A MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL GROUP STUDY ON THE SAFETY AND EFFICACY OF ISTAROXIME FOR PRE-CARDIOGENIC SHOCK (SEISMIC)

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A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study on the Safety and Efficacy of Istaroxime for Pre-Cardiogenic Shock (SEISMiC)

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[redacted]	[redacted]

STATEMENT OF COMPLIANCE

This study will be conducted in accordance with International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (ICH E6), applicable United States Code of Federal Regulations (CFR), Title 21, as well as all applicable local, state, and federal regulations and guidelines regarding the conduct of clinical studies. In addition, this study will be conducted in accordance with the ethical principles included in the World Medical Assembly (WMA) Declaration of Helsinki, *Ethical Principles for Medical Research Involving Human Subjects* adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended most recently by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013.

Throughout this protocol, the term "Clinical Investigator" or "Investigator" will be defined, in accordance with 21 CFR 54, as any listed or identified principal investigator (PI) or subinvestigator who is directly involved in study dosing or evaluation of research subjects. PIs or subinvestigators must be listed on Form FDA 1572 or equivalent and documented as appropriate on the delegation of authority signature log. The PI will assure that no deviation from or changes to the protocol will take place without prior agreement from Windtree Therapeutics, Inc., and documented approval from the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study will have completed Human Subjects Protection and ICH GCP Training and the PI must commit to compliance with the International Committee on Harmonisation's guidance on Good Clinical Practice, revision 2 (ICH E6).

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB/IEC for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. All changes to the consent form will be IRB/IEC approved; a determination will be made by the IRB/IEC regarding whether participants who previously signed an ICF need to be notified of the changes and sign a new ICF to document re-consent.

PROTOCOL AGREEMENT

I have read the protocol specified below and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated. I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent form approved by the Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) and Windtree Therapeutics, Inc. (Windtree), and will fulfill all responsibilities for submitting pertinent information to the IRB/IEC responsible for the study.

I further agree that regulatory agencies (e.g., US Food and Drug Administration), Windtree, or their designees shall have access to any source document from which case report form information may have been generated.

I agree that I and all sub-investigators listed on the delegation of authority form and/or Form FDA 1572 (or equivalent) shall inform Windtree of any equity interest in the company prior to participating in this study. I further agree that I and all sub-investigators listed will consult with Windtree before acquiring any financial interest in the company during the study and for one year after the study's completion.

_____ _____

Investigator Signature Date

_____

Print Name and Title

Site No.: _____

Site Name: _____

Address: _____

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_____

Phone Number: _____

1 PROTOCOL SUMMARY

1.1 Synopsis

Title:	A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Study on the Safety and Efficacy of Istaroxime for Pre-Cardiogenic Shock (SEISMiC)
US IND Number:	066448
Sponsor:	Windtree Therapeutics, Inc., Warrington, PA, USA
ClinicalTrials.gov No.:	NCT04325035
EudraCT No.:	2020-000885-40
Study Description:	This is a pilot, multinational, multicenter, randomized, double-blind, placebo-controlled, 2-part safety and efficacy study. Subjects will consist of males or females 18 to 85 years of age, hospitalized for acute decompensated heart failure (ADHF) with persistent hypotension (systolic blood pressure [SBP] 70-100 mmHg for two hours) and heart rate 75 to 150 beats/minute. Part A will dose all subjects for 24 hours with either 1.0 µg/kg/min or placebo; Part B will dose all subjects for 60 hours with two different regimens of istaroxime or placebo. Enrollment of Part A and Part B will be sequential. See Section 1.3, Schedule of Activities
Study Objective:	The primary objective of this study is to assess the ability of istaroxime to increase SBP in patients with cardiogenic shock (CS) or pre-shock (predominantly Society for Cardiovascular Angiography & Intervention (SCAI) Stage B), defined as hospitalization for ADHF with persistent hypotension (SBP 70-100 mmHg for two hours) who currently, and since admission to the hospital and throughout Screening, have not received IV vasopressors, inotropes, or digoxin, or not on cardiovascular, respiratory, or renal mechanical support.
Rationale:	CS continues to be associated with high rates of morbidity and mortality. Earlier intervention that could mitigate clinical and hemodynamic deterioration to CS may stabilize and reverse

myocardial dysfunction as well as maintain favorable hemodynamics, and end organ perfusion.

Pharmacologic intervention that can stabilize the failing LV and improve myocardial contractility and hemodynamics without increasing workload or myocardial oxygen demand, and mortality, are most desirable to prevent the downward spiral from pre-CS to CS.

Istaroxime, a derivative of androstanedione, exerts its effects through dual mechanisms of action, which may be favorable addressing pre-CS and early CS: 1) the inhibition of the Na^+/K^+-ATP-ase activity, thereby causing an increase in intracellular calcium, which increases cardiomyocyte contractility (inotropy); and 2) the activation of the sarcoplasmic reticulum calcium ATPase isoform (SERCA) by modulating SERCA-phospholamban interaction, promoting sarcoplasmic reticulum calcium reuptake, thus improving both relaxation (lusitropy) and contractility, as well as potentially reducing risk for clinically significant arrythmias. There have been several clinical studies with istaroxime on heart failure patients, which have demonstrated that istaroxime has the potential to improve cardiac function without the side effects or increase in mortality that has been observed in other treatments.

Part A will study the effect of istaroxime on blood pressure and tolerability in subjects with pre-cardiogenic shock SCAI Stage B associated with 24 hours of dosing. Part B will study the efficacy and tolerability of istaroxime over a longer dosing period with different dosing regimens in a similar population with slightly wider blood pressure values to allow for enrollment of patients with labile blood pressure at the extremes of the SCAI Stage B range.

Endpoints:

Primary Efficacy Variables:

- SBP area under the curve (AUC) to 6 hours from start of infusion.

Key Secondary Efficacy Variables:

- SBP AUC to 12 hours from start of infusion for subjects in Part B.

- SBP area AUC to 24 hours from start of infusion for subjects in Parts A and B.
- SBP AUC to 48 hours from start of infusion for subjects in Part B.
- SBP AUC to 60 hours from start of infusion for subjects in Part B.

Other Secondary Efficacy Variables

- Treatment-failure score, based on death, circulatory, respiratory, or renal mechanical support or intravenous inotrope or vasopressor treatment, and changes in systolic blood pressure.
- Change from baseline in SBP at 6, 12, and 24 hours of treatment for both parts, and at 48 and 60 hours of treatment for Part B.
- Number of subjects with increases from baseline in SBP ≥ 5% and ≥ 10 mmHg at a timepoint between 4-6 hours after dosing and at least one other measurement separated by ≥ 2 hours during the 24- or 60-hours infusion.
- Number of subjects requiring treatment with intravenous vasopressors, inotropes, and/or mechanical cardiac or renal support or have died in the period from randomization to 24 or 48 hours (for subjects in Part A and B, respectively) and Day 5 (“treatment failure”).
- Changes in quality of life measured by the EQ-5D from baseline to Day 5 (96 hours) from infusion start and at Day 30 (Part A only).
- Change from baseline in creatinine clearance at 24, 48, 72 and 96 hours from infusion start.
- Change from baseline and observed heart rate measurements at 12, 24, 48, 72 and 96 hours from infusion start.
- Change from baseline and observed mean arterial pressure (MAP) at 12, 24, 48, 72 and 96 hours from infusion start.
- Change from baseline and observed brain natriuretic peptide (BNP), NT-pro-BNP, troponin (cTn; either T or I) and venous lactate at 12, 24, 48, 72 and 96 hours from infusion start.
- Time to worsening heart failure through Day 5.
- Time to HF re-admission or death through Day 30.

- Length in ICU/length of initial hospitalization.
- Days alive and out of acute care (including all intensive acute care units).
- Days alive and out of the hospital through Day 30.
- Number of subjects with hospital re-admissions through Day 30.
- Changes in invasive hemodynamic parameters (e.g., PCWP, RAP, CVP, PAP, CO) from pre-treatment to 3, 6, 12, 18, 24, 48, 54, and 60 hours.
- Number of subjects with CS SCAI stage progression through Day 30.
- Mortality and reasons for death through Day 30.
- Changes in echocardiographic measurements at 24, 36, 48, 60, and 72 hours, as applicable.

Pharmacokinetics

- PK assessments of istaroxime and its primary metabolites (PST 2915, PST 2922, and PST 3903). In addition, diastereoisomers of istaroxime, PST 2890 (E) and PST 2892 (Z), will be measured and assessed. For Part A: blood samples at 6, 12, and 24 hours after infusion start and 0.25, 0.5, 1, 6, 12, and 24 hours after infusion end. For Part B: blood samples at 6, 12, 24, 36, 48, 52, 56, 60 hours after infusion start and 12, 36, 48 hours after infusion end and at discharge.

Safety Variables

- Incidence of adverse events (AEs) and serious AEs (SAEs)
- Clinically significant arrythmias (arrythmias requiring intervention) for a total of 72 hours from the start of infusion as determined by a Holter monitor (during the infusion and after the infusion has been stopped).

Study Population: Subjects will consist of patients 18 to 85 years of age (inclusive) with CS of SCAI Stage B as a result of acute heart failure and persistent hypotension (SBP 75-90 mmHg in Part A and 70-100 for Part B for two hours) and who, since admission to the hospital and throughout Screening, have not received IV vasopressors, inotropes, or digoxin, or not on cardiovascular, respiratory, or renal mechanical support.

Subjects for Part B will receive istaroxime over a longer dosing period with differing dosing regimens in a similar population to Part A with slightly wider blood pressure values to allow for enrollment of patients with labile blood pressure at the extremes of the SCAI Stage B range.

A total of approximately 60 study subjects (approximately 30 per treatment group) will be enrolled in Part A. A total of approximately 30 study subjects (approximately 10 per group) will be enrolled in Part B. Subjects will be enrolled sequentially (Part A followed by Part B).

Phase: Phase 2a

Description of Sites: Up to 30 sites in Part A; up to 15 sites in Part B. Sites may be located in Europe, Asia, South America, and the United States.

Description of Study Interventions: **Test article**: Istaroxime in a 7 ml glass vial (10 mg istaroxime plus 50 mg lactose lyophilized powder). The dose of istaroxime will be up to 1.0 µg/kg/min.

Comparator: Matching placebo (50 mg lactose lyophilized powder).

Vials are reconstituted with 5 ml saline for injection and study treatments (test and placebo) are administered as a continuous IV infusion, preferably through a central vein (i.e., central venous line or pulmonary arterial catheter), but no more distal than an antecubital vein, via an infusion pump. Subjects enrolled in Part A will be dosed for up to 24 hours; subjects enrolled in Part B will be dosed for up to 60 hours. Subjects randomized to placebo in Part B will receive placebo for 60 hours.

All subjects will receive standard of care. All subjects in Part B will be required to have a PAC in place prior to randomization.

Inclusion Criteria: All inclusion criteria must be met in order to be enrolled in this study:

1. Clinical presentation consistent with SCAI Stage B pre-cardiogenic shock caused by acute decompensation of chronic systolic heart failure (due to arterial hypertension, ischemic heart disease or dilated cardiomyopathy), without evidence for an acute coronary syndrome.
2. Signed informed consent form (ICF);
3. Males and females, 18 to 85 years of age (inclusive);

4. An admission for an ADHF episode within 36 hours prior to randomization, defined as:
   a. Dyspnea, at rest or with minimal exertion,
   b. Congestion on chest x-ray or lung US with BNP ≥ 400 pg/mL or NT-proBNP ≥ 1400 pg/mL.

   Elective admissions for medications tune up or procedures do not qualify as an ADHF admission.
5. History of left ventricular ejection fraction (LVEF) ≤ 40%;
6. Persistent hypotension defined as
   a. SBP of 75 to 90 mmHg (Part A) or 70 to 100 mmHg (Part B) for ≥ 2 hours prior to Screening;
   b. Stable SBP, defined as no decrease in SBP by > 7 mmHg on two separate measurements during the last 2 hours prior to randomization;
7. Heart rate 75 to 150 bpm. If the subject is on a beta-blocker, the range is 60 to 150 bpm;
8. Echocardiogram during initial hospitalization confirming ejection fraction ≤ 40% and no evidence of other pathology to confound interpretation of cardiac physiology (e.g., pericardial effusion);
9. Subject is monitored by a PAC at the time of randomization (Part B only).

Exclusion Criteria: Subjects meeting any exclusion criteria must not be enrolled in this study:

1. Cardiogenic shock of SCAI stage C or worse
2. Cardiogenic shock due to any other condition besides acute decompensation of chronic heart failure.
3. Any of the following in the past 60 days: acute coronary syndrome, coronary revascularization, MI, CABG, or percutaneous coronary intervention;
4. Current (within 6 hours of Screening) or anticipated need for treatment with positive inotropic agents or vasopressors, renal support including ultrafiltration, or mechanical circulatory, ventilatory or renal support (intra-aortic balloon pump, endotracheal intubation, mechanical ventilation, or any ventricular assist device);
5. Venous Lactate > 2 mmol/L;

6. History of heart transplant or UNOS priority 1a heart transplant listing
7. Ongoing treatment with digoxin (if digoxin was stopped before signing the ICF and the digoxin plasma level is < 0.5 ng/ml, the patient may be enrolled);
8. Severe renal impairment (eGFR < 30 ml/min, calculated by the MDRD formula);
9. Hypersensitivity to the study medication and its excipients (including known lactose hypersensitivity) or any related medication;
10. Stroke or TIA within 3 months;
11. Active coronary ischemia;
12. Any significant valvular disease (including any moderate or severe valvular stenosis, moderate or severe aortic or pulmonary regurgitation, or severe tricuspid or mitral regurgitation);
13. Primary hypertrophic or restrictive cardiomyopathy or systemic illness known to be associated with infiltrative heart disease;
14. Admission for AHF triggered primarily by a correctable etiology such as significant arrhythmia (inclusive of atrial fibrillation as the main reason for admission), infection, severe anemia, acute coronary syndrome, pulmonary embolism, exacerbation of COPD, planned admission for device implantation, or over-diuresis as a cause of hypotension;
15. Pericardial constriction or active pericarditis;
16. Life-threatening ventricular arrhythmia, uncontrolled arrythmia, or implantable cardioverter defibrillator (ICD) shock or history of sudden death within 6 months;
17. Cardiac resynchronization therapy (CRT), ICD, ablation, or pacemaker implantation (or planned implantation) within the past 3 months;
18. Sustained ventricular tachycardia in the last 3 months with no defibrillator;
19. Sustained hypotension (SBP < 70 mmHg) for at least 30 minutes from the time of arrival to the hospital;
20. Severe pulmonary disease or cor pulmonale or other causes of isolated right-sided HF or not related to left ventricular dysfunction;
21. Acute respiratory distress syndrome;

22. Suspected sepsis; fever > 38° or active infection requiring IV antimicrobial treatment;
23. Body weight < 40 kg or ≥ 150 kg;
24. Laboratory exclusions:
    a. Hemoglobin < 9 g/dl,
    b. Platelet count < 100,000/µl,
    c. Serum potassium > 5.3 mmol/l or < 3.5 mmol/l;
25. A life expectancy < 3 months based on the judgment of the investigator;
26. Uncontrolled thyroid disease;
27. Pregnant or breast-feeding;
28. Ongoing drug or alcohol abuse;
29. Participation in another interventional study within the past 30 days.

Treatment Groups: For Part A, eligible patients will be randomized to the study to receive either istaroxime or placebo (lactose) in a 1:1 ratio:

Group 1: istaroxime IV infusion over 24 hours

Group 2: placebo IV infusion over 24 hours

For Part B, eligible patients will be randomized to the study to receive either of 2 istaroxime regimens or placebo (lactose) for 60 hours in a 1:1:1 ratio:

Group 1: istaroxime IV infusion at 1.0 µg/kg/min for 6 hours, 0.5 µg/kg/min for 42 hours, followed by 0.25 µg/kg/min for 12 hours.

Group 2: istaroxime IV infusion at 0.5 µg/kg/min for 48 hours, followed by placebo IV infusion for 12 hours.

Group 3: placebo IV infusion over 60 hours.

During istaroxime administration, if the subject develops tolerability issues such as significant nausea, antiemetics may be administered and the infusion rate may be decreased. The infusion rate may also be decreased at the discretion of the investigator based on the occurrence of an adverse event, the development of significant bradycardia, or greater than desired blood pressure elevation.

Informed consent must be obtained before any study procedures are carried out. Study procedures that are part of standard of care may be performed prior to signing of the informed consent.

Study Duration: Screening period: Day 0 to Day 1

In Part A: Treatment period: Day 1 (randomization to end of infusion)

Post-treatment period: Days 2 to 5

In Part B: Treatment period: Day 1-3 (randomization to end of infusion)

Post-treatment period: Days 3 to 5

Follow-up period: Day 6 to Day 30

Participant Duration: Subject participation will be from Screening to Day 30 or death, whichever occurs first.

Statistical Methodology: The statistical analysis of both the primary and secondary objectives will be based on all enrolled subjects. The modified intent-to-treat (mITT) population (defined as all randomized subjects who received study treatment and had at least one post-baseline blood pressure assessment) and the per-protocol population (subjects with no major protocol deviations) will be evaluated for efficacy, based upon the treatment group to which they were randomized. For this estimation study, the per-protocol population will be considered the primary efficacy population. For safety, all subjects who received any amount of study treatment will be evaluated, based upon the treatment they actually received. All analyses will be performed by treatment group through 96 hours and through study end (Day 30).

All continuous variables (e.g., weight, body temperature, blood chemistry) will be summarized using number (n), mean, standard deviation (SD), median, minimum, and maximum. All discrete variables (e.g., sex, AEs, number of subjects with hospital re-admissions), will be summarized using frequency (n) and percent.

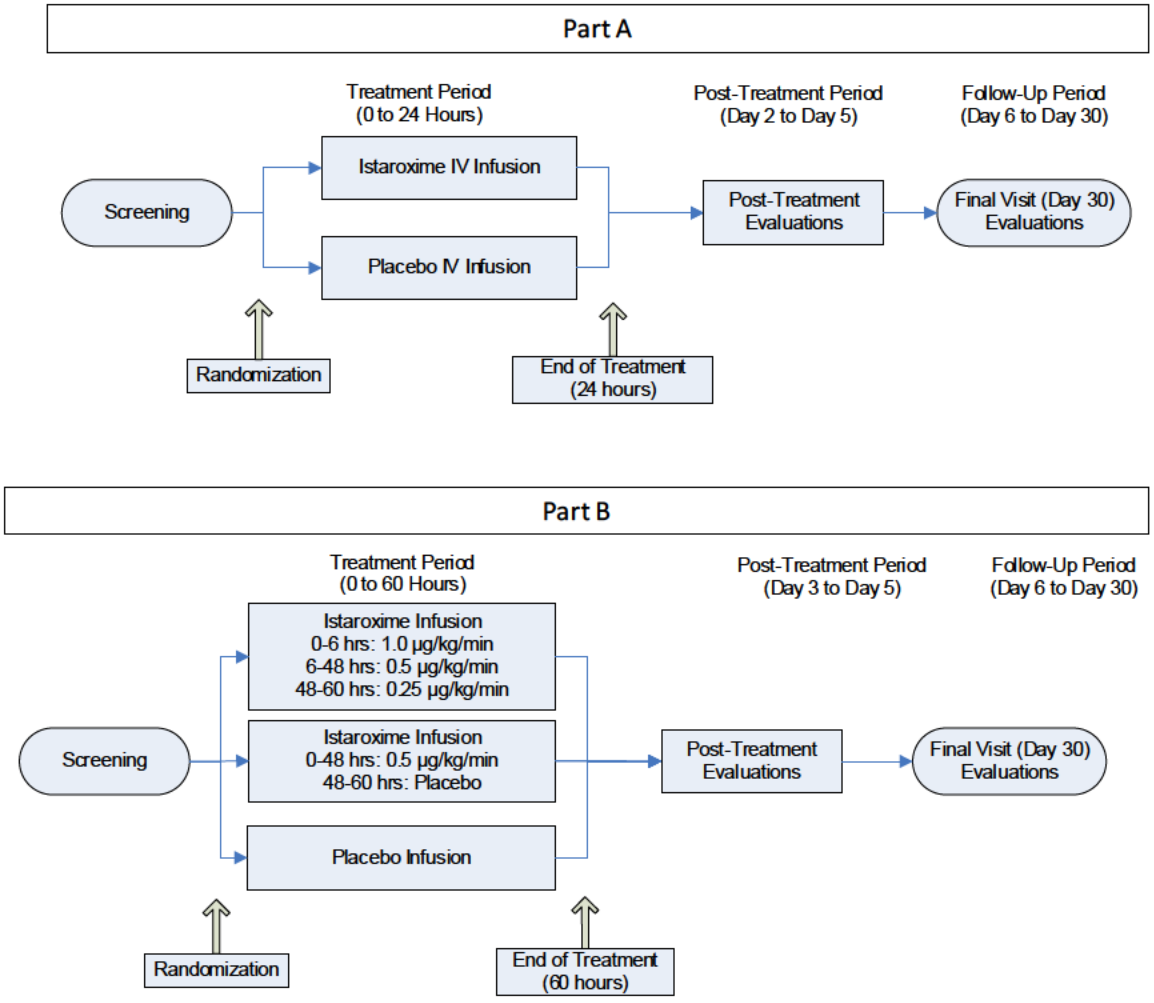
Demographic parameters will be summarized by treatment group and assessed qualitatively for homogeneity of treatment groups.

The primary endpoint ($AUC_{0\text{-}6hr}$) and key secondary endpoints will be compared between treatment groups using randomization-based ANCOVA with treatment in the model. The term ‘pooled site’ will be

included in models within Part A or Part B, not for the combined analysis.

Concomitant medications will be classified using the WHODrug dictionary and summarized using frequency and percent. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and preferred terms will be summarized by treatment group using frequency and percent (will not be compared statistically). All-cause mortality, physical examination and medical history will be summarized.

1.2 Schema



1.3 Schedule of Activities (SOA)

Assessment/Procedure	Screening Period (Day 0-1)	Treatment Period			Post-treatment Period								Follow-up Period (Day 6 to Day 30)
		Part A: Day 1 (0 to 24 hrs)			Part A: Days 2-5								
		Part B: Days 1-3 (0 to 60 hours)								Part B: Days 3-5			
Hours relative to infusion start:		**Pre-dose**	**0-12**	**13-23**	**24**	**30**	**36**	**48**	**60**	**72**	**84**	**96**	
Informed consent	X												
Demog./medical hist./baseline char.	X												
Eligibility criteria	X												
Physical examination	X				X			X		X		X	X
Local laboratories	X				X			X		X		X	X
Serum Pregnancy Test[1]	X												X
Urinalysis	X												
EQ-5D[17]		X										X	X
Echocardiography[2]	X		X		X	X[17]	X	X	X	X			
12-Lead ECG	X	X	X[4]		X			X		X		X	X
Holter monitoring[5]	X	X[6]	---->	---->	----->	----->	----->	----->	----->	-----|			
Invasive Hemodynamics[7]		X	X[8]	X[8]	X	X[17]		X[9]	X				
Randomization		X											
Study Treatment[10]			X	X	X	X	X	X	X				
Pulse, SBP, and DBP[11]	X[12]	X	X[13]	X[13]	X[13]	X[13]	X[13]	X[13]	X[13]	X	X	X	X
Oxygen saturation (pulse oximetry)	X	X	X[14]	X[14]	X	X	X	X	X	X		X	X
Weight	X				X			X		X		X	X
Body Temperature	X	X			X			X		X		X	
Urine Collection			X[15]	-------|	X[15]	----->	------|	X[15]	----->	------|			
Biomarkers/venous lactate (local)	X	X	X[4]		X			X		X		X	X
Sample collection for biomarkers		X	X[4]		X			X		X		X	X
Sample collection for PK[16]		X	X[16]		X		X	X[16]	X	X		X	X
Assessment/worsening of HF		X			X			X		X		X	
Concomitant Medications	X	X			X			X		X		X	X
Adverse Events			X	X	X	X	X	X	X	X	X	X	
Hospital Readmissions													X

Note: Day 1 is the day of randomization. Final Visit is at Day 30

1 Women of childbearing potential only.
2 Analyzed by central reader.
4 At 12 hours only.
5 From the start of screening (ICF signed), subjects who are to be included in the study should be in a unit where there is real-time telemetry and close medical supervision.
6 Continuous monitoring up to 24 hours prior to dosing, and from pre-dose through 72 hours post-dose
7 Subjects in Part B will be required to have a pulmonary artery catheter in place prior to randomization. Additional hemodynamic measurement will be required if the subjects become hypotensive, bradycardic, congested or hemodynamically unstable.
8 Measured at 3, 6, 12, and 18 hours.
9 Measured at 48 and 54 hours.
10 If no central venous line, administration should be no more distal than antecubital vein.
11 BP measured as described in Study/Pharmacy Manual.
12 Measured hourly during screening and at pre-randomization. Change in SBP in last two pre-randomization measurements cannot have decreased by > 7 mmHg in order to qualify.
13 For Part A: Measured at 0.5 and 1 hours, then hourly to 30 hours after infusion start, and then at 32, 34, 36, 40, 44, 48 hours. For Part B: measured hourly from pre-dose to 24 hours after infusion start, and then every 2 hours through 32 hours; every 4 hours from 32 to 48 hours; hourly from 48 to 66 hours; and at 72, 84, and 96 hours.
14 Measured at 1, 2, 3, 4, 5, 6, 12, and 18 hours after infusion start.
15 Collected from 0 to 24 hours, from 24 to 48 hours, and from 48 to 72 hours.
16 For Part A: blood samples at 6, 12, and 24 hours after infusion start and 0.25, 0.5, 1, 6, 12, and 24 hours after infusion end. For Part B: blood samples at 6, 12, 24, 36, 48, 52, 56, 60 hours after infusion start and 12, 36, 48 hours after infusion end and at discharge.
17 Part A only.

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2 INTRODUCTION

2.1 Study Rationale

Cardiogenic shock (CS) continues to be associated with high rates of morbidity and mortality posing a therapeutic challenge for clinicians (1,2,3). Given that short-term mortality ranges from 35% to over 60% (3,4), interventions to prevent deterioration to overt CS are clearly needed. Other than revascularization of culprit coronary vessels in patients presenting with acute myocardial infarction (AMI), no other intervention has impacted short-term survival in patients with CS, and no established therapies exist for patients with non-AMI CS etiologies (3). Earlier intervention that could mitigate clinical and hemodynamic deterioration to CS may offer a window of opportunity to stabilize and reverse myocardial dysfunction as well as maintain favorable hemodynamics, and end organ perfusion.

The period of clinical and hemodynamic deterioration that precedes CS is known as "Pre-CS," or Stage B CS, using the Society for Cardiovascular Angiography and Intervention classification scheme (4). The major aim of an intervention in pre-CS is to interrupt or reverse the hemodynamic downward spiral that ultimately leads to CS and death.

Unloading the LV lowers the LV filling pressure, reduces LV end-diastolic wall stress, and decompresses the microvasculature, thereby increasing myocardial blood flow to the endocardial layers and preserves LV contractile function (5). Pharmacologic intervention that can stabilize the failing LV and improve myocardial contractility and hemodynamics without increasing workload or myocardial oxygen demand are most desirable to prevent the downward spiral from pre-CS to CS. To date, most pharmacologic interventions including the older inotropic agents (e.g., dobutamine, milrinone) and vasoactive agents (e.g., norepinephrine), or newer inotropes under development that do not increase intracellular calcium have not shown to reduce mortality despite transient improvements in hemodynamic status. Thus, there is an unmet need for an agent to treat patients with pre-CS to prevent the hemodynamic deterioration that leads to overt CS, without increasing mortality.

Windtree Therapeutics, Inc. (Windtree) has been studying istaroxime, a derivative of androstanedione, chemically unrelated to cardiac glycosides, which has the potential to overcome many of the issues with prior or existing therapies being used to treat patients with AHF and pre-CS/CS. Istaroxime exerts its effects through dual mechanisms of action, which may be favorable addressing pre-CS and early CS: 1) the inhibition of the Na^+/K^+-ATP-ase activity, thereby causing an increase in intracellular calcium, which increases cardiomyocyte contractility (inotropy); and

2) the activation of the sarcoplasmic reticulum calcium ATPase isoform 2a (SERCA2a) by modulating SERCA-phospholamban interaction, promoting sarcoplasmic reticulum calcium reuptake, thus improving both relaxation (lusitropy) and contractility, as well as potentially reducing risk for clinically significant arrythmias.

Part A of the current study aims to assess the effect of istaroxime dosed over 24 hours in patients with Society for Cardiovascular Angiography and Interventions (SCAI) Stage B pre-CS (4). These patients are in a relative stable situation in which acute decompensation leads to congestion and low blood pressure, but without overt signs of hypoperfusion and do not require support with rescue therapies including inotropes, vasopressors, or mechanical devices.

Part B of the study is to expand the patient pool to those with slightly wider blood pressure values, and to investigate the safety and efficacy of istaroxime 0.5 and 1.0 µg/kg/min when given over 60 hours.

2.2 Background

Left unattended, pre-CS leads to overt CS with refractory hypotension, profoundly altered cellular metabolism and end-organ failure. This transition period lacks the clinical and hemodynamic criteria that define CS: systolic blood pressure (SBP) < 90 mmHg (or vasopressor therapy to maintain SBP > 90 mmHg), cardiac index (CI) < 2.2 L/min/m^2, pulmonary capillary wedge pressure > 15 mmHg, and clinical evidence of end-organ hypoperfusion (6).

Acute MI remains the most common cause of pre-CS (3). However, in the absence of MI, steady hemodynamic deterioration may lead to decompensated heart failure (HF) with reduced ejection fraction (HFrEF); end-stage HF becomes a prevalent cause of pre-CS (7). Other common causes of pre-CS include nonadherence to a medical regimen, myocarditis, stress cardiomyopathy, the use of diltiazem, and illicit medications in patients with chronic HFrEF (8).

The pathogenesis of CS is typically associated with an underlying acute coronary syndrome (ACS) (8). Acute decompensated HF may account for up to 30% of CS cases (9). These patients often experience a decline in disease stability or often have poor adherence to guideline-based therapies, triggering an acute worsening of their chronic disease. Treatment of patients with chronic HF presenting in CS can differ substantially from the treatment of other types of CS because the hemodynamic condition and neurohormonal milieu are often strikingly different. Patients with HF often have profound upregulation of vasoconstrictor substances such as angiotensin II, endothelin-1, and norepinephrine (10,11).

2.2.1 Clinical Outcomes

Among patients with ACS-associated CS who had revascularization and who survived to hospital discharge, long-term follow-up of the SHOCK trial suggests that the majority (62%) were alive 6 years later (12). In comparison, a study of patients ≥ 65 years of age with MI-associated CS (who survived to hospital discharge) demonstrated increased risk of mortality in the first 60 days after discharge. These patients had a rate comparable to that of patients without shock thereafter (13). Despite favorable longer-term survival, CS may be associated with considerable morbidity, since registry data have reported 1-year all-cause and HF rehospitalization rates of 59% and 33%, respectively (13). There is a need for new in-hospital and post-discharge therapeutic approaches to improve outcomes for patients with CS and the need for more analyses in the non-ACS CS population.

2.2.2 Unmet Medical Need in Treatment of pre-CS

The treatment of CS is complicated by the heterogeneity of the underlying pathophysiological mechanisms causing CS, and our inability to correctly account for individual patient variability and ability to tailor treatment. There is a clear unmet need to treat patients with pre-CS to stabilize the failing LV and prevent the hemodynamic and subsequently general metabolic deterioration that leads to overt CS.

2.2.3 Istaroxime Background and Mechanism of Action

Istaroxime (PST 2744) is a derivative of androstanedione, chemically unrelated to cardiac glycosides that has the potential to overcome many of the issues with prior or existing therapies being used to treat patients with AHF. Istaroxime exerts its effects through dual mechanisms of action: 1) the inhibition of the Na^+/K^+-ATP-ase activity, thereby causing an increase in intracellular calcium, which increases cardiomyocyte contractility (inotropy); 2) the activation of the sarcoplasmic reticulum calcium ATPase isoform 2a (SERCA2a) by modulating SERCA-phospholamban interaction, promoting sarcoplasmic reticulum calcium reuptake, thus improving both and relaxation (lusitropy) and contractility, as well as potentially reducing risk for arrythmias.

The deficiency in cardiac SERCA2a activity, triggered by a variety of factors, is widely recognized as one of the most important cause of cardiomyocytes decreased relaxation with the consequent impairment of the overall cardiac pumping ability and increased susceptibility to arrhythmias in HF patients (14). Moreover, it has been demonstrated both in animal models and humans that this phenotype change can be reverted to normal by SERCA2a transfection (15,16,17,18,19). To this

end, it is worth noting that the potential energy starved failing heart status may further potentiate the consequences of the SERCA2a deficiency (20).

For more than 200 years, the inhibition of the Na^+-K^+ pump has been used to increase cardiac pumping activity in spite of some unwanted side effects (arrhythmias or long-term cardiomyocytes damage). These effects, either beneficial or detrimental, are very likely due to the increased cardiomyocytes cytoplasmic Ca^{2+} that, on one hand is useful for stimulate contraction but, on the other, it may favor the side effects that are further enhanced by the deficiency of the SERCA2a activity. Altogether these effects are responsible for the very narrow therapeutic index of digoxin where an increase in dose of only 20-30% above the beneficial therapeutic plasma concentration in humans (21,22) is associated with increased mortality risk, usually associated with development of arrythmias.

Consequently, drugs with a selective effect on these two molecular targets may be beneficial to patients, since the SERCA2a activation, by the more rapid removal of the cytoplasmic Ca^{2+}, may counteract the side effects of the Na^+-K^+ pump inhibition, and by increasing the SR Ca^{2+} content may preserve the amount of Ca^{2+} available for contraction, without increasing arrhythmogenic potential (14).

2.3 Clinical Experience with Istaroxime

There have been six industry-sponsored clinical studies of istaroxime, including 2 completed phase 2 studies: PST2744-DM-04-012 (the HORIZON-HF Study) and CVT-CV-002. Since these phase 2 studies were designed to obtain preliminary evidence of clinical safety and efficacy, the 2 phase 2 studies are, therefore, most pertinent to demonstrate the potential of istaroxime to fulfill an unmet medical need in the treatment of acute decompensated heart failure (ADHF). In addition, preliminary results for Part A of this study have been tabulated and a summary of these results are presented here.

HORIZON-HF (Study PST2744-DM-04-012)

HORIZON-HF (Study PST2744-DM-04-012) was a phase 2a, double-blind, randomized, placebo-controlled, dose escalating safety and efficacy study. This study was conducted to assess the hemodynamic effects of istaroxime in 120 subjects hospitalized with worsening HF and reduced left ventricle systolic function at escalating doses of 0.5-1.0-1.5 µg/kg/min. The primary end point of the study was the change from baseline in PCWP after 6 hours of infusion. Dose-dependent improvements in PWCP were observed with a statistically significant effects of all the 3 istaroxime doses versus placebo (– 3.21 mmHg with the 0.5 µg/kg/min dose, – 3.33 mmHg with the 1.0

µg/kg/min dose, and –4.73 mmHg with the 1.5 µg/kg/min dose). HR tended to decrease during the 6-hour infusion, but no significant differences were observed at infusion end between treatment groups. Systolic blood pressure (SBP) slightly increased dose-dependently in all istaroxime-treated groups but not in the placebo group, reaching a statistically significant difference from the dose of 1.0 µg/kg/min. Echocardiographic data showed istaroxime to improve diastolic and systolic volumes, as well as Doppler and tissue Doppler parameters of diastolic function. The study showed an increase in gastrointestinal TEAEs in the highest dose group (1.5 µg/kg/min) in which 10% and 17% of subjects experienced nausea and vomiting, respectively. No subjects experienced serious or clinically relevant episodes of arrhythmia during the infusion and post-infusion period. One subject out of 30 in the istaroxime 1.5 µg/kg/min group experienced tachycardia with angina and ST-change during infusion. The QTc interval measured at 6 hours after infusion was shortened by -25, -38 and -49 ms at the three istaroxime doses, respectively.

Study CVT-CV-002

Study CVT-CV-002 was a randomized, double-blind, placebo-controlled, parallel group study designed to evaluate the efficacy of two different doses of istaroxime (0.5 and 1.0 µg/kg/min) in comparison with placebo on the E/Ea ratio in subjects with ADHF (24). A total of 96 Chinese subjects and 24 Italian subjects were randomly assigned to one of two doses of istaroxime or placebo delivered as an IV infusion over 24 hours in a 2:1 ratio within two sequential cohorts of 60 subjects each. The rate of serious AEs did not show any significant differences in the three arms, although a numerical higher number was observed in istaroxime 1.0 µg/kg/min group (n=6, 15%). Adverse drug reactions (ADRs) were more common with istaroxime treatment with a significant increase in the istaroxime 1.0 µg/kg/min group compared to pooled placebo group (n=29, 72.5% istaroxime 1.0 µg/kg/min group vs n=17, 43.6% placebo group; p=0.012). Two subjects experienced AEs leading to death, both in the 1.0 µg/kg/min group; however, one death occurred after the end of the study at day 31. Two subjects (5%) included in the istaroxime 0.5 µg/kg/min group experienced SAEs, while n=6 (15%) subjects in the istaroxime 1.0 µg/kg/min group and 2 (5%) in the placebo group experienced SAEs. No clinically relevant differences were found between groups.

Regarding the primary endpoint, in the ITT population the mean change (SD) of E/Ea ratio at 24 hours was -4.55 (4.75) in istaroxime 0.5 µg/kg/min group versus -1.55 (4.11) in placebo cohort 1 group (p=0.029) and -3.16 (2.59) in istaroxime 1.0 µg/kg/min group versus -1.08 (2.72) in placebo cohort 2 group (p=0.009).

Part A of 04-CL-1904

Study 04-CL-1904 Part A was the first part of a 2-part pilot, phase 2a multinational, randomized, double-blind, placebo-controlled safety and efficacy study aimed to assess to assess the ability of istaroxime to increase SBP in patients with early cardiogenic shock or pre-shock (Society for Cardiovascular Angiography & Intervention (SCAI) Stage B) defined as hospitalization for ADHF with persistent hypotension. A total of 60 subjects were enrolled into Part A of the study and treated for 24 hours with either 1.0 or 1.5 μg/kg/min dose of istaroxime or placebo, 29 subjects were treated with istaroxime and 31 received placebo.

Inclusion criteria included acute heart failure-related SCAI Stage B pre CS, 18 to 85 years of age, an ongoing hospitalization for ADHF, persistent hypotension (SBP 75-90 mmHg for at least two hours), left ventricular ejection fraction (LVEF) ≤ 40%, heart rate of 75 to 150 bpm and no need at time of screening or planned use for 6 hours thereafter of mechanical support or intravenous therapy to increase blood pressure. Per criteria for Stage B SCAI classification, patients with clinical signs of peripheral hypoperfusion, venous lactate > 2 mmol/L and/or on mechanical support or treatment with intravenous vasodilators, inotropes or vasopressors were excluded. Other exclusion criteria included concomitant or planned treatment with oral digoxin (could be randomized if the plasma concentration of digoxin at screening was <0.5 ng/mL); acute coronary syndrome or stroke within the past 3 months; coronary artery bypass graft or percutaneous coronary intervention within the past month or planned in the next month; life threatening ventricular arrhythmia or implantable cardiac defibrillator (ICD) shock within the past month; sustained ventricular tachycardia in the last 3 months or uncontrolled arrhythmia; fever >38°C; estimated glomerular filtration rate (eGFR) <30 mL/min/m2; serum potassium >5.3 mmol/L or <3.5 mmol/; stroke or transient ischemic accident (TIA) within 3 months; and acute respiratory distress syndrome.

The original protocol had a target and maximum dose of istaroxime of 1.5 μg/kg/min; however, after 26 of the 60 patients were recruited, the Sponsor and Executive Steering Committee amended the protocol to limit the dose of istaroxime to 1.0 μg/kg/min, after which all patients were to receive a target and maximum dose of 1.0 μg/kg/min of istaroxime. The infusion rate could be decreased at the discretion of the investigator based on the development of tolerability issues (such as nausea), significant bradycardia, or greater than desired BP elevation. All subjects were to receive standard of care.

During the study, treatment emergent adverse events (TEAEs) were observed in 27 patients (93%) in the istaroxime group vs. 25 (81%) in placebo. There were more TEAEs of nausea in the istaroxime group: 8 (28%) vs. 2 (6%), vomiting: 4 (14%) vs. 0 (0%) and infusion site pain: 4 (14%) vs. 0 (0%) in the istaroxime patients compare to placebo, respectively. There were 6 SAEs in both

the istaroxime and placebo-treated patients. Cardiac SAEs were most common with 4 (14%) events in the istaroxime group and 5 (16%) events in the placebo group. In a post hoc analysis, there was a trend toward more SAEs in the 1.5 µg/kg/min group compared to the 1.0 µg/kg/min group. There were 5 deaths in the study (primary cause of death in parentheses) with 1 (3%) death in the istaroxime 1.0 µg/kg/min group (ventricular tachycardia), 3 (23%) deaths in the istaroxime 1.5 ug/kg/min group (worsening of heart failure 2, cardiac arrest 1), and 1 (3%) death in the placebo group (worsening of heart failure).

The primary endpoint, the adjusted area under the curve (AUC) change in SBP from time of treatment to 6 hours was significantly improved in the istaroxime group. The result for the istaroxime group was 53.1 (SE 6.88) mmHg·hr versus 30.9 (SE 6.76) mmHg·hr in placebo group ($p = 0.017$). Compared to the placebo group, the adjusted 24-hour SBP AUC ($p=0.025$) and the adjusted changes in SBP at 24 hours ($p< 0.001$) were significantly improved in the istaroxime group. In a post-hoc analysis of SBP AUC to 6 hours individually evaluating the 1.0 µg/kg/min and 1.5 µg/kg/min dose compared to placebo, results comparing the two istaroxime groups were not significantly different. Echocardiographic revealed that the change in cardiac index at 24 hours was significantly improved in the istaroxime group compared to placebo ($+0.16 \pm 0.1$ vs -0.06 ± 0.1 L/min/m^2; $p=0.016$).

This study showed that istaroxime significantly improved blood pressure in patients with SCAI stage B pre-cardiogenic shock due to acute decompensated heart failure. Echocardiography revealed that istaroxime resulted in improvement in cardiac index compared to placebo. Istaroxime was generally well tolerated with no worsening of arrhythmias or renal function, and no significant differences in changes in troponin or lactate. A post hoc analysis of SBP AUC to 6 hours evaluating the 1.0 µg/kg/min and 1.5 µg/kg/min doses individually compared to placebo revealed similar efficacy results. Regarding safety, a post hoc analysis revealed a trend toward more SAEs in the 1.5 µg/kg/min group compared to the 1.0 µg/kg/min group.

2.4 Risk/Benefit Assessment

2.4.1 Known Potential Risks

Risks for Study 04-CL-1904 must be considered within the context of treating CS and pre-CS in adults, which carries substantial risk of morbidity and mortality. Mortality for subjects hospitalized with AHF is substantial, nearly 5% during the index hospitalization and over 20% at one year (23). The highest mortality rates in AHF are seen in subjects with SBP <85 mmHg (34.8%) which

progressively decreases in relation to increases of SBP at presentation (SBP 85-110 mmHg ~29%; SBP 110-140 mmHg ~21%; SBP >140 mmHg 17.4%) (23).

2.4.2 Known Potential Benefits

All subjects enrolled in this study will receive standard of care therapy for heart failure and pre-/cardiogenic shock. Additionally, approximately half of the subjects will be randomized to receive istaroxime. Istaroxime has the potential to increase blood pressure and improve cardiac function and has not been associated with clinically important side effects such as tachyarrhythmias and/or increased mortality in the phase 2 studies completed to date.

2.4.3 Assessment of Potential Risks and Benefits

As described above, patients with CS and pre-CS are at high risk of morbidity and mortality, despite treatment in a hospital setting. Istaroxime has the potential to improve cardiac function without the side effects or increase in mortality that has been observed in other treatments (Section 2.3); thus, treatment with istaroxime outweighs the risk.

Subjects enrolled will be treated in a hospital setting and will be closely monitored. If needed, subjects will receive rescue medicine or therapy as described in Section 6.5.2.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
The primary objective of this study is to assess the ability of istaroxime to increase SBP in subjects with SCAI Stage B pre-CS, defined as hospitalization for ADHF with persistent hypotension (SBP 70-100 mmHg for two hours); for at least 6 hours prior to Screening are not on cardiovascular, respiratory, or renal mechanical support; and have not received IV vasopressors or inotropes.	Change from baseline in SBP AUC at 6 hours	Blood pressure is a predictor and determinant of end organ dysfunction and the quantitative relationship between improvements in systolic blood pressure and a drop in renal dysfunction and/or failure justify that this is a reasonable and valid surrogate for clinical endpoints. In addition, results from this study will inform the design of future trials.
Secondary Efficacy		
To assess other measures of efficacy to ensure consistency of results across multiple endpoints.	**Key Secondary Efficacy Variables:** • SBP AUC to 12 hours from start of infusion for subjects in Part B. • SBP AUC to 24 hours from start of infusion for subjects in Parts A and B. • SBP AUC to 48 hours from start of infusion for subjects in Part B. • SBP AUC to 60 hours from start of infusion for subjects in Part B.	These endpoints represent endpoints that are appropriate given the trial design and were also assessed during the conduct of the earlier istaroxime trials.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	Other Secondary Efficacy Variables: • Treatment-failure score, based on death, circulatory, respiratory, or renal mechanical support or intravenous inotrope or vasopressor treatment, and changes in systolic blood pressure. • Change from baseline in SBP at 6, 12, and 24 hours of treatment for both parts, and at 48 and 60 hours of treatment for Part B. • Number of subjects with increases from baseline in SBP ≥ 5% and ≥ 10 mmHg at a timepoint between 4-6 hours after dosing and at least one other measurement separated by ≥ 2 hours during the 24- or 60-hours infusion. • Number of subjects requiring treatment with intravenous vasopressors, inotropes, and/or mechanical cardiac or renal support or have died in the period from randomization to 24 or 48 hours (for subjects in Part A and B, respectively) and Day 5 (“treatment failure”). • Changes in quality of life measured by the EQ-5D from baseline to Day 5 (96 hours) from infusion start and at Day 30 (Part A only).	

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	• Change from baseline in Creatinine clearance at 24, 48, 72 and 96 hours from infusion start. • Change from baseline and observed heart rate measurements at 12, 24, 48, 72 and 96 hours from infusion start. • Change from baseline and observed mean arterial pressure (MAP) at 12, 24, 48, 72 and 96 hours from infusion start. • Change from baseline and observed brain natriuretic peptide (BNP), NT-pro-BNP, troponin (cTn; either T or I) and venous lactate at 12, 24, 48, 72 and 96 hours from infusion start. • Time to worsening heart failure through Day 5. • Time to HF re-admission or death through Day 30. • Length in ICU/length of initial hospitalization. • Days alive and out of acute care (including all intensive acute care units). • Days alive and out of the hospital through Day 30 • Number of subjects with hospital re-admissions through Day 30. • Mortality and reasons for death through Day 30.	

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	• Changes in invasive hemodynamic parameters from pre-treatment to 3, 6, 12, 18, 24, 48, 54, and 60 hours. • Number of subjects with CS SCAI stage progression through Day 30. • Echocardiographic measurements at 24, 36, 48, 60, and 72 hours. Changes in measurements will be assessed when data is available. **Pharmacokinetics:** • PK assessments of istaroxime and its primary metabolites (PST 2915, PST 2922, and PST 3903). In addition, diastereoisomers of istaroxime, PST 2890 (E) and PST 2892 (Z), will be measured and assessed. For Part A: blood samples at 6, 12, and 24 hours after infusion start and 0.25, 0.5, 1, 6, 12, and 24 hours after infusion end. For Part B: blood samples at 6, 12, 24, 36, 48, 52, 56, 60 hours after infusion start and 12, 36, 48 hours after infusion end and at discharge.	
Safety		
To ensure that administration of istaroxime does not lead to safety concerns.	• Incidence of adverse events (AEs) and serious AEs (SAEs) • Clinically significant arrythmias (arrythmias requiring intervention) for a	Standard measurements of safety for pre-CS and heart failure treatment.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	total of 72 hours from the start of infusion as determined by a Holter monitor (during the infusion and after the infusion has been stopped.	

4 STUDY DESIGN

4.1 Overall Design

This is a pilot, multinational, multicenter, randomized, double-blind, placebo-controlled, safety and efficacy study. Subjects will consist of males or females 18 to 85 years of age hospitalized for ADHF with persistent hypotension (systolic blood pressure [SBP] 70 to 100 mmHg for one hour), heart rate 75 to 150 beats/minute, and mild to moderate renal impairment. Subjects currently, and since admission to the hospital and throughout Screening, have not received IV vasopressors, inotropes, or digoxin, or cardiovascular, respiratory, or renal mechanical support. All subjects will receive standard of care. Sites from the United States, Europe, South America, and/or Asia will participate in this study.

For Part A, eligible subjects will be randomized into one of two groups in a 1:1 ratio: 1.0 µg/kg/min istaroxime or placebo (50 mg lactose lyophilized powder), administered via 24-hour infusion.

For Part B, eligible subjects will be randomized into one of three groups in a 1:1:1 ratio: 1.0 µg/kg/min istaroxime for 6 hours, followed by 0.5 µg/kg/min for 42 hours followed by 0.25 µg/kg/min istaroxime for 12 hours; 0.5 µg/kg/min istaroxime for 48 hours followed by placebo IV infusion for 12 hours; or matching placebo (50 mg lactose lyophilized powder) as a 60-hour infusion.

See Section 1.3, Schedule of Activities, for more details on study measurements and the timing of measurements.

4.2 Scientific Rationale for Study Design

The major aim of an intervention in pre-CS is to interrupt or reverse the hemodynamic deterioration that characterizes CS. It is well documented that patients with AHF, a lower SBP at hospital admission is associated with worse outcomes and higher mortality rates. Also, many physiological parameters may be changing simultaneously in patients with ADHF and early CS. Intravascular volume status is changing as the patient is being diuresed; this can affect cardiac preload and filling pressures. Such a change along with administration of a drug with inotropic and lusitropic effects can make interpretation of physiological data difficult. Similarly, blood pressure changes can be impacted by drug effect, volume changes or changes in systemic vascular resistance.

Earlier clinical studies in early cardiogenic shock with istaroxime showed a meaningful increase in BP mediated by improved systolic and diastolic cardiac function without increasing heart rate, risk of arrythmias or impaired renal function.

In part B of this study, more robust data collection including required hemodynamic assessments obtained using a pulmonary artery catheter, more frequent cardiac assessments via echocardiography, and frequent BP measurements, will help to elucidate the complicated cardiac physiology in these patients and the specific effects of istaroxime so the use of this novel compound can be optimized.

4.3 Justification for Dose

An istaroxime dose of 1.0 µg/kg/min as a continuous infusion up to 24-hours has been shown to be generally safe and well tolerated based on previous completed studies with istaroxime administered to patients and volunteers. In Part A of the 04-CL-1904 study, istaroxime has been administered as a continuous infusion at a dose of 1.0 and 1.5 ug/kg/min in patients with Stage B cardiogenic shock. For the Part B amendment, the infusion of 1.0/0.5/0.25 µg/kg/min will be extended to 60-hours. Part B will evaluate whether a longer period of continuous infusion of istaroxime will provide significant clinical benefit for patients with Stage B cardiogenic shock and be associated with a satisfactory safety profile. Part B will also evaluate whether dose regimen of 1.0 ug/kg/min, followed by 0.5 ug/kg/min, and finally by 0.25 ug/kg/min can provide a smoother physiological transition as the istaroxime infusion is discontinued. The justification of the dose is based on pre-clinical and clinical data.

Preclinical data

Good Laboratory Practice (GLP)-compliant 24-hour continuous IV infusion studies have been conducted in rodent (Sprague Dawley rats) and non-rodent (Beagle dogs) species for up to 14 days. The no-observed-adverse-effect-levels (NOAEL) for rat and dog provided an estimated safety margin of 14-fold and 1-fold on a body weight (BW) basis, respectively, for the 1.0 µg/kg/min dose. These NOAEL values for $AUC_{0\text{-}342hr}$ and $AUC_{0\text{-}360hr}$ were 16,739 ng·hr/mL and 154.4 ng·hr/mL in rat and dog, respectively.

The istaroxime toxicity profile appears qualitatively consistent across species, including signs of cardiac toxicity, and is likely related to the istaroxime pharmacological activity, which may be amplified in healthy animals, but needed for efficacy in cardiogenic shock or AHF patients. Istaroxime doses cannot be increased in dogs due to monitorable and reversible dose-limiting

toxicities, for which dogs appear to be particularly sensitive. In both rats and dogs, the toxicity profile of istaroxime does not change with increased infusion duration (14 days vs 24-hour), supporting the extension of istaroxime treatment duration beyond a 24-hour infusion in the clinic.

Pharmacokinetics

An integrated population PK model was developed to describe the plasma concentrations of istaroxime and formation and elimination of its primary metabolites (PST2915, PST2922, and PST3093) after single IV dose of 0.25 to 1 µg/kg/min infused 24 hours in healthy subjects, or 0.5 to 1.5 µg/kg/min infused over 6 to 24 hours in AHF patients.

Based upon modeling parent and metabolite data, istaroxime is rapidly eliminated via both metabolic and non-metabolic pathways. PST2922 generally had a similar PK profile as parent istaroxime, while PST2915 and PST3093 both exhibited slower formation and elimination relative to PST2922. A formal covariate analysis was performed, and the following parameter-covariate relationships were statistically significant: LVEF ≤30% and females had lower CL for parent istaroxime.

The final population PK model was used to simulate exposures of varying doses and infusion durations. Using this model, istaroxime exposure $AUC_{0\text{-}60hr}$ after 6 hours at 1.0 µg/kg/min (127.75 ng·hr/mL) plus 42 hours at 0.5 µg/kg/min (447.125 ng·hr/mL) plus 12 hours at 0.25 µg/kg/min (63.875 ng·hr/mL) (Part B dose) was estimated to be 638.875 ng·hr/mL. For reference, the PK model projects istaroxime $AUC_{0\text{-}24hr}$ after 24-hour infusion at 1.0 µg/kg/min to be 511 ng·hr/mL (Part A dose). Based on the above PK data, the profile of istaroxime has been appropriately characterized to support clinical dosing of istaroxime for infusion durations greater than 24 hours.

Clinical Safety

Part B to this protocol has been reviewed by the study's independent Data Monitoring Committee (DMC). This group has been closely following the emerging safety profile generated in Part A of this study and has approved extending this study with Part B.

Rationale for Extended Dosing

Six- and 24-hour infusions of istaroxime have produced reproducible and desirable physiological changes in patients with severe acute decompensated heart failure. In the 24-hour infusion study, there was evidence of dose related reductions in hospital length of stay and other clinically relevant outcome assessments. Longer dosing may enhance the impact of istaroxime on these clinical outcomes. As hospitalization is associated with increased risk for poor outcomes in these patients,

it is important to take advantage of the hospitalization to maximize the patients' condition and reverse addressable components of the underlying disease pathophysiology. Increasing the duration of istaroxime infusion may contribute to that endeavor.

Acute heart failure is characterized by both systolic and diastolic dysfunction, and improvement in both of these parameters is important in patients with acute decompensated heart failure. Istaroxime, by its novel mechanism of action, may improve both systolic and diastolic dysfunction. Dose related increases in blood pressure have also been observed in acute heart failure patients treated with istaroxime. Thus, unlike multiple other drugs used in acute heart failure, improvements in cardiac function with istaroxime have not been at the expense of an undesirable drop in blood pressure. Furthermore, renal function has been preserved or improved in the hospitalized heart failure patients studied in the phase 2 program. This is an important characteristic as critically needed diuresis for patients with severe decompensated heart failure and pulmonary edema is commonly limited by low blood pressure.

There is a strong unmet need for effective therapies for patients with Stage B cardiogenic shock. A substantial proportion of acute heart failure patients are discharged with persistent congestion (26). These patients have higher readmission and higher mortality rates than those who are discharged adequately diuresed. Stage B cardiogenic shock patients are at risk for further deterioration and progression to classic cardiogenic shock (Stage C). Longer istaroxime infusion may contribute to more effective stabilization, reversal of deterioration and more successful re-equilibration in these patients. This incremental extension to the dosing regimen will explore the safety and physiological changes associated with a longer infusion period.

4.4 End of Study Definition

A subject is considered to have completed the study if he or she has completed all phases of the study, including the last scheduled procedure at Day 30, as shown in the Study Schema (Section 1.2) and Schedule of Activities (SoA) (Section 1.3), or if the subject dies.

The end of the study is defined as completion of the last visit or procedure for the last subject in the trial (last subject/last visit).

5 STUDY POPULATION

5.1 Inclusion Criteria

To be eligible to participate in this study, each potential subject must meet the following criteria:

1. Clinical presentation consistent with SCAI Stage B pre-cardiogenic shock caused by acute decompensation of chronic systolic heart failure (due to arterial hypertension, ischemic heart disease or dilated cardiomyopathy), without evidence for an acute coronary syndrome.
2. Signed informed consent form (ICF);
3. Males and females, 18 to 85 years of age (inclusive);
4. Admission for an ADHF episode within 36 hours prior to randomization, defined as:
   a. Dyspnea, at rest or with minimal exertion,
   b. Congestion on chest x-ray or lung ultrasound with BNP ≥ 400 pg/mL or NT-proBNP ≥ 1400 pg/mL;
5. History of left ventricular ejection fraction (LVEF) ≤ 40%;
6. Persistent hypotension defined as
   a. SBP of 75 to 90 mmHg (Part A) or 70 to 100 mmHg (Part B) for ≥ 2 hours prior to Screening;
   b. Stable SBP, defined as no decrease in SBP by > 7 mmHg on two separate measurements during the last 2 hours prior to randomization;
7. Heart rate 75 to 150 bpm. If the subject is on a beta-blocker, the range is 60 to 150 bpm;
8. Echocardiogram during initial hospitalization confirming ejection fraction ≤ 40% and no evidence of other pathology to confound interpretation of cardiac physiology (e.g., pericardial effusion);
9. Subject is monitored by a PAC at the time of randomization (Part B only).

5.2 Exclusion Criteria

A potential subject meeting any of the following criteria will not be allowed to participate:

1. Cardiogenic shock of SCAI stage C or worse
2. Cardiogenic shock due to any other condition besides acute decompensation of chronic systolic heart failure.
3. Any of the following in the past 60 days: acute coronary syndrome, coronary revascularization, MI, CABG, or percutaneous coronary intervention;
4. Current (within 6 hours of Screening) or anticipated need for treatment with positive inotropic agents or vasopressors, renal support including ultrafiltration, or mechanical circulatory, ventilatory or renal support (intra-aortic balloon pump, endotracheal intubation, mechanical ventilation, or any ventricular assist device);

5. Venous Lactate > 2 mmol/L;
6. History of heart transplant or UNOS priority 1a heart transplant listing;
7. Ongoing treatment with digoxin (if digoxin was stopped before signing the ICF and the digoxin plasma level is < 0.5 ng/ml, the patient may be enrolled);
8. Severe renal impairment (eGFR < 30 ml/min by the MDRD formula);
9. Hypersensitivity to the study medication and its excipients (including known lactose hypersensitivity) or any related medication;
10. Stroke or TIA within 3 months;
11. Active coronary ischemia;
12. Any significant valvular disease (including any moderate or severe valvular stenosis, moderate or severe aortic or pulmonary regurgitation, stenosis or regurgitation);severe tricuspid or mitral regurgitation);
13. Primary hypertrophic or restrictive cardiomyopathy or systemic illness known to be associated with infiltrative heart disease;
14. Admission for AHF triggered primarily by a correctable etiology such as significant arrhythmia (inclusive of atrial fibrillation as the main reason for admission), infection, severe anemia, acute coronary syndrome, pulmonary embolism, exacerbation of COPD, planned admission for device implantation, or over-diuresis as a cause of hypotension;
15. Pericardial constriction or active pericarditis;
16. Life-threatening ventricular arrhythmia, uncontrolled arrythmia, or implantable cardioverter defibrillator (ICD) shock or history of sudden death within 6 months;
17. Cardiac resynchronization therapy (CRT), ICD, ablation, or pacemaker implantation (or planned implantation) within the past 3 months;
18. Sustained ventricular tachycardia in the last 3 months with no defibrillator;
19. Sustained hypotension (SBP < 70 mmHg) for at least 30 minutes from the time of arrival to the hospital;
20. Severe pulmonary disease or cor pulmonale or other causes of isolated right-sided HF or not related to left ventricular dysfunction;
21. Acute respiratory distress syndrome;
22. Suspected sepsis; fever > 38°C or active infection requiring IV antimicrobial treatment;
23. Body weight < 40 kg or ≥ 150 kg;
24. Laboratory exclusions:
    a. Hemoglobin < 9 g/dl,
    b. Platelet count < 100,000/µl,
    c. Serum potassium > 5.3 mmol/l or < 3.5 mmol/l;
25. A life expectancy < 3 months based on the judgment of the investigator;
26. Uncontrolled thyroid disease;
27. Pregnant or breast-feeding;

28. Ongoing drug/alcohol abuse;
29. Participation in another interventional study within the past 30 days.

5.3 Lifestyle Considerations

The use of istaroxime in women of childbearing potential not using contraceptive measures should be avoided. Women of childbearing potential must have been on effective contraception for the last three months and must also have a negative pregnancy test (β-hCG) recorded prior to administration of istaroxime and a pregnancy test will also be conducted at the end of the study. Women will be considered of childbearing potential unless they have been post-menopausal for > 12 months or are surgically sterile (e.g., hysterectomy, oophorectomy).

Men and women of childbearing potential will be required to use an acceptable method of birth control throughout the study and for 30 days following discharge from the hospital. Acceptable forms of birth control include bilateral tubal ligation (women), vasectomy (men), oral contraceptive, contraceptive patch, anovulants without estrogen, intradermal contraceptive implant with progesterone, progestogen injections every three months, or double-barrier methods (intra-uterine device, female condom, male condom). Periodic abstinence (trying to lower the chances of pregnancy by the calendar, ovulation, post-ovulation or symptothermal methods) and withdrawal are not acceptable methods of contraception.

Dosage of oral HF medications must remain stable for at least 6 hours before Screening and during the entire treatment period (24-hour or 60-hour infusion). Concomitant medications should be administered at least 2 hours prior to Screening and after at least 6 hours from the initiation of study drug infusion, if possible.

The use of treatment with intravenous positive inotropic agents or vasopressors, renal support including ultrafiltration, or mechanical ventilatory or circulatory support (intra-aortic balloon pump, endotracheal intubation, mechanical ventilation, or any ventricular assist device); at any time through Day 5 are considered rescue therapy. If rescue therapy is being administered the study drug infusion may be continued at the investigator's discretion.

5.4 Screen Failures

Screen failures are defined as patients who consent to participate in the clinical trial but are not subsequently assigned to a study intervention or entered in the study. 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 will include the reason for screen failure and may include demography, eligibility criteria, and any serious adverse event (SAE).

5.5 Strategies for Recruitment and Retention

Subjects will be recruited from each institution based upon inpatient admission for ADHF. Patients who wish to enroll will go through the informed consent process; patients will be reminded they may withdraw consent at any time.

No overall recruitment strategies are planned; any strategies employed by a site must be approved by Windtree and their IRB/IEC and be included in their site regulatory binder (with a copy sent to Windtree).

6 STUDY DOSING AND ADMINISTRATION

6.1 Treatments Administered

6.1.1 Study Intervention Description

Istaroxime and matching placebo will be provided as a lyophilized powder in glass vials and will be appropriately labeled with the investigation caution statements required by the regional health authority (e.g., FDA, EMA) to ensure that users are aware that the product is limited by federal law to investigational use only. NaCl 0.9% bags for preparation of the 24- or 60-hour infusion will be provided by the site. The infusion pumps, sets, filters, and connections will be provided by the investigational site.

Informed consent must be obtained before any study procedures are carried out; however, study procedures that are part of standard of care may be performed prior to having signed informed consent.

All subjects will receive standard of care. All subjects in Part B will be required to have a PAC.

6.1.2 Dosing and Administration

For Part A, eligible patients will be randomized to the study to receive either istaroxime or placebo (lactose) in a 1:1 ratio:

Group 1: istaroxime IV infusion over 24 hours
Group 2: placebo IV infusion over 24 hours

For Part B, eligible patients will be randomized to the study to receive either of 2 istaroxime regimens or placebo (lactose) in a 1:1:1 ratio:

Group 1: istaroxime IV infusion at 1.0 µg/kg/min for 6 hours, 0.5 µg/kg/min for 42 hours, followed by 0.25 µg/kg/min for 12 hours.
Group 2: istaroxime IV infusion at 0.5 µg/kg/min for 48 hours, followed by placebo IV infusion for 12 hours.
Group 3: placebo IV infusion over 60 hours.

After istaroxime administration is started, if the subject develops tolerability issues such as significant nausea, antiemetics may be administered and the infusion rate may be decreased. The infusion rate may also be decreased at the discretion of the investigator based on the occurrence of an adverse event, the development of significant bradycardia, or greater than desired blood pressure elevation.

In order to calculate the amount of istaroxime given to each patient in each cohort, the body weight recorded at screening will be used for calculation of the syringe infusion rate in ml/hour of istaroxime (see Appendix D in the Study/Pharmacy Manual). The study drug will be administered to the subject via a syringe pump.

Just prior to dosing (< 15 minutes), the vital signs, including blood pressure, will be collected.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

Both istaroxime and placebo will be shipped to the sites from a supply depot. Site pharmacies will be stocked with an initial supply, with re-supply based on pre-set lower limits. See the Study/Pharmacy Manual for more details. When the supply is received from the depot, the pharmacist or designee must log in to the IRT system to "receive" the study supply so that it is available to be dispensed.

All these boxes will be uniformly packaged, and the vials will be tightly covered by study special label. The clinical study, drug serial numbers, expiry date, usage method and warnings will be stated on the labels.

6.2.2 Formulation, Appearance, Packaging and Labeling

The test drug istaroxime (10 mg test drug with 50 mg lactose monohydrate) and the placebo (50 mg of lactose) are contained in glass vials of 7 ml each as lyophilized powder. Istaroxime and placebo vials are reconstituted with 5 ml saline for injection.

Istaroxime is an original derivative of androstanedione, a lipophilic steroid-based compound, chemically unrelated to cardiac glycosides or to phosphodiesterase inhibitors. IUPAC name: Androstane-3,6,17-trione (E,Z)-3-[*O*-(2-aminoethyl)]oxime hydrochloride (C_{21} H_{33} Cl N_2 O_3). The active substance is a 1:1 mixture of E and Z isomers at the oximic C(3)=N double bound.

6.2.3 Product Storage

Istaroxime and placebo vials should be stored at controlled room temperature equal to or below 25°C (77°F) in a secure location. Do not refrigerate or freeze. Protect from light.

Additional details on product storage, shipping, and administration can be found in the Study/ Pharmacy Manual.

6.2.4 Preparation

Details for study drug preparation can be found in the Study/Pharmacy Manual. Briefly, both istaroxime and placebo are reconstituted by adding 5 ml of saline for injection to the vial, after which the vial is gently inverted to mix the suspension.

Preparation of the solution for infusion will be performed by a physician, pharmacist, or trained designee, using the kit or kits assigned. A record for the preparation of the infusion will be kept for each subject according to the research center's standard operating procedure.

The test drug istaroxime (10 mg test drug + 50 mg lactose) and the placebo (50 mg of lactose) are contained in glass vials of 7 ml each as lyophilized powder. The investigational drug and placebo will be provided as dosing kits containing the study boxes needed for each Part of the study.

Refer to the study/pharmacy manual for details on the preparation, storage, and administration of istaroxime and placebo.

6.3 Measures to Minimize Bias: Randomization and Blinding

To minimize bias in subject assessments, study treatment will be blinded to the study staff. Istaroxime and placebo are both lyophilized powders and are put into identical vials. Each study box will be numbered with a unique identifier, which will not allow the study staff to ascertain which treatment is being used. Details of the blinding procedure are provided in the study Blinding Maintenance and Assurance Plan and Statistical Analysis Plan documents.

In all circumstances, subject safety takes precedence over maintenance of study blinding. If, at any point, blinding interferes with emergent patient care or knowledge of study treatment is required for patient management, then the blind should be broken. If this occurs, Windtree must be notified as soon as possible.

6.4 Study Intervention Compliance

Study treatments will be administered in an in-patient setting (i.e., hospital) by trained health care practitioners. Completion of study therapy for each treatment and re-treatment will be monitored; no other compliance measurements will be employed. The study drug will be administered in a double-blinded manner, unless unblinding by the PI is necessary for the safety of the subject.

6.5 Concomitant Therapy

Whenever possible, the generic name should be recorded; name brands can be used for combination products.

6.5.1 Concomitant Therapy

If the investigator feels it is likely that a patient will need rescue therapy (see Section 6.5.2) within 6-hours after randomization, the patient should not be randomized. All attempts should be made not to administer any treatments during the first 6 hours after randomization and especially positive inotropic agents, mechanical ventilatory, circulatory support or renal support devices, including hemofiltration**.** Dosage of intravenous diuretics and/or oral HF medications should remain stable for at least 2 hours before screening and during the entire treatment period (24-hour [Part A] or 60-hour [Part B] infusion) after the start of infusion on Day 1; unless changes are required due to clinically relevant variations of the subject's condition. Administration of medications prior to admission and concomitant medications/therapies (i.e., medications ongoing at admission and with a start date between admission and Day 30) must be reported on the appropriate medication pages of the CRF, including dosage information, dates and time of administration, and indication. The international non-proprietary name should be used for concomitant medications; however, the trade name may be used for combination products.

6.5.2 Rescue Medicine or Therapy

Any introduction of new treatment with intravenous positive inotropic agents and vasopressors, renal support including ultrafiltration, or mechanical ventilatory or circulatory support (intra-aortic balloon pump, endotracheal intubation, mechanical ventilation, or any ventricular assist device) from randomization and until Day 5 they will be considered "rescue therapy". If such therapies are administered the subject will be considered a treatment failure.

Rescue medications or therapies will be recorded on the concomitant medication page.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Study treatment may be interrupted at any time as circumstances dictate (e.g., subject becomes agitated). In all cases, emergent care of the subject takes precedence over study treatment. If study treatment is interrupted or stopped, the reason for doing so must be recorded. In case of adverse events the investigator may elect to down titrate or discontinue study medication (either temporarily or permanently). Study medication can be reinitiated at the initial dose, or a reduced dose as long as study drug is not administered beyond 24 hours (Part A) or 60 hours (Part B) from initiation of infusion.

Discontinuation from study treatment does not mean discontinuation from the study, and the remaining study procedures and assessments should be completed as indicated. If a clinically significant finding is identified after enrollment (including, but not limited to changes from baseline), the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an AE.

7.2 Participant Discontinuation/Withdrawal from the Study

The subject is free to withdraw consent from participation in the study at any time.

An investigator may discontinue or withdraw a study drug if any clinical AE or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject. Prior to study drug discontinuation, the investigator may attempt, if appropriate, to reduce the study drug infusion rate. Data collection will continue until the end of the study whenever possible unless the subject or legally authorized representative specifically requests otherwise. Subjects will not be discontinued from follow up unless they withdraw consent.

The reason for study drug dose reduction, discontinuation or subject discontinuation or withdrawal from the study will be recorded on the end of study eCRF page. Subjects who withdraw or are withdrawn/discontinued from the study will not be replaced.

7.3 Lost to Follow-Up

All efforts should be undertaken to ensure that no subject is lost to follow-up. It is expected that subjects will remain in the study site until at least Day 5, with the next scheduled visit at Day 30.

Prior to Day 30, the site will contact the subject to schedule the visit. If the subject cannot be reached, at least 3 additional attempts must be made (at least one contact using telephone and at least one using email). If the subject still cannot be reached, a certified letter should be sent to the subject. If contact cannot be re-established after the certified letter, the subject will be considered lost to follow-up 1 week before the final database lock for the study.

8 STUDY ASSESSMENTS AND PROCEDURES

A table of study procedures and activities is presented in Section 1.3.

8.1 Efficacy Assessments

8.1.1 Screening Process

Within a maximum of 24 hours before randomization (to either istaroxime or placebo), medical screening will be performed on all prospective patients to assess suitability for the study. Prior to conducting any study specific procedures, the investigator or his/her designee will explain the study fully to the patient or legally authorized representative and provide him/her with a copy of the Patient Information Sheet and Informed Consent document. If the patient is willing to participate in the study, s/he and the investigator or his/her designee will both sign the Informed Consent Document and a copy of the signed document will be kept by the patient (see Section 10.1.1).

8.1.1.1 Demographics and Baseline Characteristics

The subject's basic and demographic/anthropometric data such as age, sex, race (to estimate the eGFR parameter), height, weight, and ethnicity, and information about substance use/abuse will be recorded by direct exchange with patient or legally authorized representative.

8.1.1.2 Medical History

During Screening, medical history will be documented, including atrial fibrillation, medication history for the last 30 days. In addition, the most recent previous admission for heart failure will be collected and chronic oral treatment with digoxin and other medications (e.g., diuretic and/or vasodilator) will be recorded.

8.1.1.3 Vital Signs, ECG and Arrythmia monitoring.

At Screening, vital signs (including blood pressure, weight, body temperature and oxygen saturation), and 12-lead ECG (an ECG performed within 3 hours before the subject signed informed consent can be accepted) will be recorded. Continuous arrythmia monitoring will be collected twice: once during screening (from start of screening until randomization) and once during treatment (from start of infusion for 72 hours). Subjects will be placed on continuous arrythmia monitoring via a Holter monitor. Subjects will require real-time cardiac rhythm

monitoring in the screening period and should not be enrolled/randomized if monitoring reveals uncontrolled arrythmia.

Holter Monitoring

A digital Holter recorder is being used for this study for centralized arrythmia monitoring and to reduce the logistical complexity of acquiring the large number of ECGs that would otherwise be required. The data will be analyzed by a certified cardiography technician and reviewed by a cardiologist for arrhythmia, heart rate variability, and heart rate turbulence.

In addition, the hospital telemetry system will be used for real-time safety monitoring as well as for eligibility determination.

Blood Pressure Measurements

Blood pressure (SBP and DBP) should be assessed upon the start of screening and hourly throughout the screening process. The last two measurements before randomization will be recorded in the eCRF as "pre-randomization" and "2nd pre-randomization"; the SBP cannot have decreased by > 7 mmHg from the pre-randomization to the 2nd pre-randomization measurement.

Systolic and diastolic blood pressure should be measured as described in the Study/Pharmacy Manual (e.g., arterial line, manual sphygmomanometer).

8.1.1.4 Screening Laboratory Tests

Screening laboratory tests (at a local laboratory) will include standard blood chemistry (electrolytes [including calcium, potassium, magnesium], liver function tests, creatinine, urea or BUN, glucose, albumin, protein, estimated glomerular filtration rate [eGFR]), thyroid tests TSH, total T3, and total T4; hematology (complete blood count with differential); digoxin assay; biomarkers (levels of Troponin, NT-pro-BNP or BNP, and venous lactate); urinalysis; and serum pregnancy test (β-hCG) for females of childbearing potential.

8.1.1.5 Invasive Hemodynamic Parameters

All subjects in Part B will be required to have a pulmonary artery catheter (e.g., Swan-Ganz) in place prior to randomization (optional in Part A). Invasive hemodynamic measurements, inclusive of cardiac output, systemic vascular resistance (SVR), venous oxygen saturation, right atrial, pulmonary pressures, and wedge pressure, will be collected after randomization and before study drug administration. All hemodynamic measurements will be documented by monitor tracing of

pressures, wedging and thermodilution measurements. Those will be anonymized and sent for central adjudication by the study's medical monitor.

8.1.1.6 Echocardiography

An echocardiogram is required to qualify the subject for the study. The echocardiographic readings will be recorded and sent to a central laboratory for formal assessments. The following assessments should be recorded: LV end diastolic and systolic diameters (LVEDD and LVESD, respectively), LV end diastolic and systolic volumes (LVEDV and LVESV, respectively), LV ejection fraction (LVEF), 2D global longitudinal strain, left atrium diameter (LAD), left atrium area (LAA), left atrium volume (LAV), mitral regurgitation, ERO, cardiac index (CI), stroke volume index (SVI), ejection time, left ventricular outflow tract velocity time integral (LVOT VTI), E, A, E/A ratio, Sa, Ea (also known as e′), E/Ea (or E/e′) ratio, PAPs, TAPSE, right ventricle Sa, inferior vena cava diameter (VCD), and cardiac output (CO).

8.1.1.7 Biomarkers

Blood samples for assessment of BNP, NT-proBNP, and cTnT will be drawn just prior to dosing for possible measurement at a central laboratory.

8.1.1.8 Physical Examination

A physical examination will be conducted during Screening. Any relevant clinically significant abnormalities noted prior to randomization should be recorded in the eCRF as part of the medical history. Physical assessment will include signs of heart failure, with particular attention to evidence of congestion including dyspnea on exertion or at rest, orthopnea, rales, jugular venous pulse (JVP) and peripheral edema.

8.1.1.9 EQ-5D (Part A Only)

The EQ-5D is a standardized quality of life instrument developed by the EuroQoL Group (www.euroqol.org) and consists of a descriptive system of five dimensions – mobility, self-care, usual activities, pain/discomfort, and anxiety/depression – and a visual analog scale (VAS) on which the subject rates his or her health. The questionnaire is two pages long, which should take only a few minutes to complete (24). Published studies support the validity and reliability of the EQ-5D as an outcome measure in cardiovascular disease (24). The first page asks the subject to indicate his/her health state for each of 5 dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). The second page asks the subject to rate his/her health on a vertical visual analogue scale (EQ-VAS), where the endpoints are labeled "Best imaginable

health state" at the top and "Worst imaginable health state" at the bottom. Validated translations of the EQ-5D for self-completion are available in multiple languages. The questionnaire should be completed in Part A by the subject prior to any invasive or demanding study or routine procedures, e.g., blood draws, if possible. The questionnaire should be administered to the subject in a language in which the subject is fluent, using an official validated translation, and the same language should be used for both baseline and follow-up assessments. The EQ-5D will be collected in Part A after randomization but before study drug administration.

8.1.1.10 Randomization

Once eligibility has been confirmed (i.e., all inclusion criteria and none of the exclusion criteria met), the subject will be randomized using interactive response technology (IRT). Following randomization, infusion will proceed once the solution has been prepared (see the Study/Pharmacy Manual for reconstitution and preparation instructions).

8.1.2 Treatment and Post-Treatment Periods

Just prior to dosing (within approximately 15 minutes of infusion start), vital signs, cTn, NT-proBNP or BNP, ECG, EQ-5D (Part A only), physician assessment of heart failure, invasive hemodynamic measures, and venous lactate will be assessed. In addition, blood samples for centrally analyzed biomarkers and PK will be obtained and Holter monitoring (for 72 hours) will be started.

All assessments through Section 8.1.2.7 will be performed, if feasible, prior to any premature study drug discontinuation. The Day 30 visit should take place within 5 days of Day 30 (i.e., Day 30 ± 5 days).

8.1.2.1 Vital Signs

a. For Part A: blood pressure and heart rate will be measured at 0.5, 1, 2 hours and then hourly to 30 hours after infusion start and then at 32, 34, 36, 40, 44, 48, 60, 72, 84, and 96 hours, as well as Day 30.

   For Part B: measured hourly from the first hour to 24 hours after infusion start; then every 2 hours through 32 hours; every 4 hours from 32 to 48 hours; hourly from 48 to 66 hours; and then at 72, 84 and 96 hours, as well as Day 30.

   Systolic and diastolic blood pressure should be measured as described in the Study/Pharmacy Manual (e.g., arterial line, manual sphygmomanometer)

b. Temperature will be measured daily at 24, 48, 72, and 96 hours.

c. Oxygen saturation will be measured via pulse oximetry at 1, 2, 3, 4, 5, 6, 12, 18, 24, 30, 36, 48, 60, 72, and 96 hours post infusion initiation as well as Day 30.

d. Weight will be measured at 24, 48, 72, and 96 hours and at Day 30.

8.1.2.2 Local Laboratory Tests

Laboratory tests (at a local laboratory) will be performed daily at 24, 48, 72, and 96 hours and at Day 30. Laboratory tests include chemistry (electrolytes, liver function tests, creatinine, urea or BUN, glucose, albumin, protein, and eGFR), and hematology (complete blood count with differential).

The investigator can have unscheduled electrolytes performed at any time during the study if necessary for subject safety.

In addition, cTn, NT-proBNP or BNP, and venous lactate will be assessed at 12, 24, 48, 72, and 96 hours after infusion start and at Day 30 from randomization.

Serum pregnancy test will be performed at Day 30.

8.1.2.3 ECG and Arrythmia Monitoring.

12-lead ECG will be recorded at 12, 24, 48, 72, and 96 hours after infusion start and at Day 30.

Subjects will be placed on continuous arrythmia monitoring through a Holter monitor for 72 hours after randomization but prior to infusion start. The Holter monitor will be read centrally for significant findings and/or arrythmias (e.g., atrial fibrillation, supraventricular tachycardia, ventricular tachycardia). The individual subject data should be reviewed by the investigator and significant findings may be recorded in the eCRF as adverse events.

8.1.2.4 Invasive Hemodynamic Measurements

Invasive hemodynamic measures from the PAC will be collected at 3, 6, 12, 18, 24, 30 (Part A only), 48, 54, and 60 hours. Additional hemodynamic measurements may be recorded in subjects who develop hypotension, bradycardia, become congested, or are hemodynamically unstable, or may be obtained at the investigator's discretion.

8.1.2.5 Echocardiography

For subjects who will have a complete echocardiography performed at 12, 24, 30 (Part A only), 36, 48, 60, and 72 hours post infusion start and sent to a central laboratory for assessment. The following evaluation should be collected: LVEDD, LVESD, LVEDV LVESV, LVEF, 2D global longitudinal strain, LAD, LAA, LAV, mitral regurgitation, ERO, CI, SVI, ejection time, LVOT VTI, E, A, E/A ratio, Sa, Ea (or e′), E/Ea (or E/e′) ratio, PAPs, TAPSE, right ventricle Sa, inferior VCD, and CO. Additional echocardiograms may be performed as part of standard patient care at the investigator's discretion.

8.1.2.6 Biomarkers

Blood samples for biomarkers (cTnT, BNP, NT-proBNP) analyzed at a central lab, as well as potentially other neurohormonal or inflammatory biomarkers, will be measured at 12, 24, 48, 72, and 96 hours post infusion initiation and at Day 30.

8.1.2.7 PK Assessments

For Part A: blood samples for PK profile assessments will be drawn at pre-dose, 6, 12, and 24 hours after infusion start and 0.25, 0.5, 1, 6, 12, and 24 hours after infusion end. For Part B: blood samples for PK assessments will be drawn at pre-dose, 6, 12, 24, 36, 48, 52, 56, and 60 hours after infusion start and 12, 36, and 48 hours after infusion end, and at discharge. Blood samples for PK will be analyzed at a central lab.

8.1.2.8 Physical Examination

Physical examinations will be conducted daily at 24, 48, 72, and 96 hours and at Day 30. Any relevant clinically significant abnormalities noted prior to randomization should be recorded in the eCRF as part of medical history. Any relevant clinically significant changes noted at follow-up should be reported as an adverse event. Physical assessment will include signs of heart failure, with particular attention to evidence of congestion including dyspnea on exertion or at rest, orthopnea, rales, jugular venous pulse (JVP) and peripheral edema.

8.1.2.9 Urine Output

Urine output will be collected at 0 to 24, 24 to 48, and 48 to 72 hours after infusion start.

8.1.2.10 Concomitant Medications

Concomitant Medications and therapies required for the general care of the subject are permitted, except for investigational agents and investigational medical devices. All concomitant medication and concurrent therapies will be documented from 30 days prior to Screening until the time the subject completes the study or dies. Dose, route, unit, frequency of administration, and indication for administration will be captured on the eCRF.

8.1.2.11 Worsening Heart Failure

Worsening HF assessment will be done daily at 24, 48, 72, and 96 hours. In-hospital worsening heart failure (WHF) is defined as worsening signs and/or symptoms of heart failure since the previous assessment that require an intensification of intravenous therapy for heart failure or mechanical ventilatory, renal or circulatory support. Such treatment can include the institution or up-titration of IV furosemide, IV nitrates or any other IV medication for heart failure, or institution of mechanical support such as mechanical ventilation, ultrafiltration, hemodialysis, intra-aortic balloon pump or ventricular assist device. Any medication used to treat WHF should be recorded as a concomitant medication.

8.1.2.12 EQ-5D

The EQ-5D quality of life questionnaire will be completed at Day 5 (at 96 hours) and at Day 30 for subjects in Part A only.

8.1.2.13 Readmissions and Death

Readmissions and death will be reported through discharge and Day 30. Hospitalization will be defined as any unplanned hospitalization (including admission to a hospital or any attendance in an acute care setting [e.g., ED], or in another health care facility) of 24 hours or greater, regardless of whether the subject was admitted to the hospital.

8.2 Safety and Other Assessments

8.2.1 Tolerability of Drug Administration

During dosing, the incidence of the following will be recorded:

- Site infusion pain
- Site administration pain

In addition, information (time, reason) on location of the intravenous catheter used for study drug administration and the incidence of dosing interruptions or dosing, down titrations or discontinuations will be collected.

It is recommended that volume status prior to, during, and after study drug administration be carefully monitored. Urine output volume at 0 to 24, 24 to 48, and 48 to 72 hours will be collected.

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence associated with the use of an intervention, whether or not considered intervention related. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product [21 CFR 312.32(a), ICH E6].

8.3.2 Definition of Serious Adverse Events

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 participant 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.

8.3.3 Classification of an Adverse Event

8.3.3.1 Severity of Event

The severity of an AE is assessed by the PI using the following definitions:

- **Mild** – Events require minimal or no treatment and do not interfere with the subject'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 subject's usual activity level and may require systemic drug therapy or other treatment. Severe events may be potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious."

8.3.3.2 Relationship to Study Intervention

All AEs must have their relationship to study intervention assessed by the PI, 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** – 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, follows a known response pattern to the study intervention, and cannot be explained by concurrent disease or other drugs or chemicals. An AE that is determined to be due to the subject's underlying disease state or is common for this patient population generally should not be considered related.
- **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) and/or follows a known response pattern to the study drug. 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 "related," if appropriate. An AE that is determined to be due to the subject's underlying disease state or is common for this patient population generally should not be considered possibly related.
- **Unlikely 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 subject'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 should be an alternative, definitive etiology documented by the clinician.

8.3.3.3 Expectedness

The Windtree Medical Monitor or designee 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 intervention (i.e., as per the Investigator's Brochure).

8.3.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during the study, 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 eCRF. 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.

The site will record all reportable events with start dates occurring any time after informed consent is obtained until 7 days after the last day of study participation or Day 30 (whichever occurs first). Events will be followed for outcome information until resolution or stabilization, or for 30 days after study participation. When possible, information collection should continue for subjects that withdraw or discontinue treatment early.

8.3.5 Adverse Event Reporting

All AEs are to be assessed in all subjects throughout Day 30 (from enrollment to study withdrawal or completion) and documented in the study eCRF. Each AE should be reported spontaneously or in response to general, non-directed discussion with the attending nurse or physician (e.g., "has there been any change in subject status since the last assessment period?"). For each AE, the investigator should obtain all information required to complete the AE page of the eCRF, in accordance with eCRF completion guidelines (provided separately by Windtree).

All AEs, regardless of seriousness, severity, or relationship to study participation, must be recorded (using medical terminology) in the source document and on the AE page of the eCRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology.

All AEs must be followed until resolution or until a stable clinical endpoint is reached, or for at least 30 days after the subject's last day in the study if an AE is ongoing at the time the subject completes the study.

Documentation of AEs in the eCRF must include the following parameters; (1) duration (time of onset and resolution), (2) severity or grade, (3) outcome, (4) action taken, and (5) relationship to study drug. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

8.3.6 Serious Adverse Event Reporting

When an investigator-trained designee becomes aware of an SAE, Windtree must be notified as soon as possible (and no later than 24 hours after the event has occurred) by telephone, regardless of the relationship (or lack thereof) of the SAE to study participation.

SAEs should be reported to Windtree's reporting line. When reporting SAEs, the following information should be provided:

1. Study identifier
2. Study center
3. Subject number
4. A description of the event
5. Date of onset
6. Current status
7. Clarification on whether infusion therapy was discontinued
8. The reason why the event is classified as serious
9. The Investigator's assessment of the association between the event and study participation

All reports of SAEs must be followed up within 24 hours (or sooner at the request of the medical monitor) by the completion of the SAE form and signature by the person who completed the form and the PI.

Safety report distribution is the responsibility of Windtree or designee (i.e., CRO), which coordinates distribution of safety reports to applicable regulatory authorities and ECs. Finalized safety reports are distributed by one or more individuals, "Responsible Team," within a department that has responsibility to coordinate the distribution/submission activities, either directly or via a Local Submitting Party on behalf of Windtree.

The Responsible Team receives finalized safety reports (e.g., CIOMS-I form/MedWatch 3500A/ E2B file/DSUR/SUSAR Listing) and coordinates distribution to all applicable regulatory

authorities and ECs. Distribution will be done in accordance with project-specific documents, regulatory requirements, other applicable reference sources, and applicable Windtree or designee safety procedural documents.

In addition, Windtree coordinates translation when required for safety reporting. Windtree also coordinates transcription (e.g., manual transfer of information from one reporting format to another) when required. Project specific information is used to identify appropriate recipients. Quality checks will be performed on the distribution documents and recipient information for completeness and accuracy prior to distribution.

The names, telephone, and fax numbers of the individuals who should be contacted regarding safety issues are listed below.

FOR QUESTIONS, PLEASE CONTACT THE MEDICAL MONITOR:

Gad Cotter, MD (Medical Monitor)
[redacted]

OR

Joseph Soffer, MD, FACC (Medical Monitor)
[redacted]

FOR REPORTING SAEs, PLEASE CONTACT WINDTREE AT:

Reporting Line (24/7/365): +1 833-4WINDTX

FOR ADDITIONAL ASSISTANCE, PLEASE CONTACT YOUR CRA OR WINDTREE CLINICAL OPERATIONS.

8.3.7 Reporting Events to Subjects

Occurrences of AEs or SAEs that impact the risk to subjects in the study may result in a change in the informed consent form. Subjects who have not yet completed the study must be reconsented with the amended informed consent form.

8.3.8 Events of Special Interest

This study has no AEs of special interest.

8.3.9 Reporting of Pregnancy

The use of istaroxime in women of childbearing potential not using contraceptive measures should be avoided; women of childbearing potential must have a negative pregnancy test (β-hCG) recorded prior to administration of istaroxime and at the end of the study. Men and premenopausal women will be required to use double-barrier methods of birth control for 30 days following discharge from the hospital.

If a woman becomes pregnant during the study, the pregnancy will be followed until delivery or termination, and pertinent information about the pregnancy will be collected (e.g., AEs, concomitant medications).

9 STATISTICAL CONSIDERATIONS

9.1 Sample Size Determination

For the primary endpoint (SBP AUC at 6 hours) in the active group, we assumed the change in SBP goes from 0 to 3 mmHg at 1 hour, to 6 mmHg at 2 hours, and to 10 mmHg at 3 hours, where it stays through 6 hours. In placebo, we assumed a change in SBP of 2 mmHg at 1 hour, to 3 mmHg at 3 hours, with SBP staying at 3 mmHg through 6 hours. Value assumptions are based on the previous istaroxime study HORIZON-HF (Study PST2744-DM-04-012).

With these assumptions, the AUCs are 44 mmHg·h for active and 15.25 mmHg·h for placebo. Assuming the SD of 14.5 mmHg·h is the same at all time points and the same in both groups, and there are no dropouts, the SD for the AUC is approximately 5.7 mmHg·h. Therefore, with 30 subjects/group we would have > 99% power to detect the assumed difference in AUCs at a 1-sided α of 0.025.

Thus, 60 subjects (out of approximately 180 screened) will be enrolled for Part A.

For Part B, the primary endpoint will remain SBP $AUC_{0\text{-}6hr}$; however, assessments of E/e´ ratio and wedge pressure will be done using means and confidence intervals to determine the margin of error and to plan for future studies. Since it is not planned that statistical significance will be achieved, a sample size of 30 (10 per arm) will be sufficient to calculate means and confidence intervals.

9.2 Populations for Analyses

For the efficacy analysis, the modified intent-to-treat (mITT) population (defined as all randomized subjects who received study treatment and had at least one post-baseline assessment), the per-protocol population (subjects with no major protocol deviations), and the ITT population will be evaluated, based upon the treatment group to which they were randomized.

For the safety analysis, all subjects who received any amount of study treatment will be evaluated, based upon the treatment they actually received.

9.3 Statistical Analyses

9.3.1 General Approach

For both Part A and Part B, all continuous variables (e.g., weight, body temperature, blood chemistry) will be summarized using number (n), mean, standard deviation (SD), median, minimum, and maximum. All discrete variables (e.g., sex, AEs, incidence of hospital re-admissions), will be summarized using frequency (n) and percent.

All tests or confidence intervals will be two-tailed with 95% confidence intervals (α-level of 5%). Covariates, if any, and detailed statistical methodology will be pre-specified in the statistical analysis plan (SAP). The SAP will detail the statistical methods for the study and will be finalized prior to unblinded analysis of the study. If the details in the SAP differ from the details in this section, the SAP will prevail.

For statistical modeling, within each study part (Part A and Part B) study centers will be pooled by geographic region to help ensure that both treatments are represented at each study site. After pooling, each 'pooled site' in Part A should have a minimum of 5 subjects. For the combined analyses for Part A and B, study site will not be included in modeling.

9.3.2 Analysis of the Primary Efficacy Endpoint(s)

The primary endpoint is the area under the change from baseline curve from baseline through hour 6 ($AUC_{0\text{-}6hr}$), computed by trapezoidal rule after applying any applicable imputations. The primary endpoint will be analyzed for Part A alone (istaroxime vs placebo), Part B alone (istaroxime arms 1 and 2 vs placebo), and for Parts A and B combined (pooled istaroxime vs. placebo).

9.3.2.1 Statistical Hypothesis

The primary purpose of this study is to estimate the effects of istaroxime on SBP, and not as a confirmatory clinical trial. Nonetheless, primary and secondary efficacy endpoints will be compared statistically, employing p-values and confidence intervals. The statistical analysis of both the primary and secondary objectives will be based on the ITT, mITT, and per-protocol population sets. The primary null hypothesis to be tested is that the mean $AUC_{0\text{-}6hr}$ is the same for istaroxime and placebo treatment groups.

9.3.2.2 Analyses of the SBP $AUC_{0\text{-}6hr}$

Possible intercurrent events include (1) death, (2) requirement for rescue therapy, and (3) study drug discontinuation due to adverse event. The frequency of occurrence of these events through 6 hours is expected to be low.

The primary endpoint is the SBP area under the change from baseline curve from baseline through hour 6 ($AUC_{0\text{-}6hr}$), computed by trapezoidal rule after applying any applicable imputations, and the corresponding primary null hypothesis to be tested is that the mean $AUC_{0\text{-}6}$ is the same in all treatment groups.

The primary analysis population will be the mITT population set; values after an intercurrent event (e.g., death, infusion stopped due to an AE) will be set to missing. Missing values will be imputed using values within the same treatment group. The mean $AUC_{0\text{-}6hr}$ will be compared between treatment groups using analysis of covariance (ANCOVA) with treatment, and baseline value in the model. The term 'pooled site' will be included in models within Part A or Part B, not for the combined analysis.

For the per-protocol population, as a result of excluding subjects with major protocol violations, missing values are not expected. As with the mITT population, treatments will be compared using ANCOVA with treatment, and baseline value in the model. As subjects are more likely to drop out in the placebo group, an imbalance in treatment group sizes may result.

A treatment effect of interest is the effect of following a treatment policy starting with istaroxime on the $AUC_{0\text{-}6hr}$. For the ITT population analysis, data after the intercurrent event will be utilized, and data will be imputed recursively based on the subjects in the same treatment group. Treatment groups will be compared using ANCOVA.

For Part A only, an additional analysis will be conducted to examine composite endpoints that incorporate the intercurrent events through truncation of the distribution of $AUC_{0\text{-}6}$. For this analysis, the $AUC_{0\text{-}6hr}$ for subjects with a value less than -5 mmHg·h, who die, or who require rescue therapy will be set to -5 mmHg·h. Treatment groups will be compared using a t-test. The medians in each treatment group, which are invariant to the truncation, will be presented.

In addition, for Part A, an analysis where the $AUC_{0\text{-}6hr}$ for subjects with a value less than -5 mmHg·h, who die, or who require rescue therapy will be set to 0 mmHg·h (instead of -5 mmHg·h). Treatment groups will be compared using ANCOVA adjusted for baseline SBP. The medians in each treatment group, which are invariant to the truncation, will be presented.

9.3.3 Analysis of the Secondary Endpoint(s)

Key secondary endpoints include:

- SBP AUC to 12 hours from start of infusion for subjects in Part B.
- SBP AUC to 24 hours from start of infusion for subjects in Parts A and B.
- SBP AUC to 48 hours from start of infusion for subjects in Part B.
- SBP AUC to 60 hours from start of infusion for subjects in Part B.

9.3.3.1 SBP AUC from Baseline up to 60 Hours

The area under the change from baseline curve from baseline through hour 12 ($AUC_{0\text{-}12}$: Part B), 24 ($AUC_{0\text{-}24hr}$; Parts A and B), hour 48 ($AUC_{0\text{-}48hr}$; Part B), and hour 60 ($AUC_{0\text{-}60hr}$; Part B), will be computed by trapezoidal rule after applying any applicable imputations, as above for the primary endpoint. Treatment groups will be compared using an ANCOVA model with adjustment for baseline SBP. The term 'pooled site' will be included in models within Part A or Part B, not for the combined analysis.

In addition, for Part A only, a composite endpoint that incorporates intercurrent events will be computed. The treatment effect estimated will reflect the probability of a better clinical response with respect to blood pressure with istaroxime treatment. A rank-based approach will be used: subjects who died will be assigned the worst rank, subjects who required rescue therapy will be assigned the next worse rank, and the remaining subjects will be ranked by their $AUC_{0\text{-}24hr}$ (following recursive imputation for missing values). Treatment groups will be compared using a van Elteren's test stratified by 'pooled site'.

9.3.3.2 Treatment Failure Score

A "treatment failure" score endpoint will be generated based upon individual subject responses – all subjects will be classified for both Parts A and B. Subjects will be assigned a value based on the occurrence of the following through 24 hours (Part A) and 60 hours (Part B) from baseline:

1 = died
2 = required circulatory, respiratory, or renal mechanical support or discontinued study treatment due to an AE
3 = treated with IV inotrope or vasopressor
4 = any SBP more than 10 mmHg below baseline in two consecutive measurements
5 = any SBP between 5-10 mmHg below baseline in two consecutive measurements
6 = none of the above occurred

Treatment groups will be compared using a van Elteren's test (extension of the Wilcoxon rank-sum test) stratified by 'pooled site'.

9.3.3.3 SBP Changes from Baseline to 6, 12, and 24 Hours

Changes from baseline in SBP at 6 and 24 hours in Parts A and B will be compared between treatment groups using a mixed-effects repeated measurement model (MMRM) with baseline value, treatment, visit, and treatment x visit in the model. For Part B, change from baseline in SBP at 12 hours will also be done. The term 'pooled site' will be included in models within Part A or Part B, not for the combined analysis.

9.3.3.4 Treatment Failure

The proportion of subjects who will sustain treatment failure in Parts A and B will be summarized and compared using the Cochran-Mantel-Haenszel test.

9.3.3.5 Changes in Quality of Life

Changes in quality of life from baseline to 5 and 30 days will be assessed using the EQ-5D-5L instrument and summarized by treatment group in Part A only. Index values and visual analog scale (VAS) scores will be compared between treatments at each time point using ANCOVA with treatment in the model.

9.3.3.6 Changes in Creatinine Clearance and eGFR

Changes in creatinine clearance and eGFR from baseline to 24, 48, 72 and 96 hours in Parts A and B will be compared between treatment groups using a MMRM with baseline creatinine clearance or eGFR, treatment, visit, and treatment x visit in the model. The term 'pooled site' will be included in models within Part A or Part B, not for the combined analysis.

9.3.3.7 Changes in Heart Rate, Mean Arterial Pressure (MAP), Natriuretic Peptides, and Troponin

The change from baseline and observed values of heart rate, MAP, natriuretic peptides (BNP, NT-proBNP), troponin (cTn), and venous lactate will be presented at each time point in Parts A and B. cTn, BNP, and NT-proBNP will be log-transformed for analysis. The changes from baseline to 12, 24, 48, 72 and 96 hours will be compared between treatment groups using a MMRM for each

outcome that includes baseline value, treatment, visit, and treatment x visit in the model. The term 'pooled site' will be included in models within Part A or Part B, not for the combined analysis.

9.3.3.8 Clinical Outcomes

The time to the first occurrence of WHF, HF hospitalization or death through Day 30, the time to the first HF readmission through Day 30, time to any hospital admission through Day 30 and the time to death through Day 30 will be compared between treatment groups in Parts A and B using log-rank tests. Event times for non-fatal events will be censored for those subjects without an event or at the time of last follow-up or at the time of death.

The distribution of deaths by cause will be summarized in each treatment group.

9.3.3.9 Length of Hospital Stay, Stay in ICU/CCU, and Days Alive Out of the Hospital through Day 30

Length of stay will be defined as the index hospitalization discharge date minus the randomization date plus one. Subjects still in the hospital at Day 30 will be truncated at Day 30. Subjects who die during the initial hospitalization will be assigned a value of 31.

The days during the index hospitalization spent in the ICU/CCU will be recorded as days by the investigator.

Days alive and out of hospital will be computed as 31 days minus the days in-hospital through Day 30 (including the index hospitalization and any rehospitalizations) minus the days following a death prior to Day 30. Days in hospital will be computed for the index hospitalization as the discharge date minus the baseline date plus one, and for rehospitalizations as the discharge date minus the admission date plus one.

Treatment groups in Parts A and B will be compared using van Elteren's tests stratified by pooled 'study site'.

9.3.4 Safety Analyses

Adverse Events will be coded using MedDRA (version 22.0 or later) and will be summarized by treatment group using frequency and percent but will not be compared statistically. Concomitant medications will be classified using the WHODrug dictionary (September 2019 or later) and summarized using frequency and percent in Parts A and B. All-cause mortality, physical examination and medical history will similarly be summarized.

9.3.5 Baseline Descriptive Statistics

Demographic parameters will be summarized by treatment group in Parts A and B and assessed qualitatively for homogeneity of treatment groups. For categorical data (e.g., sex, race), data will be summarized using frequency and percent; for continuous (parametric) data, data will be summarized by n, mean, SD, median, minimum, and maximum.

No inferential statistics will be computed.

9.3.6 Planned Interim Analyses

One interim analysis, when 33% of enrollment is complete in Part A, is planned as a safety assessment to be reviewed by the DMC. Additional meetings may occur on an ad hoc basis if the DMC chair deems it necessary to address safety in the study.

9.3.7 Sub-Group Analyses

It is planned that the primary endpoint will be presented by sex and race in Part A (race categories smaller than 10% of the total will be grouped).

9.3.8 Tabulation of Individual Participant Data

Datasets will be provided as CDISC-compliant standard data tabulation modules (SDTM); no separate individual participant data listings will be provided.

9.3.9 Exploratory Analyses

An exploratory analysis based on primary and secondary efficacy endpoints in Parts A and B may be performed based upon subject responses to treatment.

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

The informed consent form (ICF) describes in detail the study intervention, study procedures, and risks. The ICF is given to the patient and written documentation of informed consent is required prior to starting administration of study intervention.

10.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to patient agreement to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB), or Independent Ethics Committee (IEC) approved, and the patient will be asked to read and review the document. Within a maximum of 24 hours before administration of study medication (istaroxime or placebo), a medical screening will be performed on all prospective patients to assess suitability for the study. The investigator will explain the research study to the patient and answer any questions that may arise. A verbal explanation will be provided in terms suited to the patient's comprehension of the purposes, procedures, and potential risks of the study and of the rights of research participants. Patients will have the opportunity to carefully review the written consent form and ask questions prior to signing. The patients should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The patient will sign the informed consent document prior to any procedures being done specifically for the study. Patients 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 patient 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 the patient that the quality of their medical care will not be adversely affected if they decline to participate in this study. No waivers will be allowed for 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 the principal investigator and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform study participants and the IRB/IEC 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
- Demonstration of efficacy that would warrant stopping
- Data that are not sufficiently complete and/or evaluable

The study may resume once the reasons for suspension are addressed, and satisfy the sponsor, IRB/IEC and/or Regulatory Agency (e.g., FDA).

10.1.3 Confidentiality and Privacy

Subject confidentiality and privacy are strictly held in trust by the participating investigators, their staff, and Windtree. 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 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 IRB/IEC, or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records 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/IEC, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be entered into a validated Electronic Data Capture (EDC) system, and will be transmitted to and stored at Windtree. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites

and by Windtree research staff will be secured and password protected and meet the requirements of 21CFR11 and the General Data Protection Regulation. At the end of the study, all study databases will be archived at Windtree.

10.1.4 Future Use of Stored Specimens and Data

Blood samples will be collected for this study for the analysis of biomarker data (cTnT, BNP, and NT pro-BNP) and for assessment of pharmacokinetics. Samples collected will be frozen at -80C. If sites do not have a -80C freezers, samples may be stored at -20C or below. Shipments will be organized periodically every 3-4 months to the central lab. Windtree will coordinate the shipment of the samples with the courier. Sites will be notified when the samples are to be shipped and will be provided with instructions for shipping. Please note thata copy of the 4 mL Tube Collection Log and 1.2 mL PK Specimen Log will need to be included in the shipment box.

The samples will be maintained in case samples need to be re-checked for 2 years after the end of study. After analysis has been completed on all samples, they will be destroyed.

Data collected for this study will be analyzed and stored at Windtree. Permission to transmit data to Windtree will be included in the informed consent. When the study is completed, access to study data will be provided through Windtree.

10.1.5 Key Roles and Study Governance

The coordinating investigator and medical monitors are listed in Table 10-1.

Table 10-1. Coordinating Investigator and Medical Monitors

Coordinating Investigator	Medical Monitors
Marco Metra, MD Università degli Studi di Brescia [redacted]	Gad Cotter, MD Momentum Research, Inc. [redacted] Joseph Soffer, MD, FACC Executive Director, Clinical Development Windtree Therapeutics, Inc.

In addition, study and overall program oversight will be provided by a Steering Committee (SC). The SC will ensure the study is conducted ethically and will ensure that the safety of study subjects is maintained. The SC members, who are experts in the field of cardiology, may also provide input on the protocol and other study-related documents.

10.1.6 Safety Oversight

Safety oversight will be under the direction of the SC and a data monitoring committee (DMC).

10.1.6.1 Data Monitoring Committee

The DMC, made up of at least 3 experts in the field of heart failure, will evaluate the degree of risk involved in study subject participation within each treatment group and to determine if study continuation in accordance with the current protocol holds the potential to institute any undue harm or threat to the safety and welfare of study subjects.

During the treatment and post-dosing phases, the chair of the DMC will receive regular reports on SAEs and DMC members will be periodically updated on the program and trial status. For Part A, a scheduled meeting of the DMC will occur when 33% of enrollment is complete and at the end of Part A. For Part B, a scheduled meeting of the DMC will occur when 10 subjects have been enrolled and at the end of Part B. An ad hoc meeting of the DMC will occur if the chair deems it necessary to address safety in the study. The DMC may recommend that study enrollment be suspended if safety concerns are identified or suspected. The meetings and actions of the DMC are described in the DMC charter.

10.1.6.2 Safety Reviews

If an ad hoc DMC meeting is called or at scheduled DMC meetings, the DMC review may consist of the evaluation of (1) all AEs, (2) case reviews of subjects with reported SAEs, (3) summary tables of all safety endpoints, and (4) any other available relevant data.

Following a meeting, the DMC will provide timely recommendations to the Windtree study team. Recommendations may consist of, but not be limited to the following:

- Continue the study as planned
- Suspend study enrollment, pending additional information
- Close study enrollment

An unmasked independent statistician, not a member of the committee, will be responsible for the statistical analysis of the data.

10.1.7 Clinical Monitoring

Windtree or their designee will perform on-site monitoring visits as frequently as it is deemed necessary to ensure quality study data capture and accurate adherence to the study protocol as outlined in the Clinical Monitoring Plan (CMP). It is also necessary to ensure that the rights and well-being of trial participants are protected, and that the conduct of the trial is in compliance with International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) and with applicable regulatory requirements.

Monitoring will be performed as in-person visits and centralized review of records. In-person visits will include source data verification to the eCRF; some source data verification will be performed unless otherwise specified in the CMP.

Before enrollment, a clinical site monitor will complete a site initiation visit (SIV) at each study site. During SIVs, clinical site monitors will provide study training to site staff, ensure study drug storage is in accordance with the study protocol, and validate all other study requirements in accordance with the study monitoring plan.

Clinical site monitors will schedule a study site visit as close as possible to the time of each site's first enrolled study subject; periodic follow-up monitoring visits will ensure on a regularly scheduled basis throughout the study, in accordance with enrollment at the study site. At these visits, the clinical site monitor will compare the data entered into the eCRF with the hospital or clinic records (source documents) and check for protocol compliance. Documentation reviews will include but not be limited to the evaluation and confirmation of the following:

1. A record of informed consent
2. Adherence to enrollment criteria
3. Completion of all required study assessments
4. Accurate and complete data capture of all AEs, concomitant medications, and safety and efficacy observations.

5. Study drug storage and dispensing records maintained in accordance with study and regulatory requirements.

Findings from these reviews will be discussed with the PI and study site staff.

The dates of the monitoring visits will be recorded by the clinical site monitor in a sign-in log to be kept at the site. The study coordinator and PI are expected to be available for questions, have all source documentation readily available, and have a suitable environment provided for review of study-related documents.

In accordance with ICH GCP, Windtree will select (either directly or through a subcontract with a company specifically trained in the monitoring of clinical studies), qualified individuals to monitor study sites to ensure the quality of study progress and the close adherence of study sites to the study protocol and all related governing documents and SOPs.

1. The clinical site monitor(s), before the initiation of each study site, will ensure each investigator and study staff understands the following:
   a) The investigational status of the study drug, placebo, and the requirements for its accountability
   b) The need to uphold all directives within the clinical protocol as it relates to study conduct and subject safety at the study site
   c) The obligation to obtain informed consent in accordance with the Declaration of Helsinki and ICH GCP guidelines before enrolling each subject in the study
   d) The obligation to obtain IRB/IEC review and approval of the study before study initiation at his/her clinical site and ensure timely updates to the IRB/IEC as mandated by local and national regulatory requirements, and to ensure timely communications to Windtree of all IRB/IEC communications (to include reviews and subsequent actions) concerning the study.
2. The clinical site monitor(s) will perform periodic visits to each clinical site during the course of the study to ensure the study protocol is being followed and that:
   a) Drug and placebo inventories are being properly maintained and documentation of vial usage is accurate and complete
   b) The assignment of responsibilities log is up-to-date and that changes in personnel are reported to Windtree.
   c) The PI is reporting all serious or fatal AEs (Section 8.3.6) as soon as possible, and in no case later than 24 hours after the event, to the medical monitor or designee at Windtree.

3. The clinical site monitor(s) will perform an end-of-study visit to each clinical site to ensure that:
    a) All drug reconciliation forms, as provided in the Study/Pharmacy Manual, are accurate and complete.
    b) All used and unused vials of study drug have been reconciled.
    c) All eCRFs are complete and all monitoring of eCRFs has been completed.

Details of clinical site monitoring are documented in a CMP. The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

10.1.8 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data collection, and documentation. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution. Following written SOPs, the monitors will verify that the clinical trial is conducted, data collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements.

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 Data Handling and Record Keeping

10.1.9.1 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.

Hardcopies of the study visit worksheets may be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded

on the source documents. Study personnel at each site will enter data from source documents into the protocol-specific eCRF within 5 days of the information becoming available. Subjects will not be identified by name in the study database or on any study documents to be collected by Windtree (or designee) but will instead be identified by a site and subject number.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) will be entered into 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.

Members of Windtree's Data Management department are responsible for data processing, in accordance with procedural documentation and a study specific Data Management Plan (DMP). Windtree's Data Management will also provide eCRF Completion Guidelines; extra training will be provided to the study sites when necessary.

Clinical data will be entered directly from the source documents. After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Corrections for an existing eCRF record will automatically be recorded by the eCRF system (audit trail capturing the time, date, and the identification of the user who entered or updated eCRF data). Recorded corrections by the eCRF will create an electronic audit trail of study documentation.

Database lock will occur once all quality assurance procedures have been completed; this will include but not be limited to the following: (1) all site-based study data have been entered into the eCRF, (2) all entered data have been reconciled and reviewed by Windtree or designee, and (3) all data related queries have been rendered and resolved.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

10.1.9.2 Study Records Retention

All records that support data entered into the eCRF of each subject must be retained in the files of the PI or the hospital for a minimum of 2 years (3 years for ICF) following notification by Windtree that all investigations have been discontinued or that the last approval of a marketing application has been obtained. 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 Windtree. It is

the responsibility of Windtree to inform the investigator when these documents no longer need to be retained.

Supporting documents will include but not be limited to the following:

1. Copies of eCRFs (given to the site on electronic media)
2. All original source documents; these may include but not be limited to the following:
   a) ICFs
   b) laboratory reports
   c) progress notes
   d) medical histories
   e) physical and diagnostic findings
   f) diagnoses
   g) dates of therapy before and during this study
   h) drug dispensing/disposition records

If the PI retires, relocates, or for other reason withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. Windtree must be notified in writing of the name and address of the new custodian.

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or Study/Pharmacy Manual 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 site investigator to use continuous vigilance to identify deviations. All deviations must be addressed in study source documents and reported to the site monitor. Protocol deviations must be sent to the reviewing IRB/IEC per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB/EC requirements. Further details about the handling of protocol deviations will be addressed in the protocol deviation process.

10.1.11 Publication and Data Sharing Policy

The preparation and submittal for publication of a manuscript containing the study results shall be in accordance with a process determined by a mutual written agreement between Windtree and participating institutions.

The publication or presentation of any study results shall comply with all applicable privacy laws, including but not limited to HIPAA. This trial will be registered in the ClinicalTrials.gov and the EU Clinical Trials Register databases, and results information from this trial will be submitted to both. In addition, every attempt will be made to publish results in peer-reviewed journals.

10.1.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence 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.

The FDA has issued regulations (21 CFR Part 54, *Financial Disclosure by Clinical Investigators*) that require sponsors to submit complete and accurate certification or disclosure statements to certify the absence of certain financial interests of clinical investigators, when clinical studies are submitted to FDA in support of marketing approval. These regulations are intended to ensure that financial interests and arrangements of clinical investigators, that could affect reliability of data submitted to FDA in support of marketing approval, are identified and disclosed by the sponsor.

Clinical investigators will be asked to disclose proprietary (e.g., patent, licensing agreement) and financial (e.g., stock options, royalty) interests as they pertain to Windtree, before participating in the study. In addition, clinical investigators will be required to consult with Windtree before acquiring any financial interest in the company and must disclose any change in their proprietary or financial interests, if it occurs during the course of the study and for 1 year following study completion. The requirement for proprietary and financial disclosure also includes any ownership by the spouse or any dependent subject of the clinical investigator.

If FDA determines that the financial interests of any clinical investigator raise serious questions about the integrity of the data, FDA will take any action it deems necessary to ensure the reliability of the data, including:

1. Initiating agency audits of the data derived from the clinical investigator in question
2. Requesting that the sponsor submit further data analyses (e.g., to evaluate the effect of the clinical investigator's data on overall study outcome)
3. Requesting that the applicant conduct additional independent studies to confirm the results of the questioned study
4. Refusing to treat the covered clinical study as providing data that can be the basis for an agency action

If the sponsor does not include certification or disclosure, or both (as required), or does not certify that it was not possible to obtain the information, the FDA may refuse to file the New Drug Application (NDA).

10.2 List of Abbreviations

Abbreviation	Description
β-hCG	Serum pregnancy test
A	A wave: peak velocity flow in late diastole caused by contraction
AE	Adverse event
CFR	Code of Federal Regulations
CO	Cardiac output
CONSORT	Consolidated standards of reporting trials
CRF/eCRF	Case report form/electronic CRF
CS	Cardiogenic shock
DBP	Diastolic blood pressure
ECG	Electrocardiogram
FDA	Food and Drug Administration
GCP	Good Clinical Practice

Abbreviation	Description
HF/AHF	Heart failure/acute heart failure
HIPAA	Health Insurance Portability and Accountability Act
E	E wave: peak velocity blood flow from LV relaxation in early diastole
E/Ea	Mitral Doppler inflow E velocity to annular tissue Doppler Ea wave velocity
ERO	Effective regurgitant orifice
eGFR	Glomerular filtration rate
ICF	Informed consent form
ICH	International Conference on Harmonisation
IEC	Independent ethics committee
IRB	Institutional review board
IND	Investigational new drug
ITT	Intent-to-Treat
IRT	Interactive response technology
LAA/LAD/LAV	Left atrial area/diameter/volume
LV/LVEF	Left ventricular/LV ejection fraction
LVEDD/EDV	Left ventricular end diastolic diameter/end diastolic volume
LVESD/ESV	Left ventricular end systolic diameter/end systolic volume
MAP	Mean arterial pressure
NDA	New drug application
PAC	Pulmonary artery catheter
PAP	Pulmonary artery pressure
PI	Principal Investigator
PK	Pharmacokinetics
SAE	Serious adverse event
SBP	Systolic blood pressure
SCAI	Society for Cardiovascular Angiography and Interventions

Abbreviation	Description
SIV	Site initiation visit
SVI	Stroke volume index
TAPSE	Tricuspid annular plane systolic excursion
UP	Unanticipated problem
US	United States
LVOT	Left ventricular outflow tract (velocity time interval)
WHF	Worsening heart failure

11 REFERENCES

1. van Diepen S, Katz JN, Albert NM, Henry TD, Jacobs AK, Kapur NK, et al. Contemporary management of cardiogenic shock: a scientific statement from the American Heart Association. Circ. 2017 October: p. e232-e268.
2. Hunziker L, Radovanovic D, Jeger R, Pedrazzini G, Cuculi F, Urban P, et al. Twenty-year trends in the incidence and outcome of cardiogenic shot in AMIS Plus registry. Circ Cardiovasc Interv. 2019 April: p. e007293.
3. Berg DD, Bohula EA, van Diepen S, Katz JN, Alviar CL, Baird-Zars VM, et al. Epidemiology of shock in contemporary cardiac intensive care units. Circ Cardiovasc Qual Outcomes. 2019 March: p. e005618.
4. Singh T, Samson R, Ayinapudi K, Motwani A, LeJemtel T. Precardiogenic Shock: A New Clinical Entity. Cardiology in Review. 2019 July/August: p. 198-201.
5. Jentzer JC, van Diepen S, Barsness GW, Henry TD, Menon V, Rihal CS, et al. Cardiogenic shock classification to predict mortality in the cardiac intensive care unit. JACC. 2019 October: p. 2117-2127.
6. Kapur NK, Paruchuri V, Urbano-Morales JA, Mackey EE, Daly GH, Qiao X, et al. Mechanically unloading the left ventricle before coronary reperfusion reduces left ventricular wall stress and myocardial infarct size. Circulation. 2013 Jul: p. 328-336.
7. Hochman JS, Sleeper LA, Webb JG, Sanborn TA, White HD, Talley JD, et al. Early revascularization in acute myocardial infarction complicated by cardiogenic shock. SHOCK Investigators. N Engl J Med. 1999 Aug: p. 625-634.
8. Savarese G, Lund LH. Global Public Health Burden of Heart Failure. Card Fail Rev. 2017 Apr: p. 7-11.
9. Harjola V, Lassus J, Sionis A, Køber L, Tarvasmäki T, Spinar J, et al. Clinical picture and risk prediction of short-term mortality in cardiogenic shock. Eur J Heart Fail. 2015 May: p. 501-509.
10. Kar B, Gregoric ID, Basra SS, Idelchik GM, Loyalka P. The percutaneous ventricular assist device in severe refractory cardiogenic shock. J Am Coll Cardiol. 2011 Feb: p. 688-696.
11. Milo-Cotter O, Cotter-Davison B, Lombardi C, Sun H, Bettari L, Bugatti S, et al. Neurohormonal activation in acute heart failure: results from VERITAS. Cardiology. 2011 Sep: p. 96-105.
12. Shah M, Ali V, Lamba S, Abraham WT. Pathophysiology and clinical spectrum of acute congestive heart failure. Rev Cardiovasc Med. 2001: p. S2-S6.
13. Hochman JS, Sleeper LA, Webb JG, Dzavik V, Buller CE, Aylward P, et al. Early revascularization and long-term survival in cardiogenic shock complicating acute myocardial infarction. JAMA. 2006 Jun: p. 2511-2515.

14. Shah RU, de Lemos JA, Wang TY, Chen AY, Thomas L, Sutton NR, et al. Post-hospital outcomes of patients with acute myocardial infarction with cardiogenic shock: findings from the NCDR. J Am Coll Cardiol. 2016 Feb: p. 739-747.
15. Bers DM. Altered cardiac myocyte Ca regulation In heart failure. Physiology. 2006 Dec; 21(6): p. 380-387.
16. del Monte F, Harding SE, Schmidt U, Matsui T, Kang ZB, Dec GW, et al. Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a. Circuilation. 199 Dec; 100(23): p. 2308-2311.
17. Samuel TJ, Rosenberry RP, Lee S, Pan Z. Correcting calcium dysregulation in chronic heart failure using SERCA2a gene therapy. Int J Mol Sci. 2018 Apr; 19(4): p. 1086.
18. Kawase Y, Ly HQ, Prunier F, Lebeche D, Shi Y, Hongwei J, et al. Reversal of cardiac dysfunction after long-term expression of SERCA2a by gene transfer in a pre-clinical model of heart failure. J Am Coll Cardiol. 2008 Mar; 51(11): p. 1112-1119.
19. Fargnoli AS, Katz MG, Yarnall C, Isidro A, Petrov M, Steuerwald N, et al. Cardiac surgical delivery of the sarcoplasmic reticulum calcium ATPase rescues myocytes in ischemic heart failure. Am Thorac Surg. 2013 Aug; 96(2): p. 586-595.
20. Hulot JS, Ishikawa K, Hajjar RJ. Gene therapy for the treatment of heart failure: promise postponed. Eur Heart J. 2016 Jun; 37(21): p. 1651-1658.
21. Pinz I, Tian R, Belke D, Swanson E, Dillman W, Ingwall JS. Compromised myocardial energetics in hypertrophied mouse hearts diminish the beneficial effect of overexpressing SERCA2a. J Biol Chem. 2011 Mar; 286(12): p. 10163-10168.
22. Adams KF, Butler J, Patterson JH, Stough WG, Bauman JL, van Veldhuisen DJ, et al. Dose response characterization of the association of serum digoxin concentration with mortality outcomes in the Digitalis Investigation Group trial. Eur J Heart Fail. 2016 Aug; 18(8): p. 1072-1081.
23. Rathore SS, Curtis JP, Wang Y, Bristow MR, Krumholz HM. Association of serum digoxin concentration and outcomes in patients with heart failure. JAMA. 2003 Feb; 289(7): p. 871-878.
24. Carubelli V, Zhang Y, Metra M, Lombardi C, Felker GM, Filippatos G, et al. Treatment with 24 hour istaroxime infusion in patients hospitalised for acute heart failure: a randomised, placeo-controlled trial. Eur J Heart Fail. 2020 January.
25. Crespo-Leiro MG, Anker SD, Maggioni AP, Heart Failure Association (HFA) of the European Society of Cardiology (ESC). European Society of Cardiology Heart Failure Long-Term Registry (ESC-HF-LT): 1-year follow-up outcomes and differences across regions. Eur J Heart Fail. 2016 Jun; 18(6): p. 613-625.
26. Gupta R, Testani J, Collins S. Diuretic Resistance in Heart Failure. Curr Heart Fail Rep. 2019 Apr; 16(2): p. 57-66.

27. Oppe M, Rabin R, de Charro F, On behalf of the EuroQoL Group. EQ-5D. [Online].; 2007. Available from: www.euroqol.org.