Title: Safety, Tolerability and Effects of a Single Subcutaneous Administration of SP16–a SERPIN-like, Small Peptide Agonist of the Low Density Lipoprotein-like Receptor 1 –on the Acute Inflammatory Response in Patients with ST-segment Elevation Myocardial Infarction (STEMI)

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Safety, Tolerability and Effects of a Single Subcutaneous Administration of SP16–a SERPIN-like, Small Peptide Agonist of the Low Density Lipoprotein-like Receptor 1 –on the Acute Inflammatory Response in Patients with ST-segment Elevation Myocardial Infarction (STEMI)

Protocol Identifying Number: 02-SP16

SP16 Inflammatory Response Inhibition Trial (SPIRIT)

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IND/IDE Sponsor: Serpin Pharma, LLC

Version Number: v.2
21 August 2020

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SUMMARY OF CHANGES

Changes to prior version 27 March 2020

Changes necessary to address COVID19 Pandemic Risk. Enrollment to resume when IRB approval obtained.

Section	Change	Rationale
Section 5.2	Updated exclusion criteria to include COVID-19.	Specify criteria for COVID-19 exclusion.
Section 7.3.6	New section	Need to explain need to comply with SARS-CoV2 screening and testing practice at VCU Health
Section 7.3.7	Table of events updated	Updated with changes
Figure 1	Updated PK times to 0, 30, 60 & 180 minutes	Updated with changes
Section 4.2.3	Updated PK times to 0, 30, 60 & 180 minutes	Updated with changes
Section 7.3.2	Updated PK times to 0, 30, 60 & 180 minutes	Updated with changes
Schedule of Events Table	Updated PK times to 0, 30, 60 & 180 minutes	Updated with changes

Changes to prior version 13 December 2019

Changes in response to COVID19 Pandemic Emergency – All changes planned to revert when emergency resolved.

Enrollment has been halted on 3/23/2020.

Section	Change	Rationale
Sections 4.2.2, 7.3.5, 7.3.7	Visits changed to virtual. Echocardiogram postponed from 90 days to 365 days.	To limit in-person visits during this pandemic.

LIST OF ABBREVIATIONS

Abbreviation	Expanded Term
λ	Terminal rate constant
AAT	α1-Anti-Trypsin
ACC	American College of Cardiology
AHA	American Heart Association
AE	Adverse event
ALT	Alanine aminotransferase
AMI	Acute myocardial infarction
AST	Aspartate aminotransferase
AUC∞	Total area-under-the-curve
CFR	Code of Federal Regulations
CRF	Case Report Forms
CK-MB	Creatine Kinase-Myocardial Band
CL_{tot}	Total clearance
c_{max}	Maximum concentration
CRP	C-Reactive Protein
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
ICH	International Council of Harmonisation
IDS	Investigational Drug Services
IND	Investigational New Drug
IRB	Institutional Review Board
LAD	Left Anterior Descending artery
LRP1	Low Density Lipoprotein Receptor-like Protein 1
MOP	Manual of Procedures
MSRD	Maximum Recommended Starting Dose
MRT_{sys}	Mean resident time
OHRP	Office of Human Research Protection National Institute of Health
NIH	National Institutes of Health
NSAIDs	Nonsteroidal anti-inflammatory drugs
PCI	Percutaneous Coronary Intervention
PI	Principle Investigator
SAE	Serious adverse event
SERPINS	Serine protease inhibitors
SP16	Serpin peptide 16
SQ	Subcutaneous
STEMI	ST-segment Elevation Myocardial Infarction
$t_{1/2}$	Half-life
t_{max}	Time to C_{max}
TIMI	Thrombolysis in Myocardial Infarction
TNF	Tumor necrosis factor
UK	United Kingdom
UP	Unanticipated problems

US	United States
VCU	Virginia Commonwealth University
Vd_{cc}	Volume of distribution of the central compartment
Vd_{ss}	Volume of distribution at steady-state
WBC	White blood cell

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with Good Clinical Practice (GCP) as required by United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812) and International Conference on Harmonisation Guidance for Industry, Good Clinical Practice: Consolidated Guidance.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator: Antonio Abbate, MD, PhD
Print/Type Name

Principal Investigator: Benjamin Van Tassell, PharmD
Print/Type Name

Signed:

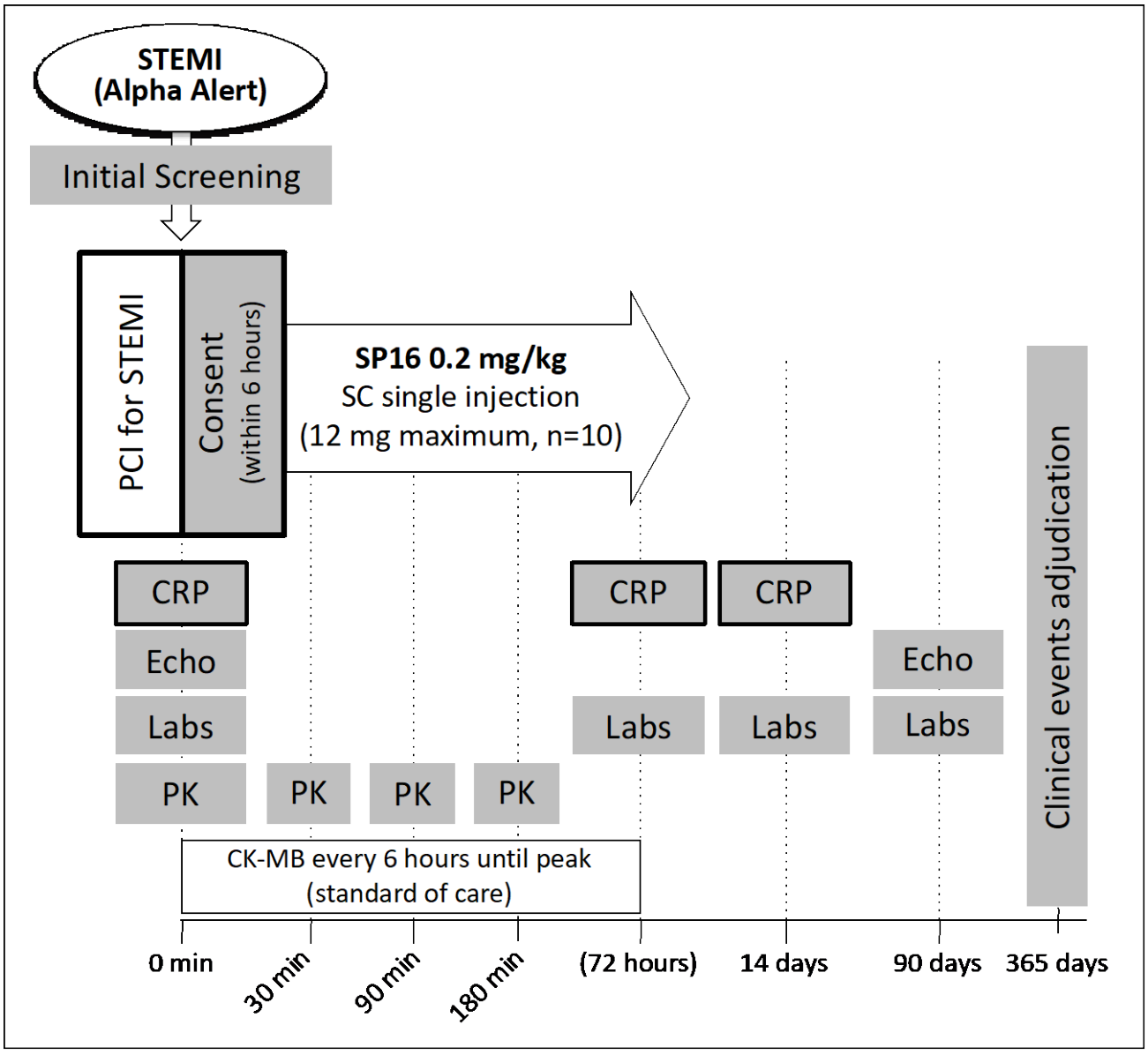
_____ _____Date/Signature

_____ _____Date/Signature

PROTOCOL SUMMARY

Title:	Safety, Tolerability and Effects of a Single Subcutaneous Administration of SP16–a SERPIN-like, Small Peptide Agonist of the Low Density Lipoprotein-like Receptor 1–on the Acute Inflammatory Response in Patients with ST-segment Elevation Myocardial Infarction (STEMI)
Design:	Single-center, single-arm, open-label, clinical trial of SP16 0.2 mg/Kg administered within 6 hours of reperfusion.
Objectives:	The primary objective is to determine the effects of SP16 on the area-under-the-curve (AUC) for C-reactive protein (CRP) at 14 days as compared with historical controls treated with placebo in the VCUART, VCUART2, and VCUART3 studies. Secondary objectives include the assessment of the effects of SP16 on AUC for creatine kinase myocardial band (CK-MB) at 72 hours.
Study Endpoints:	• AUC for CRP at 14 days [primary endpoint] • AUC for CK-MB at 72 hours • Pharmacokinetics • Safety assessments • Echocardiography at 365 days • Incidence of heart failure at 365 days
Safety Monitoring:	Subjects will be monitored directly for at least 12-hours after administration of investigational medication and then have repeated follow-up at 72 hours, 14 days, and 365 days.
Population:	Adult (>21 years of age) individuals (N=10) with acute ST-segment elevation myocardial infarction (within 12 hours of symptom onset).
Phase:	1B/2A
Number of Sites Enrolling Participants	1 (Virginia Commonwealth University)
Description of Study Agent:	SP16 is a synthetic oligopeptide based upon the structure of human alpha-1 antitrypsin. SP16 exhibits high affinity for the LRP1.
Study Duration:	Primary Endpoint: 14 days Safety Endpoint: 14 days Long-term Follow-up: 365 days from last patient enrolled
Participant Duration:	365 days

Figure 1: Schematic of the Study Design



1 KEY ROLES

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

Acute myocardial infarction remains a major cause of morbidity and mortality in the US and worldwide.[1] Despite current strategies for early reperfusion, many patients die early during the course, and those who survive are at risk for dying late from adverse cardiac remodeling, heart failure, and sudden death.

Patients presenting with ST-segment elevation (STEMI) are at particularly high risk for adverse cardiac remodeling, heart failure, and mortality.[1,2] Although there have been considerable improvements in the treatment of STEMI, the reduction in early mortality has been associated with an increasing incidence of heart failure after STEMI.[1] This likely reflects more high-risk patients surviving the index event as well as the aging of the population and the epidemics of hypertension and diabetes. Within 1 year of STEMI, more than 20% of survivors are diagnosed with heart failure, a disease associated with high morbidity, disability, and mortality.[1] Heart failure is indeed a major public health problem affecting approximately 5 million Americans with 500,000 new cases per year. In contrast to other cardiovascular disease, the incidence and prevalence of heart failure continue to increase and heart failure is now the leading cause of hospitalization for people aged ≥65 years, a segment of the population that is also rapidly growing.[1] Although survival after the onset of heart failure is also improved, current therapies may slow but not halt the progression of the disease. With the limitations to functional capacity, the progressive symptoms of dyspnea and fatigue, the frequent hospital admissions and the economic consequences of lost productivity and increasing costs of medical care, heart failure imposes a significant burden on healthcare.

There is an urgent need to develop additional treatments to prevent heart failure after AMI.[2] The current treatment in STEMI includes prompt reperfusion of the ischemic myocardium by restoration of the coronary artery patency (i.e. angioplasty or fibrinolysis), prevention of re-occlusion (i.e. antiplatelet and anticoagulants), and neurohormonal blockade (i.e. renin-angiotensin-aldosterone and adrenergic blockers). While each of these interventions provide incremental benefit and significantly reduce morbidity and mortality, the incidence of heart failure after STEMI has continued to rise, implying that the current treatment paradigm still misses one or more key pathophysiologic mechanisms. Determining the mechanisms by which unfavorable cardiac remodeling and heart failure progress despite optimal treatment is thus a critical step in the search for novel interventions, with the ultimate goal of reducing the incidence, burden, and mortality of heart failure after STEMI.[2]

A close interplay exists between inflammation, adverse cardiac remodeling and heart failure after AMI. Acute myocardial ischemia and infarction initiate an intense inflammatory response within the myocardium.[3] Leukocytes infiltrate the damaged myocardium to coordinate tissue repair and infarct healing, leading to newly formed vessels and reparative fibrosis. Thus, inflammation is necessary for infarct healing, but uncontrolled inflammation is responsible for further damage to the heart and non-functional healing which are the basis for adverse cardiac remodeling and heart failure. In experimental animal models, the degree of the inflammatory response determines adverse cardiac remodeling, independent of infarct size.[3] In patients with AMI, the intensity of the inflammatory response, reflected in levels of circulating biomarkers, predicts adverse cardiac remodeling, heart failure and death.[4] Modulation of the inflammatory response therefore represents

a target for intervention. While previous attempts to modulate the inflammatory response have failed, we propose a novel and substantially different approach to modulating the inflammatory response based upon the anti-inflammatory and cytoprotective properties of alpha-1 antitrypsin (AAT).[5]

AAT is a naturally occurring anti-inflammatory protein that is abundant in the plasma and exerts powerful cytoprotective effects in endothelial cells, cardiomyocytes and fibroblasts.[5] In an experimental model of acute myocardial infarction (AMI), AAT administered at the onset of ischemia or at time of reperfusion led to a significantly smaller infarct size and more favorable cardiac healing and remodeling.[6]

The mechanism by which AAT preserves cardiac function during AMI is mediated by the engagement of the Low Density Lipoprotein Related Protein 1 (LRP1) receptor. Serpin Peptide 16 (SP16) is a synthetic anti-inflammatory peptide developed to reproduce the -anti-inflammatory and α activities of the Serine Protease Inhibitors (SERPINs or Serpins), such as AAT. Serpins are a family of proteins characterized by the ability to inhibit plasma serine proteases such as elastase, thrombin, plasmin.[7] When Serpins bind to serine proteases with resulting inactivation of enzymatic activity, causing a conformational change by which a short peptide containing a unique motif (5-11 amino-acids) is exposed.[8,9] This motif binds to LRP1, a membrane receptor responsible for clearance of plasma proteins and for inducing an anti-inflammatory and cytoprotective signal.[9] Serpin Pharma has developed a synthetic anti-inflammatory peptide (SP16) that contains the properties of the Serpin core motif responsible for the anti-inflammatory and cyto-protective signaling, without inhibiting the plasma serine proteases (Issued Patent #8,975,224). SP16 is 17 amino acids in length, derived by excision of a 36-39 amino acid long peptide fragment to yield an active short peptide containing the unique motif. SP16 represents the shortest oligopeptide maintaining activity and a single amino acid substitution enhancing anti-inflammatory signaling and plasma stability. In vitro, SP16 did not exhibit meaningful affinity for receptors other than LRP-1.

From a safety perspective, SP16 appears to bind exclusively to LRP-1. A survey of >100 kinases and receptors showed no cross-reactivity of SP16 with other targets. SP16 appears not to be immunogenic. Antibodies to SP16 have not been detected in animal models after up to 6 months of treatment. Furthermore, deliberate attempts to produce anti-SP16 monoclonal antibodies failed to provoke an immune response.

In comparison with AAT, SP16 is a bio-superior- small peptide which retains the anti-inflammatory and cytoprotective properties of Serpins. SP16 only contains the LRP-1 binding motif, thus providing specific anti-inflammatory actions without serine protease inhibition. SP16 has demonstrated anti-inflammatory properties in several pre-clinical models. In ex vivo peripheral blood mononuclear cells derived from a collagen antibody induced arthritis mouse model, SP16 significantly reduced LPS-stimulated tumor necrosis factor (TNF)-α and Interleukin (IL)-6 production compared to vehicle and no treatment. SP16 has also been tested in db-/db diabetic mice, in which it significantly reduces serum amyloid A, an acute phase reactant, and monocyte chemotactic protein-1. Moreover, SP16 reduced IL-6 concentrations through inhibition of Myd88-mediated NF-κB activation, a key inflammatory signaling pathway. In vitro experiments have also demonstrated that SP16 inhibits NFκB activation.[10]

In an experimental reperfused AMI model in the mouse, SP16 was shown to provide a strong

cardioprotective signaling.[10] SP16 has been tested as treatment for acute myocardial infarction in the mouse. AMI was induced by temporary ligation of the proximal left coronary artery for 30 minutes. Mice which had received a single administration of SP16 immediately at reperfusion or within 30 minutes of reperfusion had smaller infarct sizes and better left ventricular systolic function at 24 hours and 7 days.

SP16 has been studied extensively in non-clinical toxicology studies. A single subcutaneous administration of SP16 at doses up to 60 mg/kg in Sprague-Dawley rats with a 14-day recovery period was well tolerated. There were no test article-related findings on clinical observation, body weights, body weight changes, food consumption and ophthalmology. There were no test article-related findings on hematology, coagulation, urinalysis, absolute and relative organ weights or macroscopic examinations. Test article- related findings were limited to increased incidence of slight edema injection site in males given 60 mg/kg, minimal decreases in albumin in males given 60 mg/kg, minimal increases in total cholesterol and triglycerides in females given ≥ 20 mg/kg, and microscopic changes at the injection site -a mild but increased severity of inflammation occurred in animals given 60 mg/kg, eosinophilic material and mild necrosis in subcutis of animals given ≥5 mg/kg). The mild necrosis in the subcutis noted in males administered 60 mg/kg and in females administered ≥5 mg/kg was considered adverse.

2.2 RATIONALE

The main hypothesis of this study is that a single subcutaneous administration of SP16 0.2 mg/kg is safe and well tolerated in patients with STEMI and associated with a reduction in the acute inflammatory response to STEMI, as measured as area-under-the-curve (AUC) for C reactive protein (CRP), the preferred inflammatory marker for cardiovascular risk prognostication. SP16 will be administered subcutaneously as this route has greater ease of administration than intravenous injection. A single dose administration has been selected based upon pre-clinical data and expected clinical use of SP16.

The dose of SP16 was chosen based on the results of a Phase I trial (NCTNCT03651089, WIRB® Protocol #20181644) in which 3 cohorts of healthy volunteers received a single subcutaneous injection of SP16 or placebo: SP16 0.0125 mg/kg (n=6), 0.050 mg/kg (n=6), 0.200 mg/kg (n=6), or placebo (n=6). Safety assessments were performed throughout 12 hours of continuous monitoring followed by return visits at 24 hours, 3 days, and 7 days. None of the SP16 doses showed any significant effects on any of the safety parameters as measured by comprehensive metabolic panel, complete blood count, liver function tests, thromboelastography, coagulation testing, electrocardiogram, and cardiac biomarkers (high sensitivity troponin I, CK-MB). All doses were well-tolerated and no patient reported injection site pain greater than 1/10 at 30 minutes after the dose.

In a Phase IIA trial of 10 patients with ST-segment elevation myocardial infarction treated with plasma derived AAT[11] the treatment was administered at a dose of 60 mg/kg, which is equimolar to an SP16 dose of 2 mg/kg. The 60 mg/kg dose of plasma-derived AAT was well-tolerated.[11] Thus, the SP16 dose of 0.2 mg/kg is less than the equivalent dose of plasma-derived AAT which was well tolerated in the 10 patient Phase 1 clinical trial. However, in experimental settings SP16 was >20-fold more powerful than plasma derived AAT at an equimolar dose and >500-fold more powerful per mg of drug.[10]

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

Allergic reaction to any drug, including SP16, is possible, and severe allergic reactions can be life threatening.

The dose of SP16 was chosen based on the results of a Phase I trial in which 3 cohorts of healthy volunteers received a single subcutaneous injection of SP16 or placebo: SP16 0.0125 mg/kg (n=6), 0.050 mg/kg (n=6), 0.200 mg/kg (n=6), or placebo (n=6). Safety assessments were performed throughout 12 hours of continuous monitoring followed by return visits at 24 hours, 3 days, and 7 days. None of the SP16 doses showed any significant effects on any of the safety parameters as measured by comprehensive metabolic panel, complete blood count, liver function tests, thromboelastography, coagulation testing, electrocardiogram, and cardiac biomarkers (high sensitivity troponin I, CK-MB). All doses were well-tolerated and no patient reported injection site pain greater than 1/10 at 30 minutes after the dose.

In clinical trials, the most common adverse reactions to plasma derived AAT (to which SP16 is related) occurring in >1% subjects were headache (7%), musculoskeletal discomfort (myalgias) (7%) and sore throat (pharyngitis) (2%). Rash, hot flushing and pruritus (itching) may also occur (<1%). All these reactions were considered mild or moderate and not severe and were self-limiting with the end of the infusion.[12] An elevation in the aminotransferase levels (ALT or AST) may occur (10%) deriving from subtle alterations in the muscles and/or liver. Such elevations were minor (less than 5-times baseline), transient (resolve within 3 months) and did not result in symptoms or any long-term consequences. Similar to any drug or intervention, the possibility of an unanticipated adverse reaction cannot be excluded.

There is a possibility that an assessment completed during the screening phase will reveal an unrecognized disease or condition (i.e. pregnancy). The results of the tests will be discussed with the subject, and if requested, forwarded to a chosen physician.

Loss of confidentiality is a potential risk. The likelihood of this occurring, however, is very low. Except when required for clinical practice or by law, subjects will not be identified by name, social security number, address, telephone number or any other personal identifier. Study related tests may be reported but subjects will not be identified.

There is minimal risk associated with the physical procedures of the study. Additional blood draws may be needed as part of the study. The blood draws may result in minor bleeding or discomfort (rare) and infection (extremely rare) at puncture site. When possible, blood will be drawn from an existing indwelling peripheral venous catheter.

2.3.2 KNOWN POTENTIAL BENEFITS

There are no guaranteed medical benefits to the study participants. There is a possibility that SP16 will result in a reduction in the inflammatory response to myocardial injury and this may portend a more favorable prognosis. All participants will be compensated for their participation.

3 OBJECTIVES AND PURPOSE

The primary objective of this study is to assess the effects of a single subcutaneous dose of SP16 on the acute inflammatory response in patients with STEMI.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

We designed a single-center, single-arm, open-label study of a single subcutaneous administration of SP16 (0.2 mg/kg) in patients with STEMI. Patients will be eligible within if undergoing PCI within 12 hours of chest pain onset and if able enrolled and treated within 6 hours of PCI. Biomarkers will be obtained at admission, and up to 72 hours (or at discharge whichever comes first), and at 14±2 days. Patients will then return to clinic at 365 days to for transthoracic echocardiography.

We will also follow the patients up to 12 months and assess the incidence of heart failure and related events.

4.2 STUDY ENDPOINTS

4.2.1 PRIMARY ENDPOINT

The primary endpoint of the study is the difference in AUC for CRP at 14 days comparing SP16-treated patients with historical controls treated with placebo in the VCUART, VCUART2, and VCUART3 studies that used identical screening and enrollment criteria.12,13

Patients presenting with STEMI have an enhanced IL-1 response as witnessed by elevation in serum markers such as CRP.4 We anticipate that patients with STEMI will have increased CRP levels and its levels will predict adverse cardiovascular outcomes. We therefore hypothesize that SP16 will blunt the CRP increase during STEMI. The rationale for measuring AUC for CRP is that it integrates measurements across multiple time points to more accurately capture the acute inflammatory response and is also an established prognostic marker in STEMI. The AUC will be calculated based upon CRP values at baseline, 72 hours, and 14 days.

4.2.2 SECONDARY ENDPOINTS

Infarct size will be based upon the time-concentration AUC of CK-MB.11,14 CK-MB will be determined by direct chemiluminescent technology at admission, immediately after PCI, and every 6 hours until 72 hours (when available). CK-MB peak value will be defined as the maximal CK-MB level measured during hospitalization. For patients with fewer than 6 recorded CK-MB measurements, CK-MBauc will be estimated by a validated log-normal model incorporating the admission CK-MB level, the CK-MB peak level, and the time-to-CK-MB peak to reconstruct the typical time course of CK-MB curve.14 The time-to-CK-MB peak, as a measure of duration of myocardial injury, will be considered as the time between the initial onset of symptoms (chest pain per patient report) and the CK-MB peak level.

A transthoracic echocardiogram will be performed at baseline and 365 days. The echocardiogram will measure left and right ventricular and atrial dimensions, left and right ventricular systolic function, transmitral flow Doppler spectra, mitral and tricuspidal valve annulus tissue Doppler spectra, ejection time and stroke volume, inferior vena cava, aorta and pulmonary artery diameters and Doppler spectra, according to the recommendations of the American Society of Echocardiography.[15] Results from clinically indicated studies will be used for baseline measurements.

4.2.3 PHARMACOKINETIC ASSESSMENT

A dedicated tube of blood will be collected to measure SP16 concentration at baseline, 30, 90 minutes, and 180 minutes. Pharmacokinetic data will be analyzed using non-compartmental analysis. This approach will yield estimates of pharmacokinetic parameters such as total clearance (CLtot), cmax (maximum concentration), tmax (time to Cmax), mean resident time (MRTsys), volume of distribution of the central compartment (Vdcc), volume of distribution at pseudo-steady-state (Vdpss), volume of distribution at steady-state (Vdss), total area-under-the-curve (AUC∞), half-life (t1/2), and terminal rate constant (λ) without having to specify a particular compartment model.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

In order to be eligible for this study, patients must meet ***ALL*** the 3 criteria:

1) Presentation to the hospital with acute STEMI defined as chest pain (or equivalent) with an onset within 12 hours and ECG evidence of ST segment elevation (>1 mm) in 2 or more anatomically contiguous leads that is new or presumably new *(for intermittent pain lasting more than 12 hours, the time from the when the pain became severe and constant);*
2) Coronary intervention planned and/or completed within 12 hours of symptom onset, and enrollment in the study within 6 hours of angiogram (max 18 hours from symptom onset)
3) Age>21years (NIH standard)

5.2 PARTICIPANT EXCLUSION CRITERIA

In order to be eligible for this study, patients must meet ***NONE*** of the Exclusion criteria.

- Inability to give informed consent
- Hemodynamic instability as defined as need for inotropic or vasoactive agents, or need for mechanical support devices (including intra-aortic balloon pump)
- Pregnancy or breastfeeding
- Preexisting congestive heart failure (AHA/ACC class C-D, New York Heart Association III-IV)
- Preexisting severe left ventricular dysfunction (EF<20%)
- Preexisting severe valvular heart disease
- Known active infections (acute or chronic) including known COVID-19 infection or symptoms consistent of COVID-19 infection or close contact with a patient with COVID-19

in the prior 3 weeks (PCR testing results from current admission can be used but are not required)
- Recent (<14 days) or active use of immunosuppressive drugs (including but not limited to high-dose corticosteroids [>1 mg/kg of prednisone equivalent], TNF-α blockers, cyclosporine) not including NSAIDs or corticosteroids used for IV dye allergy only)
- Recent (<14 days) or active use of anti-inflammatory drugs (not including NSAIDs or corticosteroids used for IV dye allergy only)
- Known chronic inflammatory disease (including but not limited to rheumatoid arthritis, systemic lupus erythematosus)
- Known active malignancy of any type, or prior diagnosis in the past 10 years
- Neutropenia (absolute neutrophil count<1,800/mm3 [or <1,000/mm3 in African American patients])
- Severe impairment in renal function (estimated glomerular filtration rate <30 ml/kg*min)
- Anticipated need for cardiac or major surgery
- Known Allergy to SP16

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

5.3.1 SCREENING

All patients with suspected STEMI at VCU Health are identified with an alert system sent out to the on-call staff and the research team, call "***Alpha Alert***". We will obtain a partial waiver for screening to prospectively identify subjects that may meet the inclusion criteria and access the electronic health records, if required by the Institutional Review Board. Every possible effort will be made to protect the privacy and confidentiality of the subjects. The information of those subjects who screen out or who decide to not enroll will be immediately discarded. Those who are found to be eligible and express interest in the study will be approached for consent.

5.3.2 CONSENTING

With the exception of screening under a waiver of consent, no other study procedures will be completed prior to the subject consenting. Those individuals unable to provide consent and in whom a legally authorized representative cannot be found to consent will be excluded from the study. Potential participants will be approached as soon as possible after screening (including before catheterization) to discuss participation and obtain informed consent. However, patients will not receive treatment with SP16 upon successful completion of catheterization and completion of all screening criteria. Only appropriately trained investigators will attempt to obtain informed consent to participate in the study.

5.3.3 STUDY DRUG ADMINISTRATION

Following screening and enrollment, the local investigational pharmacy will prepare the study drug for administration (0.2 mg/kg, up to 12 mg maximum). The drug will be administered by subcutaneous injection by the hospital nursing staff.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

Participants may withdraw voluntarily from the study or the PI may terminate a participant from the study.

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Participants are free to withdraw from participation in the study at any time. An investigator may terminate participation in the study if:

- Any clinical adverse event (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 participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

Halting criteria for study conduct are described separately in Sections **Error! Reference source not found.** and **Error! Reference source not found.**.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

Effort will be made to complete follow-up for all participants who received investigational medication to ascertain safety endpoints. Participants who withdraw from the study prior to receipt of investigational medication will be considered screen failures and this enrollment spot will be replaced by another subject. Participants who receive investigational medication but refuse all forms of follow-up will be encouraged to seek alternative medical follow-up.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. The Sponsor and Principal Investigators will jointly evaluate study progress and risks to subjects after the first 3 subjects have completed. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the Institutional Review Board (IRB) and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to determination of unexpected, significant, or unacceptable risk to participants.

Study may resume once concerns about safety, protocol compliance, and data quality are addressed and satisfy the sponsor, IRB and/or FDA. Specific halting rules are described in Section **Error! Reference source not found.**.

6 STUDY AGENT

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

SP16 will be supplied by the Sponsor Serpin Pharma LLC to the Investigational Drug Services (IDS) Pharmacy of Virginia Commonwealth University.

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Investigational SP16 will be prepared by Serpin Pharma LLC. The pharmacy at the clinical site (VCU) will transfer the drug (SP16) from the vial into a tube in preparation for the injection.

6.1.3 PRODUCT STORAGE AND STABILITY

The drug is stored in 4.5 mL, brown vials in -20ºC. The drug is formulated in water, pH 5.8-6.2, at 3 mg/mL. Final volume in each vial is 2.5 mL. The drug was manufactured for phase II clinical trial on August 25, 2017. The API stability after 12 and 24 months since manufacturing was found to be essentially unchanged. The expiration date was set as 12/31/2021.

SP16 Solution for SQ injection **[3mg/ml] 2.5ml** Dosage and use: See prescribing information. Single Use Only. Keep frozen at -20°C. Protect from light. Sponsor: Serpin Pharma Caution: Limited by federal (USA) law to investigational use only.	IND 127746 LOT #:17243 Use By 12/31/2021

6.1.4 PREPARATION

SP16 API will be re-suspended in double distilled water and pH was adjusted to pH 5.9 ± 0.02 by adding 0.1M hydrochloric acid and/or 0.1M sodium hydroxide solution slowly. For complete details of formulation and sterile-fill-finish, please see MTA 172-2 and BMR-991.

6.1.5 DOSING AND ADMINISTRATION

The drug can be thawed up to 6 hours from the time of injection and can be stored afterwards at 4ºC until injection. Unused drug should be discarded and not be used for additional patients. Each subject will receive a single, weight-based dose of SP16 administered by subcutaneous injection (0.2 mg/kg, up to a maximum of 12 mg).

6.1.6 ROUTE OF ADMINISTRATION

SP16 will be administered by subcutaneous injection.

6.1.7 DOSE

SP16 will be administered at a dose of 0.2 mg/kg (up to a maximum of 12 mg). SP16 is formulated as a 2-mL syringe containing 6 mg of SP16 (3 mg/mL), such that all patients will require 2 separate subcutaneous injections to deliver the total dose.

6.1.8 DURATION OF THERAPY

All subjects will receive a single dose of SP16 (consisting of 2 injections given in the same session). Additional doses will not be administered.

6.1.9 TRACKING OF DOSE

Investigational drug storage will be recorded and maintained by the VCU IDS Pharmacy. Dose administration will be recorded electronically by the nurse in the catheterization laboratory.

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

The VCU Investigational Drug Services Pharmacy will prepare all doses of SP16. After preparation of each SP16 dose, dose administration will be tracked by the electronic Medication Administration Record at VCUHealth. Tracking of all incoming doses, dispensed doses, administered dose and wasted doses will be handled by the IDS Pharmacy according to local and national regulations. A single shipment of all investigational doses will be delivered by Serpin Pharma LLC to the IDS Pharmacy prior to the start of enrollment. Any unused syringes will be returned to Serpin Pharma LLC at the end of the study period.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

The following research procedures will be performed.

- Medical history through patient interview, and if available, medical record review by a licensed study physician.
- Medication history as reported by the patient, and if available, through pharmacy refill records by a licensed study physician.
- A complete review of systems and physical examination will be performed by a licensed study physician.
- A 12-lead electrocardiogram (ECG) will be reviewed by the investigators.
- Blood sample collection for comprehensive metabolic panel, complete blood cell count, coagulation tests, cardiac injury markers and pharmacokinetics will be performed by a registered nurse. Blood samples will be drawn from an indwelling venous catheter when possible.

7.1.2 STANDARD OF CARE STUDY PROCEDURES

Patients will receive the standard of care for STEMI, and these procedures will be charged to the patient or insurance, as applicable.

This may include:

- Coronary angiogram and angioplasty.
- Echocardiography and cardiac magnetic resonance.
- Laboratory tests including CK-MB
- Monitoring in the Intensive Care Unit.
- Medications.

7.2 LABORATORY PROCEDURES/EVALUATIONS

A Schedule of Procedures is included in Section 7.3.6.

7.2.1 CLINICAL LABORATORY EVALUATIONS

The following laboratory evaluations will be performed.

- Hematology: hemoglobin, hematocrit, white blood cells (WBC) with differential count, platelet count.
- Biochemistry: sodium, potassium, carbon dioxide, chloride, blood urea nitrogen, creatinine, total bilirubin, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase.
- High-sensitivity CRP
- Pharmacokinetics: SP16 concentration
- Pregnancy test (if indicated)

7.2.2 OTHER ASSAYS OR PROCEDURES

None.

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

Laboratory testing will be handled by the VCU Health Department of Pathology.

Blood samples for pharmacokinetics will be drawn from a venous catheter and stored at -80 C prior to analysis. Each sample will be labeled with the subject's unique study identifier.

7.2.4 SPECIMEN SHIPMENT

The drug will be shipped on dry ice using GMP procedures (Global Courier) from the manufacturing site in the UK to the pharmacy at VCU. The entire drug supply for phase I would arrive in one shipment. The boxes and vials are labelled (see label sample).

SP16 Solution for SQ injection **[3mg/ml] 2.5ml** Dosage and use: See prescribing information. Single Use Only. Keep frozen at -20°C. Protect from light. Sponsor: Serpin Pharma Caution: Limited by federal (USA) law to investigational use only.	IND 127746 LOT #:17243 Use By 12/31/2021

7.3 STUDY SCHEDULE

7.3.1 SCREENING

- Review medications history to determine eligibility based on inclusion/exclusion criteria.
- Perform medical examinations needed to determine eligibility based on inclusion/exclusion criteria.
    - Physical examination, including vital signs
    - ECG
- Review of all study procedures and answer any participant questions
- Obtain informed consent
- Collect demographic information
- Collect blood for baseline pharmacokinetics, comprehensive metabolic panel, CRP, complete blood cell count, and pregnancy test (if appropriate).

7.3.2 DOSING (after PCI)

- Re-verify that subject meets inclusion/exclusion criteria upon completion of reperfusion.
    - Review coronary angiogram
- Administer dose of SP16 (0.200 mg/kg, up to 12 mg) within 6 hours of reperfusion
- Blood samples for pharmacokinetics will be collected at 30 +/- 15 min, 90 +/- 15 min, 180 +/- 15 min and after SP16 administration

7.3.3 DISCHARGE (or 72 hours, whichever comes first)

- Blood samples for hsCRP, safety labs (CBC, CBMP) and pharmacokinetics will be collected at 72 hours (or at discharge, whichever comes first).
- Assess patient for adverse events
- Record adverse events as reported by participant

7.3.4 VISIT 4: FOLLOW-UP VISIT (Day 14+/- 2)

- History and physical exam
- Blood sample for safety laboratory tests (CBC, CMP)
- Blood sample for hsCRP
- Assess patient for adverse events
- Record adverse events as reported by participant.

7.3.4 VISIT 5: FOLLOW-UP VISIT 2 (Day 90+/-14)

- History and physical exam
- Blood sample for safety laboratory tests (CBC, CMP)
- Assess patient for adverse events
- Record adverse events as reported by participant.

7.3.5 VISIT 6: FOLLOW-UP VISIT 3 (Day 365 +/- 30)

- History and physical exam
- Assess patient for adverse events
- Transthoracic echocardiography
- Record adverse events as reported by participant.

7.3.6 SARS-CoV2 SCREENING AND TESTING

Screening and testing for SARS-CoV2 infection will performed as indicated by current VCU Health guidelines. These guidelines are frequently updated and are available to the PI and study physicians via the VCU Health intranet. Screening procedures include questions targeted at assessing symptoms and exposure risk. Testing may be performed by means of nasopharyngeal or oropharyngeal swab and polymerase chain reaction assay. As of August 21, 2020, every patient admitted to the hospital will be screened by specific questions and tested by a nasopharyngeal swab at time of admission. Patients being seen in the outpatient clinics will be screened by history only.

7.3.7 SCHEDULE OF EVENTS TABLE

Procedures	Screening	Dosing	Initial Inpatient (6 hours)	Discharge (or 72 hours)	Follow-up (14 days)	Follow-up 2 (90 days)	Follow-up 3 (365 days)
Informed consent	X	X					
Verify subject meets inclusion/exclusion criteria	X	X					
Demographics	X						
Screening (and testing) for SARS-CoV2 virus*	X				X	X	X
Medical history and physical examination	X			X	X	X	X
CBC w/diff, plts[a]	X			X	X	X	
Comprehensive metabolic panel[a]	X			X	X	X	
Serum Pregnancy test[b]	X						
ECG[a]	X						
Administer SP16 or matching placebo		X					
hsCRP	X			X	X		
Pharmacokinetic analysis	X		X[c]				
Echocardiography			X[a]				X
Subject interview for adverse events				X	X	X	X
Record adverse events if and as reported by participant			X	X	X	X	X

*per VCU Health standard of care
[a]If not already performed for routine care
[b]If applicable
[c]Drawn at 0, 30 min, 90 min and 180 mins post-dose

7.4 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

The patients will receive standard of care for STEMI. There will be no restrictions.

7.5 JUSTIFICATION FOR USE OF PLACEBO

Not applicable.

7.5.1 PRECAUTIONARY MEDICATIONS, TREATMENTS, AND PROCEDURES

None.

7.6 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

There will be no restriction to medications, treatments, or procedures.

7.7 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

Prophylactic medications, treatments or procedures will not be administered.

7.8 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

Rescue medications, treatments and procedures, such as anti-histamines, topical or systemic corticosteroids may be administered at the discretion of a licensed study physician in order to treat problems that emerge during the conduct of the study such as rash or allergic/anaphylactic reactions. There will be no restrictions with respect to the type or nature of rescue medications, treatments and procedures, which will be chosen at the discretion of a licensed study physician.

7.9 PARTICIPANT ACCESS TO STUDY AGENT AT STUDY CLOSURE

The investigational drug will not be available outside the study.

8 ASSESSMENTS OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

All adverse events (AEs) that occur during the study will be recorded. At each study visit, subjects will be interviewed to identify any AEs that have occurred. In addition, physical examinations, laboratory tests and other pre-specified assessments will be evaluated to identify AEs.

Risks to subjects will be minimized by restricting study procedures to appropriately trained personnel.

8.1.1 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)).

Note: Laboratory/ECG/vital signs abnormalities are not an AE in themselves unless clinically relevant or meet the definition of an AE.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

Serious adverse event or serious suspected adverse reaction. An 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 patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The office of human research protection (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 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.

This study will use the OHRP definition of UP.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

For 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.

8.2.2 RELATIONSHIP TO STUDY AGENT

For all collected AEs, the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **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 drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge 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 drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfill this definition.
- **Possibly 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 drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) 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 drug 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.

8.2.3 EXPECTEDNESS

The PIs will be responsible for determining whether an 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 agent.

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

The occurrence of an 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. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (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.

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.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 days after the administration of investigational medication. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

Adverse (non-serious) events will be reported to the IRB on an annual basis and to the Sponsor at designated Safety Review milestones.

8.4.2 SERIOUS ADVERSE EVENT REPORTING

The investigators will report all adverse events to the Sponsor and local IRB. Serious, unexpected adverse events will be reported to the local IRB and to the Sponsor within 5 business days. All other AEs (unexpected but not serious or expected) will be reported to the Sponsor as a summary during follow up and at the completion of the study. All other AEs will be reported to the IRB at the time of regular (i.e. yearly) continuing review submissions.

The Sponsor will report all serious, unexpected adverse events as an IND safety report to the FDA no later than 15 calendar days after the sponsor's initial receipt of this information. Fatal or life-threatening unexpected experiences for which there is a possibility that the experience may have been caused by the drug will be reported by the Sponsor to the FDA by telephone or facsimile transmission no later than 7 calendar days after receipt of this information. An annual report which includes summaries of all IND safety reports will be submitted to the FDA each year in the annual report by the Sponsor.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic. Other supporting documentation of the event may be requested by the study sponsor and should be provided as soon as possible.

The study Sponsor will be responsible for notifying FDA 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.

8.4.3 UNANTICIPATED PROBLEM REPORTING

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the Site Investigator's responsibility to report UPs to their IRB and to the Study Sponsor.

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

- UPs will be reported to the IRB and to the study Sponsor within 5 business days of the Investigator becoming aware of the problem.

8.4.4 EVENTS OF SPECIAL INTEREST

Not applicable.

8.4.5 REPORTING OF PREGNANCY

In the event that a subject becomes pregnant after screening and before enrollment, that subject will be excluded from participation. Pregnancy that occurs after receipt of investigational medicine will prompt reporting to the patient's primary physician for appropriate monitoring and follow-up.

8.5 STUDY HALTING RULES

Administration of study agent will be halted under the circumstances described below. The Principal Investigators will halt enrollment immediately and notify the Sponsor within 24 hours. The Study Sponsor will inform the Data Safety Monitoring Board (DSMB) members within 24 hours of becoming aware and will provide the DSMB with AE listing reports. The DSMB will convene an ad hoc meeting by teleconference or in writing as soon as possible. The DSMB will provide recommendations for proceeding with the study to the Study Sponsor and Principal Investigators. The Study Sponsor will inform the FDA of the temporary halt and the disposition of the study.

Enrollment in the study will be terminated if the any of following occur:

- Serious adverse event, if determined to be probably or definitely related to the investigational medication in a patient who have received SP16 or is unexpected in nature.

Enrollment in the study will be halted until joint review by the PIs and Sponsor if any of the following occur:

- Two identical unexpected serious adverse events in two separate subjects.

In addition, the Principal Investigators may choose to halt enrollment, if in their opinion, continued enrollment would pose an unacceptable risk to subjects. Additional description of halting and stopping is provided in Premature Suspension or Stopping of the Study Section 5.5.

8.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of the Principal Investigators and Sponsor. The Principal Investigators and Sponsor will review safety data on an ongoing basis. The occurrence of any adverse events specified in Section 8.5. will prompt a safety review of the Principal Investigators and Sponsor in consideration to Halting Rules.

8.7 ADJUDICATION OF CLINICAL EVENTS

8.7.1 Event-adjudicating committee

The event-adjudicating committee is composed of a general cardiologist, a cardiologist with training in heart failure, and a general internal medicine specialist. The committee will meet at the end of the study and adjudicate all the events. The committee will be blinded to treatment allocation. In order to favor allocation concealment, the committee will also be blinded to C-reactive protein levels, which may be affected by treatment.

8.7.2 Definition of the events

The events adjudicated will include:

- Death;
- Cardiac death (in which a direct cause attributable to cardiac disease is present);
- Sudden cardiac death (in which cardiac death occurred out of the hospital and suddenly; or in the hospital due to ventricular arrhythmias unrelated to other concomitant cardiac conditions);
- Non-cardiac death (in which the event of death is considered not to be a direct consequence of cardiac disease);
- Incidence of heart failure during the index hospitalization (defined as dyspnea beginning or persisting >24 hours after admission and meeting both of the following criteria:
    - physical signs of heart failure - including 2 or more of the following: edema, crackles/rales, jugular vein distention, hepatojugular reflex, tachypnea, rapid weight gain, S3 gallop, abdominal distension/ascites, radiologic evidence of worsening edema, pulmonary artery occlusive pressure (wedge) >18 mmHg or cardiac output <2.2 l/min-m2;
    - need for additional/increased heart failure therapy - including one of the following: initiation or significant increase of oral diuretics, requirement of intravenous diuretics, inotropes or vasodilators, need for ultrafiltration due to decompensated heart failure;
- Re-hospitalization for any cause;
- Re-hospitalization for heart failure (meeting all criteria listed above for heart failure during index hospitalization)
- Incidence of heart failure (not hospitalized) defined as new or worsening dyspnea and meeting one of the two of the following criteria:
    - physical signs of heart failure - including 2 or more of the following: edema, crackles/rales, jugular vein distention, hepatojugular reflex, tachypnea, rapid weight gain, S3 gallop, abdominal distension/ascites, radiologic evidence of

worsening edema, pulmonary artery occlusive pressure (wedge) >18 mmHg or cardiac output <2.2 l/min-m2;
    - need for additional/increased heart failure therapy - including one of the following: initiation or significant increase of oral diuretics, requirement of intravenous diuretics, inotropes or vasodilators, need for ultrafiltration due to decompensated heart failure;
- Acute myocardial infarction, as defined by the WHO consensus statement
- Unstable angina, or need for coronary revascularization
- Cardiac tachy- or brady-arrhythmias leading to a new hospitalization or to prolongation of hospital stay;
- Acute renal failure (defined as in increase in plasma creatinine levels of 50% or 0.5 mg/L);
- Acute respiratory failure (not due to heart failure);
- Sepsis or other serious infection requiring antibiotic therapy;
- Acute stroke.

The analysis will consider time to first event and time to each event. It will also consider event rates at 3 and 12 months, in order to favor comparisons with other studies. The number of days free of hospitalization during the first 1, 3, 6 and 12 months will also be measured and compared between groups.

8.7.3 Implications of the findings of the event-adjudicating committee

The events will be adjudicated only after the completion of the study, and therefore the findings by the committee will have no implications on the conduct of the study.

9 CLINICAL MONITORING

Monitoring for this study will be performed by the Principal Investigators to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s). Complete review of Case Report Forms will be performed by the Principal Investigators during Safety Milestones as described in Section **Error! Reference source not found.**.

10 STATISTICAL CONSIDERATIONS

10.1 ANALYSIS DATASETS

- All analyses will be conducted on the Safety Analysis Dataset (e.g., participants who received the investigational product).

10.2 DESCRIPTION OF STATISTICAL METHODS

10.2.1 GENERAL APPROACH

Baseline measurements and demographic characteristics will be summarized with median and

interquartile range. Descriptive summaries of categorical measurements will consist of frequencies and proportions. The summaries for each measurement will be provided separately for the treatment group and in comparison with placebo-treated patients of VCUART, VCUART2 and VCUART3 clinical trials. Between group comparisons between the two treatment groups will be conducted with the chi-square test for categorical variables and the Mann-Whitney test for continuous variables. An assessment of sample size or power analyses is not performed for this pilot Phase IB/IIA study. A two-sided p-value < 0.05 will be considered statistically significant.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Virginia Commonwealth University will maintain appropriate medical and research records for this trial, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of participants.

12 QUALITY ASSURANCE AND QUALITY CONTROL

The PIs will oversee all data collection and recording on Case Report Forms. They will periodically review Case Report Forms.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

Principal Investigators will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

13.2 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), 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 previously consented participants need to be re-consented. Western Institutional Review Board (Puyallap, WA) will serve as the IRB of record for this study.

13.3 INFORMATED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

All study procedures will be completed after to the subject consenting with the exception of screening under a waiver of consent. Those individuals unable to provide consent and in whom a legally authorized representative cannot be found to consent will be automatically excluded from the study. Only appropriately trained investigators will attempt to obtain informed consent to participate in the study. Considering, the emergency nature of the condition, we will request the

Institutional Review Board to approve a short consent form to be followed by a full-length consent form.

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is an ongoing process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. 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. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. When a potential candidate is identified, he or she will be provided with a consent form that explains the purposes of the study, the study procedures, the potential risks of participation, and all other requisite information as required by law. The consent form will also explain that continuation in the study is "voluntary" and that the patient has "the right to withdraw from the study at any time". A copy of the informed consent document will be given to the participants for their records.

13.4 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples 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.

- Coded identifiers will be attached to data or samples.
- The key to coded identifiers will be stored in a password-protected database compliant with local standards and will be accessible only to the research team. Case Report Forms and written informed consent forms will be stored in a locked cabinet within a locked office with access restricted to the study team.
- Coded data and samples, but not the key, will be shared with the Sponsor.

The study participant's contact information will be securely stored at the 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 local IRB and Institutional regulations.

13.4.1 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS OR DATA

- Intended Use: Samples and data collected under this protocol may be used to accomplish the objective of this study. No genetic testing will be performed.
- Storage: Access to stored samples will be limited using physical restrictions to locked storage areas and coded identifiers. Samples and data will be stored using codes assigned by the investigators. Data will be kept in password- protected computers. Only investigators will have access to the samples and data.

13.5 FUTURE USE OF STORED SPECIMENS

Not applicable.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. 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. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered onto Case Report Forms.

14.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 5 years beyond completion of this study or 2 years after the last approval of a marketing application in an 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 investigational product, whichever is later. 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.

14.3 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or 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 S7A, B:[16,17]

- 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 site to use continuous vigilance to identify and report deviations within 3 working days of identification of the protocol deviation. All deviations must be addressed in study source documents, reported to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

14.4 PUBLICATION AND DATA SHARING POLICY

This study will be registered on clinicaltrials.gov. The results of this study will be presented at conferences and published in a peer-reviewed medical journals in a way that no identifiers are included.

15 STUDY ADMINISTRATION

15.1 STUDY LEADERSHIP

The PIs and Sponsor will serve as study leadership.

16 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 trial.

17 APPENDIX

Version	Date	Significant Revisions
v.1	12/13/2019	Initial version
v.2	03/27/2020	Suspension of enrollment and modification of follow up procedures in response to COVID-19 pandemic

18 LITERATURE REFERENCES

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