiality of records

The original data collection forms will be kept in secure file cabinets, for USC patients forms will be kept in the Clinical Investigations Support Office (CISO).

Patient medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the ICF (or separate authorization to use and disclose personal health information) signed by the patient or unless permitted or required by law.

Medical information may be given to a patient's personal physician or other appropriate medical personnel responsible for the patient's welfare for treatment purposes.

Data generated by this study must be available for inspection upon request by representatives of the FDA and other regulatory agencies, national and local health authorities, VasGene representatives and collaborators, and the IRB/Ethics Committee (EC) for each study site, if appropriate.

## 8.6 Obligations of Investigators

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 Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator 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 and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

This clinical research study will be monitored both internally by the PI and externally by the USC IRB. In terms of internal review, the PI will continuously monitor and tabulate AEs. Appropriate reporting to the USC IRB will be made. The PI of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

- Interim analyses occur as scheduled,
- Stopping rules for toxicity and/or response are met,
- Risk/benefit ratio is not altered to the detriment of the subjects,
- Appropriate internal monitoring of AEs and outcomes is done,
- Over-accrual does not occur,
- Under-accrual is addressed with appropriate amendments or actions, and
- Data are being appropriately collected in a reasonably timely manner.

Routine monitoring will be carried out via a periodic team conference among investigators during which toxicity data, including all SAEs, will be reviewed and other issues relevant to the study such as interim assessment of accrual, outcome, and

compliance with study guidelines, will be discussed. Monitoring will be carried out on an ongoing basis. The severity, relatedness, and whether or not the event is expected will be reviewed.

## 8.7 Study Medication Accountability

Recipient of study drug will acknowledge receipt of the drug by returning the INDRR-1 form indicating shipment content and condition. Damaged supplies will be replaced.

Accurate records of all study drug dispensed from and returned to the study site should be recorded by using the institution's drug inventory log or the National Cancer Institute drug accountability log.

All partially used or empty containers should be disposed of at the study site according to institutional standard operating procedure. Return unopened, expired, or unused study drug with the Inventory of Returned Clinical Material form as directed by VasGene. Data Collection forms and submission schedule

- If a treatment trial, protocol data will be entered into eCRFs in MediData;
- Within two weeks of registration, the data manager will complete the initial set of On Study forms and baseline Toxicities;
- Within two weeks of completion of each course of treatment, the data manager must complete the Course Assessment, Toxicities, and if appropriate Response data; and
- After Off Treatment, within two weeks of each follow up, complete the Follow Up forms.

The study coordinator and investigators are responsible for ensuring that the eligibility checklist is completed in a legible and timely manner for every patient enrolled in the study, and that data are recorded on the appropriate forms and in a timely manner. Any errors on source data should be lined through, but not obliterated, with the correction inserted, initialed, and dated by the study coordinator or PI. All source documents will be available for inspection by the FDA and the USC Health Sciences IRB.

## 8.8 Data Management and Monitoring/Auditing

### 8.8.1 Active Monitoring Program details

a. **Adherence to Protocol/Per Patient:** It is the responsibility of the USC Principal Investigator (PI) to ensure that patient recruitment and enrollment, treatment, follow-up for toxicities and response, and documentation and reporting at USC are all performed as specified in the protocol. When a study is opened at two or more institutions, the PI at each institution will assume the responsibilities for the day-to-day monitoring of the trial, as described below.

b. **Day-to-Day Monitoring – Eligibility**: At USC, the Study Coordinator will assist the Investigator in reviewing eligibility and will assemble the required source

documents, and do a final review by completing an Eligibility Registration Worksheet. When a study is opened at two or more institutions, the PI at each institution will review the patient eligibility in accordance with that institution's policy. For all institutions, the Eligibility Registration Worksheet with a copy of Informed Consent and supporting source

c. **Day-to-Day Monitoring – Informed Consent**: Prior to registering the patient on study, the Study Coordinator will review the informed consent, to ensure that the patient has signed and dated the most current IRB-approved form, and that the form has been signed and dated by the person obtaining the consent as well as appropriate witnesses. A copy of the ICF will also be provided to CISO QA for review. CISO SOP 3.3 will be followed.

d. **Day-to-Day Monitoring – Treatment**: The PI and co-investigators are responsible for ensuring that treatment is given per protocol. The Study Coordinator will review the treatment orders with the treating investigator. Regardless of who the treating physician is, there will be only one responsible Study Coordinator for each study at each of the hospitals affiliated with the USC Norris Cancer Center. The treating investigator will review the status of each patient on-study, with the Study Coordinator and treating physicians, on an on-going basis. When a study is opened at two or more institutions, CISO QA will periodically audit medical records for the subjects on study at other institutions to ensure compliance and adherence to the protocol.

e. **Data Management – Patient Charts**: When a study is opened at two or more institutions, the policy in place at each institution will be followed for maintaining medical and research related records. Such policies will be provided to the CISO QA prior to enrolling 1st patient. At USC, All written source documents not associated with the study research are maintained in the patient chart, which is stored in the Department of Medical Records at the appropriate hospital. At the Norris Hospital, the official medical record is the Electronic Patient File (EPF). Radiographical images are stored in the Department of Radiology and in an electronic system called Synapse. At Los Angeles County General Hospital the official medical record is called Affinity. These are the permanent, official documents for each patient on-study. A copy of the signed informed consent, physician's notes, orders, test results and pathology notes are maintained in the patients' hospital charts. It is the responsibility of the research staff to ensure that the patient chart contains the required documents and work closely with treating investigators to ensure all protocol-related assessments are carefully documented.

f. **Data Management – Research Charts**: When a study is opened at two or more institutions, the policy in place at each institution will be followed for maintaining medical and research related records. Such policies will be provided to the CISO QA prior to enrolling 1st patient. At USC, to facilitate adherence to the protocol schedule and data management, research charts are created to collect copies of the relevant notes, orders and results, that are in the Patient Chart. In Addition, all source documents related to the research, such as original informed consent

forms, HIPAA Forms, AE assessment worksheets, disease response worksheets and NTFs are maintained in the Research Charts. Protocol calendars, worksheets, and checklists, are also kept in the research chart. These are maintained in the Clinical Investigation Support Office until the study is completed and the results are published and no further need is anticipated. These are then stored off-site. It is the responsibility of the Data Manager to ensure that the research chart contains all the required documents.

g. **Data Management – Case Report Forms:** It is the responsibility of the Data Manager to complete the required case report forms. For in-house trials, case report forms are developed for each trial; these are used to finalize the data entry screens in the Cancer Center clinical trials database. It is the responsibility of the PI to review the Off-Study Summary form which summarizes pertinent toxicity, response and adherence information, once the patient has completed treatment.

## 8.9 Quality Assurance Monitoring Committee (QAMC) Oversight

The Quality Assurance and Monitoring Committee (QAMC) of the NCCC has the responsibility for study auditing and monitoring for protocol compliance, data accuracy, performance of audits and monitoring of accrual. QAMC procedures are detailed in the NCCC Data Safety and Monitoring Plan available on CISO Website.

## 8.10 QAMC Annual Patient Audits

The QAMC is responsible for conducting audits and providing the initial review of the audits, for all open institutional (i.e. USC initiated), CCCP-sponsored trials, and any trials identified by the CIC. These trials are audited by the QAMC once a year. Faculty and staff at the Cancer Center involved in clinical research – but not directly involved in the research under evaluation – are asked to serve as auditors. Twenty percent of patients accrued during the past 12 months – and a minimum of 2 patients – are selected at random; however, additional patients may be selected for audit if there is some indication that there might have been a problem or unusual circumstance (possibly related to compliance, toxicity, response or some indication of an irregularity). The audit involves a review of the research chart, hospital medical record (i.e., source documentation) and evaluates the following: documentation of eligibility (including failure to obtain appropriate informed consent) and baseline status of the patient; documentation of adherence to protocol-specified treatment and follow-up; evaluation of toxicity; and evaluation of response or other outcome. In addition, for investigative agents, a drug audit is also performed for these patients by the Research Pharmacist. In addition, for Institutional, Investigator Initiated Trials, Data in the CAFÉ database are compared to the information in the medical record.

## 8.11 QAMC Annual Protocol Review

All open trials are reviewed at least once a year by the QAMC (or more often if stipulated by the CIC). This annual review includes the following: evaluation of the current accrual relative to the planned total accrual; examination of gender and minority accrual; examination of all reported violations; review of past audits and

correspondence with the PI; review of results of current audit (by an outside agency or by the NCCC QAMC); review of previous correspondence between the PI and the QAMC/DSMC. The QAMC review process is detailed in USC NCCC DSM Plan available on the CISO website.

### 8.12 Data and Safety Monitoring Committee (DSMC) Oversight

The Data and Safety Monitoring Committee (DSMC) is an independent body responsible for the safety of study subjects through the review of new protocols to ensure an adequate adverse event assessment/reporting plan, study stopping rules and through the real-time and periodic monitoring of severe adverse events (SAEs) or those AEs that require expedited reporting. The DSMC performs quarterly and annual safety reviews as well as interim efficacy/futility analyses on institutional trials. DSMC procedures are detailed in USC NCCC DSM Plan available on the CISO website.

### 8.13 Phase I Committee Oversight (for Phase I or I/II IIT only)

The USC Norris Comprehensive Cancer Center Phase I DLT committee reviews all open institutional phase I studies at regularly scheduled intervals. The committee reviews the adverse events, serious adverse events, and treatment administration for each patient during the DLT observation period as specified per protocol. The committee will determine whether a patient is evaluable for DLT and whether an AE meets the DLT definition or not. Decisions regarding dose escalation, de-escalation and cohort expansion are made by the committee in coordination with the PI.

### 8.14 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.

### 8.15 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, an IRB modification form must be completed within five (5) business days of making the change.

### 8.16 Non-Emergency departures from protocol

A protocol deviation is any variance from an IRB approved protocol.

If the deviation meets all of the following criteria, it is considered a minor protocol deviation that:

- Is generally noted or recognized only after it occurs;
- Has no substantive effect on the risks to research participants;

- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected; or
- Did not result from willful or knowing misconduct on the part of the investigator(s).

If the deviation meets any of the following criteria, it is considered a protocol violation:

- Has harmed or increased the risk of harm to one or more research participants;
- 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); or
- Demonstrates serious noncompliance with federal regulations, State laws, or University policies.

**Protocol Deviations:** personnel will report to any sponsor or data and safety monitoring committee in accordance with their policies.

**Protocol Violations:** All protocol violations will be entered in the clinical trial database by the Research Coordinator. In addition, Research Coordinator and Investigator should report all protocol violations within one (1) week of the knowledge of the event using iStar.

## 8.17 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB as well as to all the sponsoring agencies (FDA, NCI, etc.) for review and for approval prior to implementation. It is the responsibility of the study PI to ensure that the appropriate agencies have been informed of the proposed amendments and that these have been reviewed and approved.

## 8.18 Retention of Records

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an

International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

FDA regulations (21 CFR §312.62[c]) and the ICH Guideline for GCP (see *Section 4.9* of the guideline) require that records and documents pertaining to the conduct of clinical trials and the distribution of investigational drug, patient records, consent forms, laboratory test results, and medication inventory records, must be retained for 2 years after the last marketing application approval in an ICH region or after at least 2 years have elapsed since formal discontinuation of clinical development of the investigational product. All state and local laws for retention of records also apply.

For studies conducted outside the U.S. under a U.S. IND, the Principal Investigator must comply with the record retention requirements set forth in the FDA IND regulations and the relevant national and local health authorities, whichever is longer.

## 9. Correlative Studies

### 9.1 Blood/Bone Marrow Collection for measurement of EphB4 on leukemia blasts

Bone marrow aspirate and peripheral blood specimen (where circulating blasts are present) will be evaluated for expression of EphB4 and pAKT levels by flow cytometry. Measurements will be performed at baseline and during treatment per the schedule outlined below. Clinical responses to therapy will be correlated with baseline EphB4 expression.

Bone Marrow:

| TIME POINT | TUBES TO BE DRAWN |
|---|---|
| Screening | 1 EDTA tube (3 ml) |
| Cycle 3 Day 1 | 1 EDTA tube (3 ml) |
| Any unscheduled bone marrow biopsy performed for clinical reasons (eg. suspicion of progression) | 1 EDTA tube (3 ml) |

Peripheral Blood:

| TIME POINT | TUBES TO BE DRAWN |
|---|---|
| Cycle 1: day 1, pre-treatment (within 2 hr) | 1 EDTA tubes (7.5 ml) |
| Cycle 1: day 2 and day 5, when circulating blasts (>500 blasts/dl) are present | 1 EDTA tubes (7.5 ml) |
| Cycle 1: Day 15, pre-treatment (within 2 hr) | 1 EDTA tubes (7.5 ml) |
| Cycle 2: day 1, pre-treatment (within 2 hr) | 1 EDTA tubes (7.5 ml) |
| All subsequent cycles: day 1, pre-treatment (within 2 hr) | 1 EDTA tubes (7.5 ml) |

### 9.2 Collection of unstained bone marrow biopsy slides

A second paraffin embedded bone marrow core or 10 unstained slides (4 microns each) should be collected from all bone marrow biopsies.

Each specimen should be labeled using a Sharpie pen with the following information:

Protocol #:

CISO Patient #

Study Cycle #/Study Day #

Date and time of collection

### 9.3 Blood collection for immunophenotyping of immune response:

| **time point** | **tubes to be drawn** |
|---|---|
| cycle 1: day 1, pre-treatment (within 2 hr) | 2 EDTA tubes (15 ml) |
| cycle 1: Day 15, pre-treatment (within 2 hr) | 2 EDTA tubes (15 ml) |
| cycle 2: day 1, pre-treatment (within 2 hr) | 2 EDTA tubes (15 ml) |
| all subsequent cycles: day 1, pre-treatment (within 2 hr) | 2 EDTA tubes (15 ml) |

1. Bone Marrow aspirate should be placed in ETDA tube immediately to prevent clotting

2. Blood samples should be collected using standard venipuncture technique. Do not centrifuge the tubes. If brief storage is required, please store at room temperature out of direct sunlight.

3. Label each tube with the patient ID#, patient initials, date and time of collection, and protocol time point collected.

4. After collection, the tubes of venous blood should be sent to Dr. Merchant's lab. SPECIMENS MUST BE DRAWN AND SENT TO THE LAB WITHIN 30 MINTUES OF BEING DRAWN.

**Supplies & Contacts:**

NOTE: Ms. Lisa Harton, Dr. Noah Merin, or Dr. Akil Merchant should be notified at least 24 hours in advance via email or phone prior to an upcoming collection.

**Merchant Lab:**

Contact person: Lisa Harton

Phone: 323-442-7828

Address:

1450 Biggy Street, Norris Research Tower 3509

USC/Norris cancer center

Los Angeles, CA 90033

Backup contact: Akil Merchant

akil.merchant@med.usc.edu

Phone: 832-524-5152

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## APPENDIX 1 – MYELODYSPLASTIC SYNDROME*: INTERNATIONAL WORKING GROUP (IWG) MODIFIED RESPONSE CRITERIA

The IWG criteria define 4 aspects of response based on treatment goals: (1) altering the natural history of disease, (2) cytogenetic response, (3) hematological improvement (HI), and (4) quality of life.

Proposed modified IWG response criteria for altering natural history of MDS

| Category | Response criteria (response must last at least 4 weeks) |
|---|---|
| Complete remission | Bone marrow $\leqslant$ 5% myeloblasts with normal maturation of all cell lines<br>Persistent dysplasia will be noted<br>Peripheral blood:<br>Hb $\geqslant$ 110 g/l,<br>Platelets $\geqslant$ 100 x$10^9$/L,<br>Neutrophils $\geqslant$ 1.0 x$10^9$/L<br>Blasts 0%. |
| Partial remission | All CR criteria if abnormal before treatment except:<br>Bone marrow blasts decreased by $\geqslant$ 50% over pre-treatment but still > 5%<br>Cellularity and morphology not relevant |
| Marrow CR | BM $\leqslant$5% myeloblasts and decrease by $\geqslant$ 50% over pre-treatment<br>Peripheral blood: if HI responses, they will be noted in addition to marrow CR |
| Stable disease | Failure to achieve at least PR, but no evidence of progression for > 8 wks |
| Failure | Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of BM blasts, or progression to a more |

| | |
|---|---|
| | advanced MDS subtype than pretreatment |
| Relapse after CR or PR | At least one of the following:<br>Return to pretreatment BM blast percentage<br>Decrement of ⩾ 50% from maximum remission/response levels in granulocytes or platelets<br>Reduction in Hb concentration by ⩾ 15 g/L or transfusion dependence |
| Cytogenetic response | Complete: Disappearance of the chromosomal abnormality without new ones<br>Partial: At least 50% reduction of the chromosomal abnormality |
| Disease progression | ⩾ 50% increase in blasts<br>Any of the following:<br>At least 50% decrement from maximum remission/ response in granulocytes or platelets<br>Reduction of Hb by ⩾ 20g/L<br>Transfusion dependence |
| Survival | Endpoints:<br>Overall: death from any cause<br>Event free: failure or death from any cause<br>PFS: disease progression or death from MDS<br>DFS: time to relapse<br>Cause-specific death: death related to MDS |

Proposed modified IWG response criteria for haematological improvement:

| Hematological improvement | Response criteria (response must last at least 8 weeks) |
|---|---|
| Erythroid response (pre-treatment<110 g/L) | Hb increase by $\geqslant$ 15g/L<br>Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for Hb $\leqslant$ 90g/L pre-treatment will count in the RBC transfusion evaluation |
| Platelet response (pre-treatment<100 $x10^9$/L) | Absolute increase of $\geqslant$ 30 x $10^9$/L for patients starting with > 20 x $10^9$/L<br>Increase from < 20 x $10^9$/L to > 20 x $10^9$/L and by at least 100% |
| Neutrophil response (pre-treatment<1.0 $x10^9$/L) | At least 100% increase and an absolute increase > 0.5 x $10^9$/L |
| Progression or relapse after HI | At least 1 of the following:<br>At least 50% decrement from maximum response levels in granulocytes or platelets<br>Reduction in Hb by $\geqslant$ 15g/L<br>Transfusion dependence |

## APPENDIX 2: MODIFIED 2003 INTERNATIONAL WORKING GROUP (IWG) ACUTE MYELOID LEUKEMIA RESPONSE CRITERIA

| Response[a] | Peripheral Blood (PB) | Bone Marrow (BM) |
|---|---|---|
| CR | ANC ≥1000/μL, Platelets ≥100,000/μL, independence from RBC and platelet transfusions over the past week, no leukemic blasts[b] | <5% leukemic blasts |
| CRp | ANC ≥1000/μL, Platelets <100,000/μL, independence from RBC transfusions over the past week, no leukemic blasts[b] | <5% leukemic blasts |
| CRi | ANC <1000/μL, no leukemic blasts[b] | <5% leukemic blasts |
| Partial response | ANC ≥1000/μL, Platelets ≥100,000/μL, no leukemic blasts[b] | Decrease of ≥50% in leukemic blasts to level of 5% to 25% |

[a] Responses are based on both PB and BM conditions.

[b] For the purpose of response assessment and according to published IWG criteria, blasts may be seen in PB as rare PB blasts may be identified during regeneration, but the subject is in CR if BM blasts are <5% with no Auer rods (Cheson et al 2003).

ANC=absolute neutrophil count; CR=complete response; CRp=complete response with incomplete platelet recovery; CRi=CR with incomplete blood count recovery.

Source: Cheson et al 2003

## APPENDIX 3: Revised IPSS (Greenberg 2012)

### MDS Cytogenetic Scoring System

| Prognostic subgroups, % of patients | Cytogenetic abnormalities | Median survival,* y | Median AML evolution, 25%,* y | Hazard ratios OS/AML* | Hazard ratios OS/AML† |
|---|---|---|---|---|---|
| Very good (4%*/3%†) | −Y, del(11q) | 5.4 | NR | 0.7/0.4 | 0.5/0.5 |
| Good (72%*/66%†) | Normal, del(5q), del(12p), del(20q), double including del(5q) | 4.8 | 9.4 | 1/1 | 1/1 |
| Intermediate (13%*/19%†) | del(7q), +8, +19, i(17q), any other single or double independent clones | 2.7 | 2.5 | 1.5/1.8 | 1.6/2.2 |
| Poor (4%*/5%†) | −7, inv(3)/t(3q)/del(3q), double including −7/del(7q), complex: 3 abnormalities | 1.5 | 1.7 | 2.3/2.3 | 2.6/3.4 |
| Very poor (7%*/7%†) | Complex: > 3 abnormalities | 0.7 | 0.7 | 3.8/3.6 | 4.2/4.9 |

### IPSS-R prognostic score values

| Prognostic variable | 0 | 0.5 | 1 | 1.5 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|
| Cytogenetics | Very good | — | Good | — | Intermediate | Poor | Very poor |
| BM blast, % | ≤ 2 | — | > 2%- < 5% | — | 5%-10% | > 10% | — |
| Hemoglobin | ≥ 10 | — | 8- < 10 | < 8 | — | — | — |
| Platelets | ≥ 100 | 50-< 100 | < 50 | — | — | — | — |
| ANC | ≥ 0.8 | < 0.8 | — | — | — | — | — |

— indicates not applicable.

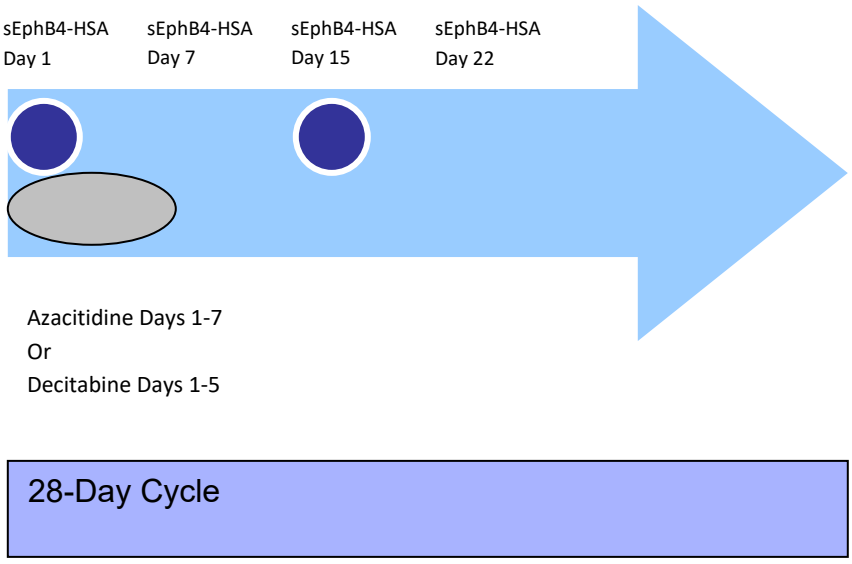
## IPSS-R prognostic risk categories/scores

| Risk category | Risk score |
|---|---|
| Very low | ≤ 1.5 |
| Low | > 1.5-3 |
| Intermediate | > 3-4.5 |
| High | > 4.5-6 |
| Very high | > 6 |

## IPSS-R prognostic risk category clinical outcomes

| | No. of patients | Very low | Low | Intermediate | High | Very high |
|---|---|---|---|---|---|---|
| Patients, % | 7012 | 19 | 38 | 20 | 13 | 10 |
| Survival, all* | | 8.8 | 5.3 | 3.0 | 1.6 | 0.8 |
| | | (7.8-9.9) | (5.1-5.7) | (2.7-3.3) | (1.5-1.7) | (0.7-0.8) |
| Hazard ratio | | 0.5 | 1.0 | 2.0 | 3.2 | 8.0 |
| (95% CI) | | (0.46-0.59) | (0.93-1.1) | (1.8-2.1) | (2.9-3.5) | (7.2-8.8) |
| Patients, % | 6485 | 19 | 37 | 20 | 13 | 11 |
| AML/25%*† | | NR | 10.8 | 3.2 | 1.4 | 0.73 |
| | | (14.5-NR) | (9.2-NR) | (2.8-4.4) | (1.1-1.7) | (0.7-0.9) |
| Hazard ratio | | 0.5 | 1.0 | 3.0 | 6.2 | 12.7 |
| (95% CI) | | (0.4-0.6) | (0.9-1.2) | (2.7-3.5) | (5.4-7.2) | (10.6-15.2) |

## APPENDIX 4- STUDY SCHEMA: PHASE II



## APPENDIX 5 – CURRENT NATIONAL CANCER INSTITUTE COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS (NCI CTCAE)

Please use the following link to the NCI CTCAE website:

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

## APPENDIX 6 – EASTERN COOPERATIVE ONCOLOGY GROUP (ECOG) PERFORMANCE STATUS SCALE

| Grade | Description |
|---|---|
| 0 | Fully active, able to carry on all pre-disease performance without restriction |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework or office work |
| 2 | Ambulatory and capable of all self-care but unable to carry out any work activities; up and about $>$ 50% of waking hours |
| 3 | Capable of only limited self-care, confined to a bed or chair $>$ 50% of waking hours |
| 4 | Completely disabled; cannot carry on any self-care; totally confined to bed or chair |
| 5 | Dead |

## APPENDIX 7 – ANAPHYLAXIS PRECAUTIONS

### EQUIPMENT NEEDED

Tourniquet;

Oxygen;

Epinephrine for subcutaneous, intravenous, and/or endotracheal use in accordance with standard practice;

Antihistamines;

Corticosteroids; and

Intravenous infusion solutions, tubing, catheters, and tape.

### PROCEDURES

In the event of a suspected anaphylactic reaction during study drug infusion, the following procedures should be performed:

1. Stop the study drug infusion.
2. Apply a tourniquet proximal to the injection site to slow systemic absorption of study drug. Do not obstruct arterial flow in the limb.
3. Maintain an adequate airway.
4. Administer antihistamines, epinephrine, or other medications as required by patient status and directed by the physician in charge.
5. Continue to observe the patient and document observation.

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## APPENDIX 8 – FDA MEDWATCH 3500A FORM

http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM163919.pdf

## APPENDIX 9: Description of Correlative Studies

**Measurement of EphB4 expression at study entry and during therapy with sEphB4-HSA.**

EphB4 expression is highly variable on the surface of leukemic blasts. Our preclinical data suggest that response to sEphB4-HSA requires surface expression of EphB4, but the level of EphB4 expression does not seem to correlate with response. Using our xenograft model we have shown that residual blasts after treatment with sEphB4-HSA have lower levels of EphB4 expression. (Fig. 1) This appears to be both a marker of the efficacy of sEphB4-HSA as well as a potential mechanism of therapeutic resistance. We will measure the expression of EphB4 on the surface of leukemia blasts using flow cytometry at study entry and various points during the study and correlate this with treatment response. If response is limited to patients with a certain level of EphB4 expression, this can be used as a biomarker for selection of patients in subsequent studies.

**Measurement of intracellular signaling activity (pAKT/pS6) during therapy with sEphB4-HSA**. We have shown that EphB4 signaling activity is dependent on activation of pAKT and the treatment with sEphB4-HSA leads to decreases in intracellular pAKT levels. We will profile leukemia blasts for their pAKT and pS6 levels before and during treatment, including MRD cells, and correlate these with patient responses. Unlike traditional methods of western blotting, our method of flow cytometric measurement of intracellular signaling pathways allow us to look at signaling in rare populations (up to 0.01%) of residual leukemia. Such measures will serve as pharmacodynamic measures of sEphB4-HSA activity and suggest mechanisms of resistance.

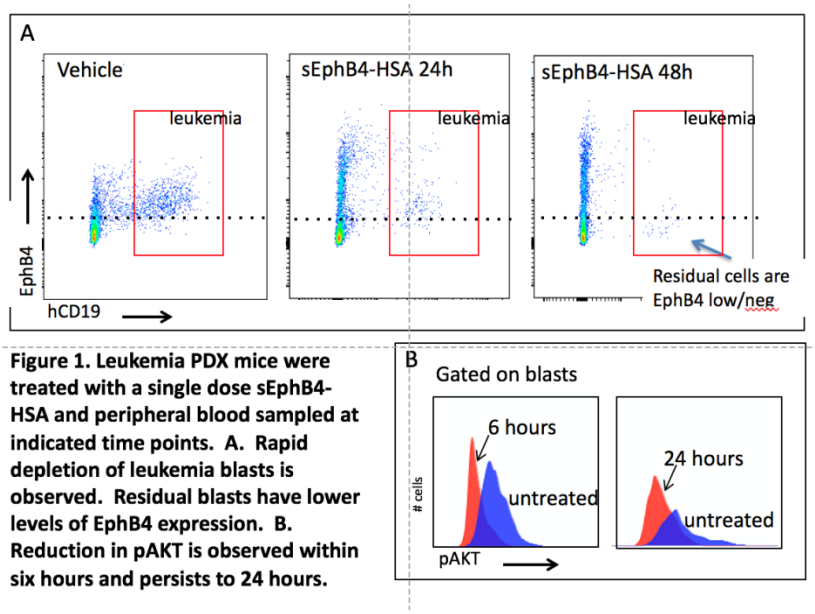


**Figure 1. Leukemia PDX mice were treated with a single dose sEphB4-HSA and peripheral blood sampled at indicated time points. A. Rapid depletion of leukemia blasts is observed. Residual blasts have lower levels of EphB4 expression. B. Reduction in pAKT is observed within six hours and persists to 24 hours.**

**Profiling of patient immune responses to sEphB4-HSA by assessing induced T regulatory cells, exhausted T cells, and myeloid-derived suppressor cell populations before and after treatment, and correlation with clinical outcome.**

We will perform immune monitoring using multi-parameter flow cytometry antibody panels that we have developed to characterize the phenotypic and functional status of patient immune cell subsets. Peripheral blood will be collected and be diluted 1:1 with PBS before separation of peripheral blood mononuclear cells (PBMCs) by density gradient centrifugation. Cells will be frozen and stored in liquid nitrogen. When assays are performed, frozen cells will be thawed and washed in staining buffer. Cells will be then incubated with directly conjugated monoclonal antibodies for markers of T cell differentiation, NK cell activation, T regulatory cells, and myeloid-derived suppressor cells. Flow cytometry will be performed in Dr. Akil Merchant's lab on a BD FACSVerse with subsequent analysis using FlowJo Version 10.1 software. Analysis will be performed after gating on live singlet cells. Correlation between changes in immune cell subsets and response to therapy will be analyzed.

Regulatory T cells are a subset of CD4+ T cells that suppress immune responses and are believed to play a role in autoimmunity, cancer immune-evasion, stem cell transplantation, GHVD, and graft acceptance. Induced T regulatory cells (iTregs) expand in response to tumor antigens and a presumably responsible for the suppression of anti-tumor immune responses.

T cell exhaustion is a state of T cell dysfunction developed during chronic exposure to antigen. Exhausted CD8+ T cells lose capacity to produce activating cytokines, proliferate and kill, and eventually undergo apoptosis and deletion. Signaling via inhibitory surface receptors including PD-1, 2B4, TIM-3 and CD160 play key roles in T cell exhaustion.

Myeloid-derived suppressor cells (MDSC) are abnormal myeloid cells that inhibit lymphocyte proliferation, and cytotoxic T lymphocyte induction and activity. These cells lack membrane markers for mature T cells, B cells, NK cells, or macrophages. Studies have revealed that circulating MDSC numbers correlate with a poor prognosis and tumor evasion of immune responses.

Measurement of EphB4, EphrinB2, PD1, PDL-1 and infiltrating immune cells on bone marrow biopsy specimens.

**Bone Marrow Biopsy**

Bone marrow biopsy slides will be obtained and evaluated for EphB4, ephrinB2, PDL-1 expression on blasts. Characterization of stroma for ephrinB2 and infiltrating immune cells will be performed.

Appendix 10

# SERIOUS ADVERSE EVENT
# E-MAIL COVER SHEET

*Complete and email Medwatch 3500A within 24 hours of learning of a new SAE*

To: Vasgene Therapeutics Inc.
Email: Linda@vasgene.com

**USC Principal Investigator:** **Casey O'Connell, MD**

Phone: 323-865-3944
Fax: 323-865-0061
Email: OConnel_C@med.usc.edu

**DSMC Coordinator:**

**Grace Kim**
Phone: 323-865-3122
Fax: 323-865-0089
Email: grace.kim@med.usc.edu

**PROTOCOL**: 9L-16-6 A pilot/safety study of sEphB4-HSA in combination with a hypomethylating agent (HMA) for patients with relapsed or refractory myelodysplastic syndrome (MDS) and AML previously treated with a hypomethylating agent.

Site Name: Name of Submitter:

Phone #: Date of submission:

**Please Complete:**

Subject #: Subject Initials:

____ NEW SAE

____ FOLLOW- UP TO PREVIOUSLY REPORTED SAE

## APPENDIX 11: Study Calendar: sEphB4-HSA + HMA

| | | **Cycles 1 & 2** | | | | | | | | | **Subsequent Cycles 3+ (patients in PR/CR)** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Screen | C1D1 | C1D8 | C1D15 | C1D22 | C2D1 | C2D8 | C2D15 | C2D22 | | C3D1 | C3D8 | C3D15 | C3D22 | |
| Informed consent | X | | | | | | | | | | | | | | |
| Medical history | X | | | | | | | | | | | | | | |
| ECOG PS | X | X | | | | X | | | | | | | | | |
| Vital signs* | X | X | X | X | X | X | X | X | X | | X | | X | | |
| Weight, Height, BSA calculation | | X | | | | X | | | | | X | | X | | |
| CXR | X | | | | | | | | | | | | | | |
| EKG* | X | X | | X | | X | | X | | | | | | | |
| Pulse oximetry | X | X | | | | X | | | | | X | | | | |
| Prior/Con Meds | X | X | | X | | X | | X | | | X | | X | | |
| RBC & Platelet Transfusion Record | X | X | | X | | X | | X | | | X | | X | | |
| Labs & Monitoring | | | | | | | | | | | | | | | |
| CBC | X | X | X | X | X | X | | X | | | X | | X | | |
| CMP | X | X | X | X | X | X | | X | | | X | | X | | |
| Pregnancy Test | X | X | | | | X | | | | | X | | | | |
| Coags (PT/apt) | X | X | X | X | X | X | | X | | | X | | X | | |
| Amylase & Lipase | X | | | | | | | | | | | | | | |
| Mag, Phos | X | X | X | X | X | X | | X | | | X | | X | | |
| Urinalysis | X | X | X | X | X | X | | X | | | X | | X | | |
| TSH | X | X | | | | | | | | | | | | | |
| Viral hepatitis serologies* | X | X | | | | X | | | | | | | | | |
| BM biopsy w/ slides | X | | | | | | | | | X | | | | | X |
| BM aspirate | X | | | | | | | | | X | | | | | X |
| BM Immunophenotype | X | | | | | | | | | X | | | | | X |
| BM Cytogenetics | X | | | | | | | | | X | | | | | X |

Vital signs*: Temperature, pulse, respiratory rate, blood pressure, oxygen saturation
ECG*: ECG should be performed before and after sEphB4 dosing during cycles 1 and 2; hold therapy if QTc>480s
Viral hepatitis serologies*: Hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antigen, hepatitis C IgG

| | | **Cycle 1** | | | | **Cycle 2** | | | | | **Subsequent Cycles 3+ (patients in PR/CR)** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Screen | C1D1 | C1D8 | C1D15 | C1D22 | C2D1 | C2D8 | C2D15 | C2D22 | | C3D1 | C3D8 | C3D15 | C3D22 | |
| STUDY TREATMENTS | | | | | | | | | | | | | | | |
| sEphB4-HSA | | X | | X | | X | | X | | | X | | X | | |
| Azacitadine | | X (D1-7) | | | | X (D1-7) | | | | | X (D1-7) | | | | |
| Decitabine | | X (D1-5) | | | | X (D1-5) | | | | | X (D1-5) | | | | |
| CORRELATIVES | | | | | | | | | | | | | | | |
| BM aspirate for EphB4, pAKT expression | X | | | | | | | | | X | | | | | X |
| Blood Sample EphB4, pAKT expression | X | X | | | | X | | | | X | | | | | X |
| Blood Sample characterization of circulating immune cells | X | X | | | | X | | | | X | | | | | X |
| Bone Marrow aspirate for characterization of infiltrating immune cells | X | | | | | | | | | X | | | | | X |
| Bone Marrow biopsy for measurement of PD-L1, PD-L2 and infiltrating immune cells | X | | | | | | | | | X | | | | | X |
| ADVERSE EVENT MONITORING | | | | | | | | | | | | | | | |
| Survival | | X | X | X | X | X | X | X | X | | X | X | X | X | X |
| Subsequent Therapies | | | | | | | | | | | | | | | X |
| Transformation to AML (date) | | | | | | | | | | | | | | | X |
| Recurrence of transfusion dependence | | | | | | | | | | | | | | | X |

[*]Final Response Assessment to be completed after C2D15 and prior to C3D1, to include: bone marrow sample for EphB4 expression; blood sample EphB4 expression, blood sample for T cells, bone marrow for T cells, blood samples for PD-L1 & PD-L2, buccal swab

[Π]Patients with circulating blasts (absolute blast count above 500 cell/dl) will have samples collected Day 2 and 5 of cycle 1.