Subarachnoid hemorrhage and soluble epoxide hydrolase inhibition: a phase 1b trial

Protocol Number: SUSHI-1

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and applicable United States (US) Code of Federal Regulations (CFR). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent forms, recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Signed:_____Date:_____

Ross Martini MD, Principal Investigator

LIST OF ABBREVIATIONS

AE	adverse event
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
BUN	blood urea nitrogen
CRF	case report form
CSF	cerebrospinal fluid
CT	computed tomography
DCI	delayed cerebral ischemia
DDI	drug drug interaction
DHET	dihydroxyeicosatrienoic acids
DPOME	dihydroxyoctadec-12-enoic acid
ECG	electrocardiogram
eCRF	electronic case report form
EETs	epoxyeicosatrienoic acids
EF	ejection fraction
EMR	electronic medical record
EPOME	epoxyoctadecenoic acid
EVD	external ventricular drain
FDA	food and drug administration
GCP	good clinical practice
GLP	good laboratory practice
GMP	good manufacturing practice
GOSE	Extended Glasgow Outcome Scale
HDL	high density lipoprotein
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review board
LAR	legally authorized representative
LDL	low density lipoprotein
LV	left ventricle
MCA	middle cerebral artery
MOP	manual of procedures
MR	magnetic resonance
mRS	modified Rankin scale
NSICU	Neurosciences Intensive Care Unit
NSTEMI	non-ST elevation myocardial infarction
OHRP	office for human research protections
OHSU	Oregon Health and Science University
PCP	primary care provider
PI	principal investigator
QC	quality control
SAE	serious adverse event
SAH	subarachnoid hemorrhage
sEH	soluble epoxide hydrolase
SOA	Schedule of activities
SOP	standard operating procedure
STEMI	ST elevation myocardial infarction
UP	unanticipated problem
US	United States

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: **Subarachnoid hemorrhage and soluble epoxide hydrolase inhibition: a phase 1b trial**

Study Description: Soluble epoxide hydrolase (sEH) is the metabolizing enzyme of epoxyeicosatrienoic acids (EETs), which may play a role in reducing neuroinflammation and regulating cerebral blood flow after subarachnoid hemorrhage (SAH). We hypothesize that pharmacologic inhibition of the sEH enzyme is safe and will result in increased EETs availability at the neurovascular unit, and a measured increase in the EET/DHET ratio in the serum and cerebrospinal fluid. This study is a double-blind, placebo-controlled, phase 1b randomized trial to evaluate the safety and of GSK2256294, an inhibitor of soluble epoxide hydrolase, in patients with aneurysmal SAH.

Objectives:

Primary Objective:
Determine the safety of administration of GSK2256294 in patients with aneurysmal SAH.

Secondary Objective:
Determine the pharmacodynamics effect of administration of GSK2256294 in patients with aneurysmal SAH on reducing EETs metabolism and biomarkers of cerebrovascular inflammation and endothelial injury.

Tertiary Objective:
Provide preliminary estimates of clinical endpoints to inform the design of a larger trial

Endpoints:

Primary Endpoints:
Determination of safety

Secondary endpoints:

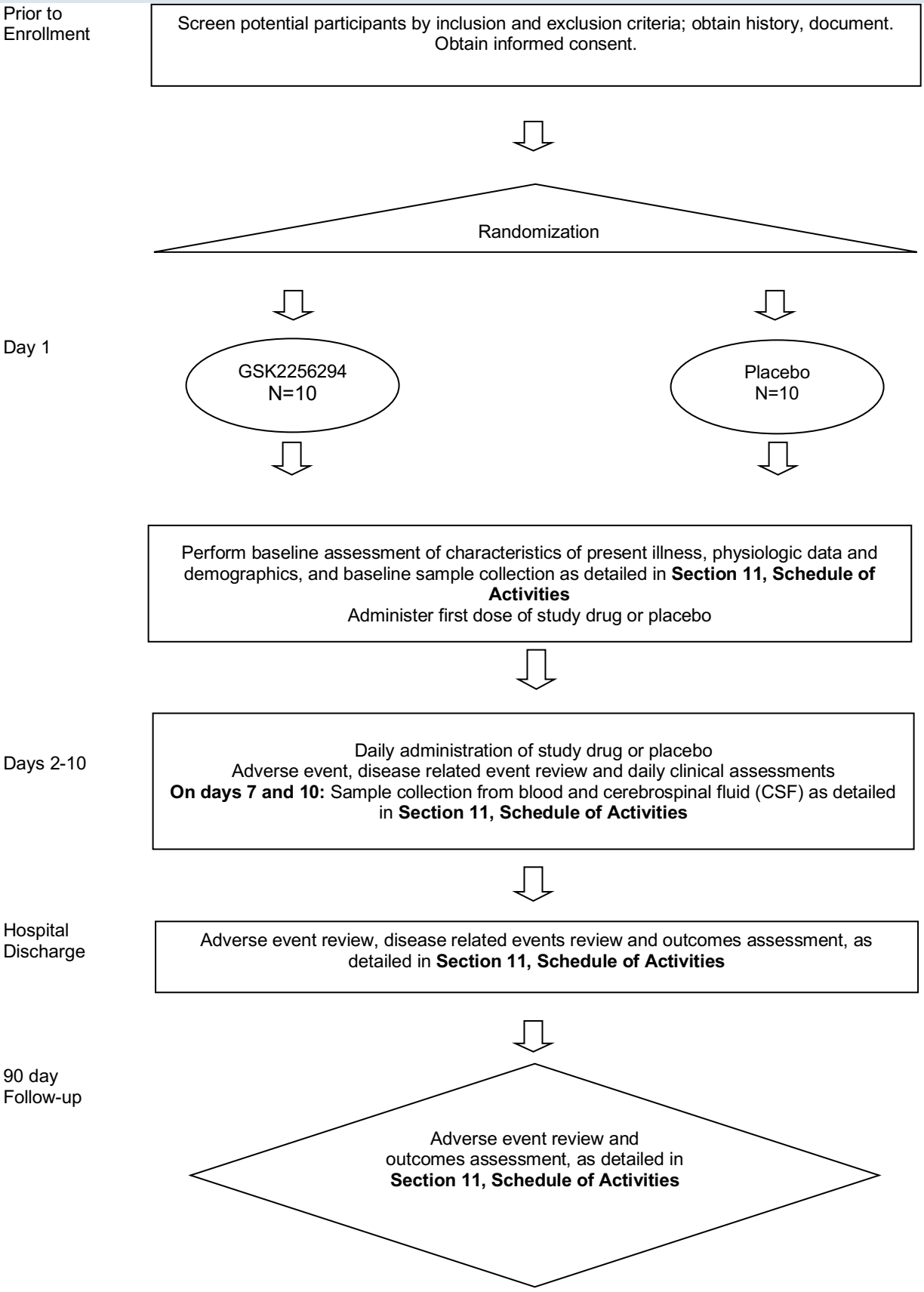
1. Study days 7 and 10 serum EET/DHET ratios
2. Study days 7 and 10 cerebrospinal fluid (CSF) EET/DHET ratios
3. Study days 7 and 10 serum EPOME/DPOME ratios
4. Neuroinflammatory and endothelial injury biomarker levels from the blood and CSF at day 7 and day 10.

Tertiary, exploratory endpoints:
Clinical outcomes associated with SAH including neurologic status, disposition, vital status and incidence of delayed cerebral ischemia.

Up to 30 subjects will be randomized. Patients age 18 or above with confirmed ruptured will be approached to provide written informed consent. In order to be eligible to participate in this study, an individual must meet all of the following criteria: non-contrast CT with evidence of subarachnoid hemorrhage, and digital subtraction cerebral angiography or CT angiogram documenting the presence of

	a cerebral aneurysm. An individual who meets any of the following criteria will be excluded from participation in this study: evidence of prolonged QTc >500 milliseconds, baseline AST and ALT > 2x normal reference values, administration of strong inducers/inhibitors of CYP3A4, symptom onset compatible with SAH of ≥ 3 days prior to admission to Oregon Health and Science University Neurosciences Intensive Care Unit (OHSU NSICU), unable to speak or understand English or Spanish, current pregnancy based on urine HCG positive pregnancy test, preexisting severe neurologic deficit or condition, chronic renal failure requiring dialysis, severe terminal disease with life expectancy <6 months, or refusal of informed consent.
Phase:	Phase 1B
Description of Sites/Facilities Enrolling Participants:	The study will take place at Oregon Health & Science University Hospital, with enrollment of patients admitted to the OHSU NSICU, a part of a comprehensive stroke center certified by the American Heart Association and Joint Commission for Accreditation of Healthcare Organizations, with a catchment area including the state of Oregon, Southwest Washington and Northern California. Approximately 80-100 patients with aneurysmal SAH are admitted each year.
Description of Study Intervention:	Up to 30 patients will be equally randomized to receive once daily either 10 mg dose of GSK2256294 or placebo enterally for a duration of 10 days.
Study Duration:	24 months
Participant Duration:	90 days

1.2 SCHEMA


Prior to Enrollment
Screen potential participants by inclusion and exclusion criteria; obtain history, document. Obtain informed consent.
Randomization
Day 1
GSK2256294 N=10
Placebo N=10
Perform baseline assessment of characteristics of present illness, physiologic data and demographics, and baseline sample collection as detailed in **Section 11, Schedule of Activities**
Administer first dose of study drug or placebo
Days 2-10
Daily administration of study drug or placebo
Adverse event, disease related event review and daily clinical assessments
On days 7 and 10: Sample collection from blood and cerebrospinal fluid (CSF) as detailed in **Section 11, Schedule of Activities**
Hospital Discharge
Adverse event review, disease related events review and outcomes assessment, as detailed in **Section 11, Schedule of Activities**
90 day Follow-up
Adverse event review and outcomes assessment, as detailed in **Section 11, Schedule of Activities**

1.3 KEY ROLES AND CONTACT INFORMATION

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2 INTRODUCTION

2.1 STUDY RATIONALE

SAH due to the rupture of an intracranial aneurysm is a life threatening medical emergency with a mortality of 25-40%. In survivors of the initial bleeding, endothelial injury and blood in the subarachnoid space induce an intense inflammatory response, leading to vessel wall thickening and caliber reduction over the first 10-14 days following the bleed. This inflammatory process may cause delayed cerebral ischemia (DCI). DCI is the clinical result of a complex neurovascular inflammatory response characterized by blood brain barrier breakdown, impaired blood vessel autoregulation, direct inflammatory injury, thrombosis, and cerebral edema. The ultimate result is decreased cerebral blood flow and altered neurovascular communications. The inflammatory and ischemic injury associated with DCI may result in long-term cognitive dysfunction and functional disability in >50% of survivors.

In an effort to prevent and treat DCI, patients with SAH require intensive care unit monitoring of hemodynamics and neurologic function for 10-14 days after the primary hemorrhage. Monitoring of the tone of intracranial blood vessels is routinely carried out daily with transcranial Doppler flow velocities, and cerebral angiography when indicated. Clinical deteriorations that are accompanied by signs of decreased cerebral blood flow are treated with blood pressure augmentation and/or intra-arterial vasodilators, with limited therapeutic effectiveness. The only recommended pharmacological therapy is the prophylactic administration of a 21-day course of the calcium channel blocker nimodipine.[1]] There are currently no other effective therapies to directly target microvascular blood vessel dysregulation or neuroinflammation.

2.2 BACKGROUND

Epoxyeicosatrienoic acids (EETs) are arachidonic acid metabolites that are emerging as important regulators of cerebral blood flow and neuroinflammation after brain injury, including SAH.[2, 3] EETs are synthesized by cytochrome P450 epoxygenases in response to cellular insults including ischemia. The beneficial properties of EETs, which have been demonstrated *in vitro* and *in vivo,* include: vasodilation, promotion of angiogenesis, reduced microvascular thrombosis, inhibition of platelet aggregation, decreased apoptosis, and decreased inflammation. All these processes are disturbed following SAH. EETs have multiple sites of action: on the endothelium where they likely regulate inflammatory response, on vascular smooth muscle where they are involved in regulating vasomotor tone, and more broadly throughout the neurovascular units regulating neurovascular communication.

Multiple biomarkers have been identified that characterize neuroinflammation after SAH, and many have been associated with EETs signaling (Table 1). EETs are primarily metabolized to inactive dihydroxyeicosatrienoic acids (DHETs) by the enzyme soluble epoxide hydrolase (sEH), which is expressed in cerebral endothelial and smooth muscle cells as well as astrocyte cell bodies and foot processes.[4]

Several polymorphisms in the gene responsible for sEH transcription, designated EPHX2, alter the structure and function of the enzyme.[5, 6] One variant conferring increased sEH activity and inactivation of EETs, known as K55R, has been linked to hypertension, ischemic stroke and coronary artery disease.[7-9] In patients with SAH, K55R genotype sEH is associated with

increased mortality and incidence of stroke.[10] Conversely, pharmacologic inhibition of sEH reduces infarct size after transient cerebral ischemia in animal models.[11]

Inhibition of sEH in humans has recently become feasible due to the availability of a drug under development by GlaxoSmithKline, GSK2256294.

Pharmacokinetics and product metabolism
In human and animal studies, the oral bioavailability of GSK2256294 was generally high, indicating good absorption, low plasma clearance and a half-life of 20-30 hours. Steady state was achieved within 6-8 days after daily repeat dosing in humans.[12] GSK2256294 has low to moderate protein binding, high passive permeability, and a moderate volume of distribution. Structural information on GSK2256294 metabolism in humans following both single and repeat oral doses in both healthy volunteers and obese smokers indicated GSK2256294 was the major drug related component in human plasma with biliary and urinary metabolites being formed by either oxidation with or without subsequent glucuronidation and N-demethylation. Similar metabolites were detected in human and nonclinical hepatocytes.

In vitro data indicated limited potential for GSK2256294 to be a perpetrator of cytochrome P450 -mediated drug interactions. Population-based physiologically based pharmacokinetics model simulations, evaluating the risk of an interaction between GSK2256294 and a strong CYP3A4 inhibitor, predicted a moderate increase (3.2 fold) to the mean systemic exposure of GSK2256294 when administered together. Co-administration of strong inhibitors and inducers of CYP3A4 has been recommended to be prohibited.

GSK2256294 inhibited the human breast cancer resistance protein and organic anion transporting polypeptide 1B1 in vitro, suggesting the potential for drug interactions with statins that have their disposition modulated by these transporters. However, a physiologically based pharmacokinetic population based drug-drug interaction modelling simulation between GSK2256294 and sensitive substrates (including rosuvastatin) has predicted the clinical interaction risk to be negligible (<1.05 fold). Hence monitoring of adverse events is recommended with the concomitant use of GSK2256294 with statins.

Toxicology
Administration of GSK2256294 was well tolerated at doses up to 600 mg/kg/day in the rat and 1000 mg/kg/day in the monkey. A dose of 1000 mg/kg/day in the rat was generally less tolerated, with adverse clinical signs of reduced food consumption and body weight loss along with macroscopic and microscopic gastric and renal changes and urinalysis effects. In addition, increased liver weight and hepatocyte hypertrophy at 1000 mg/kg/day were noted in rats, along with associated thyroid follicular cell hypertrophy, effects which are often secondary to drug metabolizing enzyme induction in rats (systemic exposure was also reduced at Days 14 and 28 in rat as compared to Day 1). Minimal to mild atrophy of mammary glands was also seen in male rats at 1000 mg/kg/day. At >100 mg/kg/day in rats, there were increased serum cholesterol concentrations along with elevated high-density lipoprotein (HDL) and low-density lipoprotein (LDL) fractions. These were considered non-adverse. GSK2256294 was negative in a battery of genotoxicity assays.

In reproductive and developmental toxicity studies to date, assessment of male rat fertility in 13 week studies has identified no test article-related effects on male mating, fertility or male mediated fetal developmental toxicity after 2 and 11 weeks of dosing at up to 1000 mg/kg/day.

Human Studies

The pharmacokinetics, pharmacodynamics and safety of GSK2256294 has been evaluated in three Phase I studies: an ascending single and repeat dose study of GSK2256294 in healthy male volunteers and otherwise healthy adult male overweight and moderately obese smokers, a single dose study in healthy young and elderly subjects, and a repeat dose study in healthy volunteers. When administered with food (high fat meal), there was, on average, a 22% increase in AUC of GSK2256294 with similar maximum plasma concentration. The median time to peak concentration was delayed by approximately 2 hours when GSK2256294 was administered with food (3.0 hrs versus 1.0 hr). These differences were not deemed to be clinically significant and GSK2256294 can be administered without any meal restrictions in future clinical studies.

GSK2256294 was eliminated mainly by direct biliary secretion and metabolism with metabolites being excreted in both urine and bile. Renal elimination of unchanged parent represented <5% of the dose. In the single dose cohorts AE reporting was higher following GSK2256294 than placebo, but there was no evidence of increased reporting with increased dose.

Pharmacodynamics and Dose Finding
The inhibitory effect of GSK2256294 on exogenous deuterium-labeled EETs was estimated in an ex vivo assay in healthy volunteers and otherwise healthy overweight to moderately obese smokers following single doses of 2-20mg, and following 14 days of once daily repeat dosing at 6mg and 18mg.[12] The sEH activity was evaluated by the formation of 14,15 dDHET, corrected for non-sEH mediated hydrolysis and subsequently the % inhibition of sEH activity was defined. >95% enzyme inhibition was observed for up to 48 hours following a single 10mg dose. >98% enzyme inhibition was observed to be sustained for up to 48 hours following 14 days of daily administration.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

GSK2256294 has been studied in several clinical cohorts. The most commonly reported AEs in healthy volunteers were contact dermatitis and headache, and in healthy adult male overweight and moderately obese smokers were headache and fatigue. Most AEs were of mild or moderate intensity. In the repeat dose cohorts of healthy adult male overweight and moderately obese smokers, AE reporting was similar in frequency overall following repeat administration of GSK2256294 compared with placebo. The most frequently reported AEs were headache and nasopharyngitis. All AEs were considered to be of mild or moderate severity. There was one AE leading to withdrawal (presyncope) reported in heathy volunteers and one SAE in healthy adult male overweight and moderately obese smokers (nephrolithiasis) reported. Neither of these events were deemed likely to be related to the study drug.

Clinically significant abnormalities in clinical chemistry and hematology parameters were infrequent and most were present at baseline. Following repeat dosing with GSK2256294, there was a statistically significant decrease in HDL compared to baseline. The ratio to placebo of change from baseline in HDL was not statistically significant, though there was a trend for a dose-dependent difference. There were no changes in vital signs of clinical importance. A clinically significant abnormality (nodal rhythm) was reported for one subject with presyncope.

There were no fatal or non-fatal SAEs and no adverse event leading to the withdrawal. Treatment with GSK2256294 did not increase the room air pulmonary artery systolic pressure and did not augment the normal hypoxic pulmonary vasoconstrictive response in healthy subjects. No clinically significant changes in any of the clinical laboratory parameters, electrocardiograms (ECGs) and vital signs were observed.

GSK2256294 has been generally well tolerated in the clinic, although most studies with GSK2256294 have been of relatively short duration and the safety database is not extensive. Due to limited experience in human subjects, there is currently not enough information available about the relationship between administration of GSK2256294 and adverse events to assign causality. It is possible that there are other long-range risk associated with drug administration that have not been identified in previous trials.

No efficacy studies have been conducted to date. There is a potential that administration of the study drug will improve EETs signaling and reduce neuroinflammation and endothelial injury. Patients in the treatment group may experience less vasoconstriction, fewer delayed ischemic complications after SAH, and improved neurologic outcomes.

This study will also involve sample collection from the blood and CSF. Blood samples will be collected whenever possible from indwelling venous or arterial access. In the absence of an available access site, venipuncture will be performed by the bedside RN. CSF samples will be collected by accessing the EVD, which will have been placed as part of the patient's routine medical care for SAH. The EVD will be accessed in the same fashion as for routine, clinically-directed sampling, and wherever possible at the same time. EVD access may very slightly increase the risk of introduction of bacteria and the development of ventriculitis.

2.3.2 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Based on previous phase 1 trials, the adverse event profile and pharmacologic interactions of GSK2256294 are minimal.[12] There is a lack of detailed, long-term event tracking, and this will be the first study of GSK2256294 in a critically ill patient population, so it is possible that there will be unanticipated adverse events. The drug exposure period will be relatively short, in line with previous phase 1 trials, and the monitoring will be significantly more intensive, which limits the additional risk.

In addition, patients admitted to the intensive care unit for the treatment of SAH will routinely undergo numerous blood draws, sampling of CSF and diagnostic procedures as part of the accepted standard of care. Our proposed study will not change the risks inherent to specimen sampling. Blood and CSF samples will be obtained at the same time (whenever feasible) and in the same fashion as routine and standard of care samples are obtained.

Study participation will not influence the clinical care of patients other than as defined in Section 11 within the Schedule of Activities. The potential benefit of reduced neuroinflammation and improved outcome in patients with an otherwise highly morbid, high mortality disease process outweighs these potential risks. Future patients with SAH may be benefited by the knowledge gained.

3 OBJECTIVES AND ENDPOINTS

- **Primary Objective:** Determine the safety of administration of GSK2256294 in patients with aneurysmal SAH.

  Frequencies of AEs and SAEs: We will compare AEs and SAEs between the drug and placebo groups. We will also compare daily safety labs (serum sodium, blood glucose, blood urea nitrogen (BUN), creatinine, hematocrit, peripheral white blood cell count) between the groups. Specific to this patient population, we will also specifically screen for disease related events including seizure, new onset cardiomyopathy, NSTEMI/STEMI, hyponatremia, hospital acquired infection, mechanical ventilation, and thromboembolic events. We will describe the severity of headache (on a daily scale of 0-10.

  Justification: Prior phase 1 studies of GSK2256294 have been in healthy volunteers with limited physiologic monitoring. The most common adverse event was headache. We will assess safety in this critically ill patient population with special focus on common sequelae of subarachnoid hemorrhage.

- **Secondary Objective:** Determine the pharmacodynamic effects of administration of GSK2256294 in patients with aneurysmal SAH.

  To evaluate the effect of enzymatic inhibition on circulating EETs levels, we will measure day 7 and day 10 serum EET/DHET ratios. EETs levels will be measured using liquid chromatography and mass spectroscopy.

  To evaluate the possible effect of enzymatic inhibition on CSF EETs levels we will measure day 7 and day 10 CSF EET/DHET ratios, and compare the drug and placebo groups. We will also measure other lipid metabolic substrates of sEH at days 7 and day 10, specifically serum epoxyoctadecenoic acid (EPOME) and dihydroxyoctadec-12-enoic acid (DPOME), to assess sEH enzyme activity.

  To evaluate more downstream effects on measures of endothelial injury and neuroinflammation, we will measure multiple biomarkers in the blood and CSF at day 7 and day 10 (Table 1). The biomarkers chosen are surrogate endpoints representing pathways of the inflammatory cascade associated with clinical endpoints in SAH.

  Justification: EETs are degraded to inactive byproducts DHETs by the enzyme sEH. sEH activity has previously been measured after exposure to the study drug, and found to reach steady state level of >95% drug inhibition at 7 days. Prior evaluations of drug efficacy have used indirect means to measure drug effectiveness in inhibiting exogenous EETs degradation. We will directly measure *in vivo* EETs levels after drug administration to determine the degree by which sEH inhibition results in measurable increases in systemic EETs. Endogenous EETs have a very short half-life and require rapid sample processing and meticulous storage conditions.

  We have chosen to measure the EET/DHET ratio due to the normal fluctuations of EETs levels in the post-bleed period after SAH. The drug will be deemed effective if we measure higher EET/DHET ratio in the treatment group compared to placebo, i.e. higher EETs suggests decreased metabolism by sEH. It is possible that serum EET/DHET ratio will not be measurably increased. EETs have a very short serum half-life (minutes), and the most plausible site of action is at the neurovascular unit.

To more precisely estimate sEH enzyme activity in plasma, we will also measure the metabolism of more stable lipid substrates of sEH: EPOME, and the metabolic byproduct DPOME. Serum EPOME and DPOME levels have previously been used to measure the hydrolase activity of sEH.[13]

We will also determine whether there are CNS effects of the drug, either via direct measurement of increased EET/DHET ratio in the CSF, or via downstream effects on the inflammatory profile in the CSF and on the markers of endothelial dysfunction. This is particularly important because in healthy subjects and animals, the drug does not cross the blood brain barrier. It is possible that EETs have a localized action on the endothelium and vascular smooth muscle, without crossing the blood brain barrier. sEH inhibition may exert anti-inflammatory and vasodilatory effects at this site without changing measurable CSF EETs levels. If this is the case, we expect to detect differences in CSF inflammatory markers.

We anticipate a possible effect of the study drug in CSF for the following reasons: 1) There is breakdown of the blood brain barrier following SAH and both lipid and non-lipid soluble molecules may be able cross the neurovascular unit due to increased permeability; and 2) Endothelial-derived cytokines and mediators may be transported across compartments, including blood, perivascular space, and CSF. It will be important to confirm the site of action of the study drug is only at the endothelium level or if its effects on the central nervous system

We will collect biomarkers at multiple time points because eicosanoid levels, endothelial dysfunction, and the inflammatory response vary significantly over the natural course of subarachnoid hemorrhage. We may control for significant variations at baseline when interpreting the results.

- **Tertiary Objective:** Provide preliminary estimates of clinically relevant endpoints to inform the design of a larger trial.

  We will collect clinical measurements on outcomes throughout the treatment course, at hospital discharge and at 90-day follow-up. To assess DCI directly, we will measure daily middle cerebral artery (MCA) transcranial Doppler flow velocities and the degree of angiographic vasospasm. We will also measure acute changes in neurologic exam, the need for therapeutic interventions for DCI, and new stroke on imaging at discharge. We will also measure more objective surrogate outcomes at hospital discharge and 90-day follow-up including: hospital length of stay, discharge disposition, modified Rankin Scale (mRS), and the extended Glasgow Outcome Scale (GOSE).

  Justification: This study is not powered to detect an effect of drug administration on these endpoints, but these data will provide information for sample size calculations in larger subsequent studies.

4 STUDY DESIGN

4.1 OVERALL DESIGN

The study is a single site double-blind, placebo-controlled, phase 1b randomized trial to evaluate the safety and preliminary pharmacologic activity of GSK2256294 in patients with

aneurysmal SAH. We hypothesize that inhibition of the sEH enzyme will be safe in patients with SAH, and result in increased EETs availability at the neuroendothelium, eventually leading to reduced neuroinflammation. Up to 30 patients will be equally randomized to receive once daily enteral administration of either 10 mg dose of GSK2256294 or placebo for a duration of 10 days.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This is a safety and proof of concept study. A randomized trial is needed to determine the effect of GSK2256294 on EETs metabolism, because EETs levels vary over the natural course of recover after SAH, and a placebo group is needed for comparison. Randomization and double blinding additionally limit the influence of potential confounders and selection bias.

4.3 JUSTIFICATION FOR DOSE

GSK2256294 exposures are approximately dose proportional over the range of 6 mg to 20 mg. High levels of enzyme inhibition was observed with single doses of 6 to 20 mg, and sustained at greater than 95% for 24 hours at a dose of 10 mg in repeat dosing trials. A 10-day administration schedule was chosen because this represents the highest risk time period for development of neuroinflammatory changes related to delayed cerebral ischemia.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit shown in the SoA, Section 11.

The end of the study is defined as completion of the last visit or procedure shown in the SoA in the trial globally.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

Age $\geq$ 18
Head CT evidence of subarachnoid hemorrhageDigital subtraction cerebral angiography or CT angiogram documenting the
presence of a cerebral aneurysm.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

Symptom onset compatible with SAH of > 3 days prior to admission to OHSU

Evidence of QTc prolongation > 500ms on admission
Baseline AST and ALT > 2x normal reference range
Administration of any of the following inducers/inhibitors of CYP3A4: ritonavir, indinavir, nelfinavir, saquinavir, clarithromycin, telithromycin, chloramphenicol, ketoconazole, itraconazole, nefazodone, cobicistat or enzalutamide.
Current pregnancy, based on urine HCG positive pregnancy test
Preexisting severe neurologic deficit or condition
Chronic renal failure requiring dialysis
Severe terminal disease with life expectancy <6 months
Unable to read or understand written or spoken English or SpanishRefusal of informed consent

5.3 SCREEN FAILURES

Screen failures are defined as participants who are eligible to participate based on inclusion criteria, but meet any of the exclusion criteria. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes screen failure details, eligibility criteria and reason for patient exclusion.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

The study population will draw from all adult patients admitted to the OHSU NSICU, part of a comprehensive stroke center certified by the American Heart Association and Joint Commission for Accreditation of Healthcare Organizations, with a catchment area including the state of Oregon, Southwest Washington and Northern California. Approximately 80-100 patients with aneurysmal SAH are admitted each year. All patients admitted with SAH have cerebral angiography or CT angiogram early after admission. Research personnel will review patients from the ICU census for eligibility on a daily basis.

To ensure completeness of follow up procedures, we will collect contact information from the patient, the patients legally authorized representative (LAR), the primary care provider (PCP), and the disposition location (facility) contact information at hospital discharge. The CareEverywhere electronic medical record network and obituary screening will be used to identify deaths.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

GSK2256294, a potent and selective inhibitor of soluble epoxide hydrolase, will be administered to the treatment group, and a matched placebo will be administered to the placebo group for a total treatment course of 10 days.

6.1.2 DOSING AND ADMINISTRATION

Subjects will receive either 10mg of GSK256294 or matched placebo. The first dose of drug or placebo will be administered after informed consent, randomization and collection of baseline laboratory samples. Subsequent doses will be administered daily for a total treatment course of 10 days. Subjects who are able to swallow will receive the capsule formulation orally. Subjects not able to swallow will receive the contents of the capsule suspended in normal saline, administered via orogastric or nasogastric enteral access by the bedside nurse. Missed doses will be administered as soon as feasible. No restriction to food intake is necessary.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

GlaxoSmithKline will supply the drug capsule and matched placebo to the OHSU investigational pharmacy. At the end of the trial, unused product will be returned to GlaxoSmithKline or destroyed.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

GSK2256294 is available in capsule form. The capsules are opaque Swedish orange hard gelatin, containing a white powder. Matched placebo capsules which are also opaque Swedish orange containing white powder will be administered to the placebo group. The capsules are packaged into white, opaque, high density polyethylene bottles with child-resistant closures. The label for the clinician administering the drug will identify the medication as the “Study Drug”. The label will contain the study number, subject number, quantity of product, and directions for use.

6.2.3 PRODUCT STORAGE AND STABILITY

The capsule will be stored at room temperature, and the drug is stable up to 30^{o}C.

6.2.4 PREPARATION

Capsule packaging will be labeled as described above and dispensed from the OHSU investigational pharmacy to the OHSU NSICU on a daily basis. At the time of administration, the bedside nurse will administer the capsule formulation orally in patients able to swallow. If patients are unable to swallow, the bedside nurse will open the capsule and suspend the contents in 10mL of 0.9% normal saline, and administered immediately after suspension via orogastric or nasogastric tube.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Participants who meet all eligibility criteria and consented for study participation will be randomized by notifying the research pharmacy service. A randomization list will be generated by computer generated sequence and will determine whether the subject receives active drug or matched placebo. The randomization procedure will require confirmation of the eligibility criteria to ensure that the integrity of the randomization is maintained. Subjects will be randomized to one of the two treatment arms based on an unrestricted or “fair-coin” randomization procedure.

Randomization will be carried out in STATA using a uniform distribution with a specific random number seed so that the results are reproducible. The research pharmacy service will maintain the randomization list and provide the study drug as appropriate. All participants who complete the informed consent process will be assigned a study identification number. Numbers will be assigned in consecutive order starting with the number 100. This number will be followed with another two-digit number representing the randomization sequence starting at 01. Participants will be referenced by the study identification number.

6.4 STUDY INTERVENTION COMPLIANCE

Study drug administration will be recorded in the electronic medical record (EMR), which will be used as source documentation, and administration verified daily. Missed doses and reason will be recorded on a study log. The drug accountability record will be maintained by the investigational pharmacy.

6.5 CONCOMITANT THERAPY

Relevant concomitant medications prescribed during the study drug administration period will be recorded from the EMR in the following classes: vasopressors, antihypertensives, hyperosmolar therapy, nimodipine, insulin, statins and antibiotics.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from drug or placebo administration does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. All patients are expected to complete the treatment course unless they experience an SAE that is thought to be related to the study drug, die, are discharged early, or withdraw early from the study.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study if any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she is unable to be contacted by the study site staff within 2 weeks of the 90-day follow-up visit.

The following actions must be taken if study personnel are unable to contact participant for follow-up:

- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, contact with PCP. We will also screen obituaries and check the EMR for potential deaths.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING, CONSENT, AND RANDOMIZATION

Screening: Research coordinators will survey the NSICU census on a daily basis to determine which patients are potentially eligible for enrollment. Patients who have cerebral angiography demonstrating ruptured intracranial aneurysm who satisfy the inclusion/exclusion criteria will be approached to provide informed consent. For women of childbearing potential, baseline urine pregnancy test will be performed at admission as part of the standard of care. If not already performed as part of standard medical care, liver function tests will be sent to determine subjects' baseline liver function.

Consent: We have developed a written consent to be reviewed and signed by the study participant or surrogate decision making. The recruitment process may take place in-person or by phone. A phone script is attached with this submission. Either the PI, coinvestigators or research staff will explain the objectives, risks and benefits of the study to the patient. We will allow adequate time to answer questions and address concerns.

Some patients will not be able to provide informed consent due to incapacity. Legally authorized surrogate decision makers will be approached to provide consent in these scenarios. There will be no financial compensation or incentive provided to study participants. Study candidates must sign the informed consent form before any study-specific tests or procedures are performed.

Enrollment/randomization: Eligible patients are defined as participants meeting all inclusion criteria and none of the exclusions and who have provided a written informed consent. Participants must be screened, consented, and enrolled in the study within 48 hours of admission to the OHSU NSICU. Participants will be randomized as described above. All participants who complete the informed consent process will be assigned a study identification number. Numbers will be assigned in consecutive order starting with the number 100. This number will be followed with another two-digit number representing the randomization sequence starting at 01. Participants will be referenced by the study identification number.

8.2 BASELINE EVALUATION

Baseline subject evaluation and testing will be performed in conjunction with routine intensive care unit treatment and monitoring. Data will be collected from the medical record or patient/surrogate interview.

- **Patient Demographics:** Ethnographic characteristics (age, sex, race/ethnicity, weight, height, BMI), level of education, and detailed contact information for follow-up

- **Characteristics of present illness:** Hunt and Hess Score, Fisher grade, location of aneurysm, surgical vs interventional treatment (completed or planned) and Glasgow Coma Scale (GCS) will be collected at the time of randomization.
- **Baseline Physiologic Data**: The first laboratory results and vital signs obtained at the time of ICU admission will be recorded, and detailed in the variable list in section 12. We will also collect details about history of preexisting medical comorbidities using the Charlson Comorbidity Index Score, screening for tobacco use, and targeted questions about neurologic history. A baseline EKG will be performed as part of the standard of care, and reviewed for evidence of conduction abnormalities or acute myocardial ischemia.
- **Admission medications:** Selected classes of medications administered from the time of ICU admission to randomization will be recorded**.** We will also record selected classes of home medications the patient may have been taking prior to hospitalization.

8.3 STUDY DRUG ADMINISTRATION SCHEDULE

The investigational pharmacy will maintain blinding, labeling and dispensing to healthcare providers of the study drug as described above. The first dose of study drug will be administered immediately after randomization. Subsequent doses will be administered daily in the morning for a total of 10 days. The preferred route of administration is orally, but patients with SAH are often neurologically compromised and unable to safely swallow. A suspended formulation will be administered via the orogastric or nasogastric route for patients deemed unsafe to swallow.

8.4 SAFETY ASSESSMENTS

8.4.1 DAILY ASSESSMENTS

Daily assessments will be performed beginning on the randomization day (day=1), and on subsequent study days that subjects receive study drug (days 2-10). The randomization day will be defined as the time from randomization to the next 6:59 AM. All subsequent study days will be defined as a 24-hour window between 7:00AM and 6:59 AM the following day. Study staff will perform daily assessments from data collected from the medical record as part of patients' routine intensive care unit treatment and monitoring, or by patient interview**.** These include:

- **Vital Signs:** every four hour vital signs including blood pressure from noninvasive and arterial sources, heart rate, body temperature, and the presence of absence of mechanical ventilation;
- **Cardiac Monitoring:** daily QTc interval, and the presence of cardiac arrhythmias on continuous EKG monitoring.
- **Intake and Output:** total fluids administered and total drain and urine output;
- **Laboratory Assessments:** serum sodium, blood glucose, BUN, creatinine, hematocrit, peripheral white blood cell count;
- **Neurologic Assessments:** GCS, MCA transcranial Doppler velocities and Lindegaard ratios, and headache severity;
- **Concomitant medications:** Daily administration of selected classes of hospital administered concomitant medications will be reviewed for all study subjects during the first 10 days.

8.4.2 DISEASE RELATED AND ADVERSE EVENT TRACKING

Daily disease related event log and adverse event log forms will be completed. Specific complications common in patients with SAH will be abstracted from the medical record with the date and time of onset and resolution. These include:

- **Seizure:** Electrographic or generalized tonic clonic seizure activity;
- **Cerebral angiography** results and interventions, including the distribution and severity of cerebral vasospasm;
- **Neurologic deteriorations attributable to DCI**: Defined as the development of new focal neurological signs or decreased level of consciousness that resulted in a decrease in the Glasgow Coma Scale (GCS) score of 2 points, lasting at least 1 hour, and not attributable to infection, changes in sedation, or non-ischemic cerebral pathology such as acute hydrocephalus);
- **The need for intervention to manage DCI**: Defined as use of augmented blood pressure goals, cardiac index monitoring, or intra-arterial vasodilator therapy;
- **New onset or previously undocumented myocardial dysfunction:** left ventricular ejection fraction (LVEF) $\leq$ 45%, or a > 10% change from baseline LVEF, LV contractile pattern consistent with neurogenic cardiomyopathy at any measured LVEF, or new wall motion abnormalities;
- **NSTEMI/STEMI:** ST segment changes on 12 lead ECG suspicious for myocardial ischemia, or any elevation of serum troponin above reference range;
- **Hospital acquired infection:** Culture data resulting in the use of antibiotics for > 72 hours;
- **Liver dysfunction:** Liver function tests will be assessed on study day 7. AST or ALT > 3x above baseline values will be identified as an adverse event
- **Thromboembolic events:** Imaging-documented presence of extracranial venous or arterial thrombus.

8.5 PHARMACODYNAMIC EFFECT ASSESSMENTS

8.5.1 SAMPLE COLLECTION

Blood samples will be collected from study participants after randomization but prior to administration of the first dose of study drug or placebo, and again on study day 7 and study day 10. For patients with an external ventricular drain (EVD), cerebrospinal fluid (CSF) will be collected at each timepoint as well.

10 mL of blood and 5 mL of CSF will be collected at each time point. If the indwelling external ventricular drain is removed prior to study day 10, CSF samples will be collected on the day of planned removal. In the event of inability to collect CSF due to obstructed catheter, or low intracranial CSF volume, one additional attempt will be allowed within a period of 24 hours.

Blood samples will be collected by the bedside nurse and processed for storage by study personnel. CSF samples will be collected by physician medical staff, from external ventricular drainage access, and then processed for storage by study personnel.

8.5.2 EICOSANOID PROFILE AND SEH ACTIVITY ASSAY

Samples will be spun down to remove cellular components, and 1mL of the resulting plasma and 2 mL of CSF will be reserved for eicosanoid profile determination. An antioxidant will be added to the plasma and CSF, and these aliquots will be frozen at -80°C in opaque glass vials. Vials will be labeled with the participant ID, type of sample, and date and time of collection. At the end of the enrollment period after the last sample collection, liquid chromatography and mass spectroscopy will be used to determine EET and DHET levels and EPOME and DPOME levels. All samples will be batch processed based on protocols developed and validated by the OHSU Bioanalytical Core Lab.

8.5.3 INFLAMMATORY AND ENDOTHELIAL INJURY SAMPLE PROCESSING

The remaining plasma and CSF obtained at each sample collection time point will be frozen at -80°C and reserved for batch processing of the following biomarkers below. Luminex assays for the biomarkers will processed in the OHSU Anesthesiology Core Lab at the end of the enrollment period after the last sample collection.

TABLE 1: Neuroinflammatory and endothelial injury biomarkers associated with EETs signaling

Biomarker	Role	Blood	CSF
Drug Action			
Eicosanoid Profile (EETs and DHETs)	Drug effect marker[12]	X	X
Inflammatory Biomarkers			
Tumor necrosis factor alpha (TNF-α)	Neuroinflammatory marker, apoptosis, decreased in EETs knockouts[4]		X
Interleukin 1β (IL-1β)	Neuroinflammatory marker[14]		X
Interferon gamma (IFN-γ)	Neuroinflammatory marker, decreased in sEH knockouts[4]		X
Interleukin 6 (IL-6)	Neuroinflammatory marker, associated with vasospasm,[14] decreased in sEH knockouts[4]		X
Interleukin 8 (IL-8)	Neuroinflammatory marker, associated with vasospasm[14]		X
Monocyte chemoattractant protein 1 (MCP-1)	Neuroinflammatory marker, associated with vasospasm[14]		X
Endothelial Injury			
e-selectin	Leukocyte adhesion marker[15]	X	
p-selectin	Platelet adhesion marker[16]	X	
Vascular cell adhesion marker (VCAM-1)	Vascular inflammation, blood brain barrier breakdown, hydrocephalus[17]	X	
Platelet endothelial cell adhesion marker (PECAM-1, CD31)	Leukocyte, platelet, monocyte adhesion, angiogenesis[16]	X	
Intercellular adhesion molecule (ICAM-1)	Endothelial surface marker for inflammation [16]	X	

8.5.4 EPHX2 POLYMORPHISM ANALYSIS SAMPLE PROCESSING

sEH activity variation as a result of genetic polymorphisms of EPHX2 have the potential to exist in the study population and act as confounders. We will determine sEH EPHX2 genotype at the end of the enrollment period after the last sample collection. DNA will be extracted and purified from peripheral leukocytes isolated from collected blood samples. Allelic discrimination of

EPHX2 will be performed by TaqMan amplification and quantitative PCR (qPCR). We will use probes for 2 of the most common EPHX2 genotypes, K55R and R287Q, as these are the most common and demonstrated to have a clinical effect in patients with SAH. We have previously validated this method with DNA sequencing.

TERTIARY ENDPOINT ASSESSMENTS

Discharge and Follow-up Assessments:
At hospital discharge and 90-day follow-up, we will also measure objective clinical surrogate endpoints. The hospital discharge assessment will be abstracted from the patient medical record and obtained from patient interview. The 90-day follow-up assessment will be obtained from phone interview with the patient or their surrogate. We will collect the following information:

- **At Hospital Discharge:**
  - **Hospital length of stay,** in days
  - **New stroke on imaging:** We will review the last head CT or other brain imaging to detect the presence of a new area of cerebral infarction. We will define a cerebral infarction identified on hospital discharge that was not present on imaging between 24–48 hours after aneurysm occlusion, and not attributable to other causes such as surgical clipping or endovascular treatment. Hypodensities resulting from extraventricular drains or residual intraparencyhmal hematomas will not be considered new strokes.
  - **Discharge disposition:** We will record the disposition from the hospital in one of the following categories: home, home with services, rehab, LTAC, SNF, hospice, death
  - **Modified Rankin Scale:** We will determine and record the mRS score from where 0 – no symptoms, 1 – no significant disability, able to carry out all usual activities despite some symptoms, 2 – slight disability. Able to look after own affairs without assistance, but unable to carry out all previous activities, 3 – moderate disability, requires some help, but able to walk unassisted, 4 – moderately severe disability, unable to attend to own bodily needs without assistance, and unable to walk unassisted, 5 – severe disability, requires constant nursing care and attention, bedridden, incontinent, 6 – deceased.
- **At 90-day follow up**
  - **Modified Rankin Scale**
  - **Extended Glasgow Outcome Scale (GOSE):** At 90 day follow up, we will determine the GOSE based on a structured interview of 19 questions detailed in the variable list and assessment tools. We will record the GOSE on a scale of 1-8 where 1 – deceased, 2 – vegetative state, 3 – low severe disability, 4 – upper severe disability, 5 – low moderate disability, 6 –upper moderate disability, 7 – low good recovery, 8 – upper good recovery.

8.6 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.6.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.6.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.6.3 CLASSIFICATION OF AN ADVERSE EVENT

8.6.3.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.6.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

OR

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be

clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.6.3.3 EXPECTEDNESS

The principal investigator and independent safety monitor will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention or reported in the investigator brochure

8.6.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study drug (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Research staff will record all reportable events with start dates occurring any time after informed consent is obtained until 28 days post randomization or hospital discharge, whichever comes sooner. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed until resolution or stabilization.

8.6.5 ADVERSE EVENT REPORTING

Summaries of adverse events will be recorded for each study visit, and reported to the independent safety monitor, the institutional review board, and the study sponsor annually, in accordance with regulatory requirements. Expected disease-related events common in the study population which will not be reported per the standard process for reporting include: seizure, need for intervention to manage DCI, new onset cardiomyopathy, hypotension, hypertension, NSTEMI/STEMI, hyponatremia, hospital acquired infection, respiratory failure requiring mechanical ventilation, therapeutic antimicrobial use and indication, thromboembolic events, and disease related death.

8.6.6 SERIOUS ADVERSE EVENT REPORTING

The investigators will report within 72 hours of knowledge to the independent safety monitor, any unexpected serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the independent safety monitor and to the IRB

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable.

The investigators will be responsible for notifying the Food and Drug Administration (FDA) and GlaxoSmithKline of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

8.6.7 REPORTING EVENTS TO PARTICIPANTS

Participants who experience adverse events will be followed until resolution, and will be referred for consultation as needed.

8.7 UNANTICIPATED PROBLEMS

8.7.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.7.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the Institutional Review Board (IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the study sponsor within 72 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the study sponsor within 7 days the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within of the IRB's receipt of the report of the problem from the investigator.

8.7.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Participants who experience adverse events related to unanticipated problems will be followed until resolution, and will be referred for consultation as needed.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL ENDPOINTS

Primary Endpoint: Safety

- Determination of safety

Secondary Endpoints: Blood and CSF biomarkers of drug pharmacodynamic effect

- Day 7 serum EET/DHET ratio
- Day 7 CSF EET/DHET ratio
- Day 7 CSF inflammatory cytokines
- Day 7 serum endothelial injury biomarkers
- Day 7 serum EPOME/DPOME ratio
- Day 10 serum EET/DHET ratio
- Day 10 CSF EET/DHET ratio
- Day 10 CSF inflammatory cytokines
- Day 10 serum endothelial injury biomarkers
- Day 10 serum EPOME/DPOME ratio

Tertiary Endpoints: Clinical endpoints

- Incidence of neurologic changes attributable to DCI
- Incidence of angiographic vasospasm
- Incidence of new stroke at hospital discharge
- Hospital length of stay
- Hospital discharge Modified Rankin Scale score
- Hospital discharge disposition
- 90-day follow-up Modified Rankin Scale score
- 90-day follow-up Glasgow Outcome Scale Extended score

9.2 SAMPLE SIZE DETERMINATION

The total sample size was selected *a priori* based on the need to evaluate and describe the safety profile of the study drug in this patient population.

9.3 POPULATIONS FOR ANALYSES

Any patient who receives a single dose of study drug will be included in safety analyses as part of the safety population. Only patients who compete 10 days of study drug administration will be included in the secondary analyses as part of the per-protocol population.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

The statistical analysis performed for this study will be presented for all patients who receive at least 1 dose of the study drug. There will be no *a priori* stopping rules, since the entire sample is required to evaluate safety. However, the study can be stopped at any time for reasons related to safety concerns. The statistical analyses will be performed using STATA® version 13 or later (STATACorp, College Station TX).

The number of participants included in each analysis set will be summarized, along with the reason for any exclusions. Participants discontinuing from study treatment and/or withdrawing from study participation the primary reason for discontinuation will be summarized.

Descriptive summaries of demographic and baseline characteristics will be tabulated for all participants.

9.4.2 MISSING VALUES

Study investigators will monitor the study to maximize completion of data collection and samples. Every effort will be made to retain subjects to follow up. Patterns of missing data will be evaluated for the presence of informative missingness or if missing data are missing completely at random or at random.

9.4.3 ANALYSIS OF THE PRIMARY ENDPOINT

Safety will be assessed by clinical review of all relevant parameters including vital signs, laboratory values, adverse events, serious adverse events, disease related events, and mortality. Unless specified otherwise, the safety analyses will be conducted for the safety population.

Summary tables and listings will be provided for all reported adverse events, defined as adverse events that start on or after the first administration of study drug. The reported adverse event term will be assigned a standardized preferred term.

Adverse events will be summarized based on the number and percentage of patients experiencing the event. In the event a patient experiences repeat episodes of the same adverse event, then the event with the highest severity grade and strongest causal relationship to study treatment will be used for purposes of incidence tabulations. Tabular summaries will be provided for all adverse events, the relationship to study drug treatment the maximum severity grade, action taken, and whether the event qualified as SAE or not.

Daily safety laboratory test results will be summarized in a descriptive manner by calculating the mean, standard deviation, median, and range. Directional shifts (comparing baseline grade with worst post-baseline grade) will be analyzed using standard shift tables. The shift table will present directional shifts from baseline to above or below the laboratory standard normal range using the maximum increase and/or decrease observed throughout the course of treatment/observation.

Vital signs will be summarized in a descriptive manner by calculating the mean, standard deviation, median, and range in the same manner described for laboratory values.

All deaths will be reported in a patient listing, which will include the primary cause of death and the number of days between the date of the last dose of study drug and death.

9.4.4 ANALYSIS OF THE SECONDARY AND TERTIARY ENDPOINTS

Secondary endpoints will be compared between the study drug and placebo groups using the per-protocol population. Serum and CSF EET/DHET ratios and biomarkers will be presented as

the mean and standard deviation, and differences evaluated with two sample Student's t-test with a two-sided type 1 error rate of 0.05%.

Tertiary endpoints will be compared descriptively between the groups based on the mean, median, standard deviation and range.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol: Informed consent form and complete variable list.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to <study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and

will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).]

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

10.1.4 SAFETY OVERSIGHT

Safety oversight will be under the direction of an independent safety monitor. The ISM will be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. Unless requested by the safety monitor for the evaluation of the event and in order to ensure an objective evaluation, the safety monitor will be blinded to group assignment. The safety monitor will not be involved in the analysis of data. The safety monitor will not have financial, proprietary, professional, or other interests that may affect impartial, independent decision-making. All SAEs, AEs, and unanticipated problems related to the study will be monitored by the study investigators on an ongoing basis and AEs will be summarized annually after enrollment is complete.

10.1.5 QUALITY ASSURANCE AND QUALITY CONTROL

Quality management of study conduct, data and biological specimen collection, documentation and completion will be performed by the PI.

10.1.6 DATA HANDLING AND RECORD KEEPING

10.1.6.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into the case report forms, consisting of an electronic data capture system APOM OCEAN, a 21 CFR Part 11-compliant data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.6.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.7 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the principal investigator to use continuous vigilance to identify and report deviations within 14 working days of identification of the protocol deviation. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

10.1.8 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale

11 SCHEDULE OF ACTIVITIES

See attached

12 REFERENCES

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