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Project N0. CVT-CV-002

General Protocol Amendment N 2
Final Version with SC and DSMB Members

CFDA Drug Clinical Trial **Original number: 20130181**
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CLINICAL STUDY PROTOCOL OF ISTAROXIME

The clinical study of the safety and efficacy of Istaroxime in Treatment of Acute Decompensated Heart Failure

- A multicenter, randomized, double-blind, placebo controlled, parallel group clinical study

CONFIDENTIAL

Final Version, 7 February, 2013

Amended Final Version 08 April 2013

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Amended Final Version 30 November 2017

Approved and Signed by: **Date**

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I, the undersigned _____
Last Name *First Name*

Principal Investigator at the following site:
SITE _____

declares:

to have read carefully and thoroughly the Study Protocol:

CODE: CVT- CV-002

TITLE: The clinical study of the safety and efficacy of Istaroxime in Treatment of Acute Decompensated Heart Failure - A multicenter, randomized, double-blind, placebo controlled, parallel group clinical study

VERSION: Amended Final Version 30 November 2017

Approved by the Sponsor: **CVie Therapeutic Company Limited,** and by the Chair of Steering Committee: **Dr. JUN Huang**

- to agree to the above-mentioned Protocol
- to conduct the Study in accordance with Study Protocol and in agreement with the Sponsor's and the Study Chairman's requests.

Signing this document I declare to have read the paragraph relevant to Study acknowledgement and confidentiality and authorise **CVie Therapeutics Company Limited**, to record my data on a computerised archive containing all the data pertinent to the Study.

Signature _____ Date: ___/____/___
(dd/mm/yy)

GENERAL INFORMATION

Protocol No.:	CVT-CV-002
Title:	The clinical study of the safety and efficacy of Istaroxime in Treatment of Acute Decompensated Heart Failure—A multicenter, randomized, double-blind, placebo controlled, parallel group clinical study
Date and Version:	Amended Final Version 30 November 2017
Study Sponsor:	CVie Therapeutics Company Limited Unit110-111, Bio-informatics center, No2. Science ParkWestAvenue, Hong Kong Science Park, Shatin, N.T. HK
Product:	Istaroxime (PST2744)
Pharmaceutical form:	lyophilized powder for intravenous infusion
Dosage:	0.5-1.0 μg/kg/min
Control Drug:	Placebo
Pharmaceutical form:	lyophilized powder for intravenous infusion

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The information in this protocol is confidential. It is provided to every potential Investigator and his/her team, as well as to the Institutional Review Board, that must statute on the trial. No information can be provided to a third party without the written agreement of CVie Therapeutics, except for the medical personnel directly concerned by the trial and for the information needed by the patient and his/her family.

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LIST OF ABBREVIATIONS

A	Pick mitral flow velocity during atrial contraction
Aa	Atrial myocardial contraction velocity
ADHF	Acute decompensated heart failure
AE	Adverse event
A_e	Amount excreted unchanged into urine
A_e%	Percent of the dose excreted unchanged into urine
ALAT	Alanine aminotransferase
ANOVA	Analysis of variance
ASAT	Aspartate aminotransferase
AUC	Area under the curve
$AUC_{0-\infty}$	Area under the concentration-time curve extrapolated to infinity
AUC_{0-t}	Area under the curve over the time interval 0 to the time of last quantifiable concentration
AUMC	Area under the first moment versus time curve
BNP	Brain natriuretic peptide
BP	Blood pressure
Bpm	Beats per minute
cTnT	Cardiac Troponin T
CHF	Chronic Heart Failure
CI	Confidence interval
Cl	Clearance
Cl_R	Renal clearance
C_{max}	Maximum concentration
CMH	Cochran-Mantel-Haenszel
CRF	Case Report Form
CRO	Contract Research Organization
CRT	Cardiac Resynchronization Therapy
C_t	Last quantifiable concentration
CV	Coefficient of variation
Da	Diastolic myocardial velocity
DBP	Diastolic blood pressure
DSMB	Data Safety Monitoring Board
DRQ	Data request Query
E	Pick mitral flow velocity during early rapid filling
Ea	Early diastolic myocardial relaxation velocity
E/Ea	ratio for estimation of LV filling pressure
E/A	ratio of the early over late filling velocity
EC	Ethics Committee
ECG	Electrocardiogram
e-CRF	Electronic case report form
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
GCP	Good Clinical Practice
Hb	Hemoglobin
HF	Heart failure
HR	Heart rate
ICD	Implantable Cardioverter Defibrillator
IUPAC	International Union of Pure and Applied Chemistry

i.v.	Intravenous
ITT	Intention-to-treat
λ_z	Terminal rate constant
LC/MS/MS	Liquid chromatography coupled to mass spectrometry
LVEF	Left ventricular ejection fraction
MBP	Mean blood pressure
MedDRA	Medical Dictionary for Regulatory Activities
MRT	Mean residence time
NT-proBNP	N-terminal brain natriuretic peptide precursor
PCWP	Pulmonary capillary wedge pressure
PD	Pharmacodynamics
PK	Pharmacokinetics
PP	Per protocol
PR	Interval between P and R waves
QRS	Interval between the beginning of Q wave and the end of S wave
QT	Interval between the beginning of Q wave and the end of T wave
RR	Interval between R and R waves
Sa	Systolic myocardial velocity
SAE	Serious adverse event
SAS	Safety analysis set
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
SERCA2a	Sarcoplasmic reticulum calcium ATPase isoform 2a
SV	Stroke Volume
SVR	Systemic vascular resistance
$t_{1/2}$	Terminal half-life
T_{inf}	Infusion duration
t_{last}	Time of last quantifiable concentration
t_{max}	Time to maximum concentration
TSH	Tyroid Stimulating Hormone
URL	Upper Reference Limit
V_{ss}	Volume of distribution at steady-state
VAS	Visual Analog Scale
V_z	Volume of distribution during the terminal phase
WHO	World Health Organization
WHO-DD	World Health Organization Drug Dictionary

SYNOPSIS

Title	The clinical study of the safety and efficacy of Istaroxime in Treatment of Acute Decompensated Heart Failure - A multicenter, randomized, double-blind, placebo controlled, parallel group clinical study.
Indication	Acute Decompensated Heart Failure (ADHF)
Objective	To Assess the safety, tolerability and efficacy of two different doses of istaroxime (0.5 and 1.0 µg/kg/min), a new agent with lusitropic and inotropic activities that improves the cardiac contraction-relaxation cycle. The 2 doses of istaroxime (0.5 and 1.0 µg/kg/min) will be infused i. v. for 24 hours in comparison with placebo, in treatment of Chinese and Italian patients with Acute Decompensated Heart Failure. In a subset of Italian patients and in a subset of Chinese patients pharmacokinetics and metabolism of istaroxime shall also be studied.
Study Design	A multicenter, randomized, double-blind, placebo-controlled, parallel group study.
Study Period	This study includes a screening period (Days -1), a treatment period (Day 1), a post-treatment period (Days 2-4), and a follow-up period (which includes one patient visit on Day 30).
Subject Selection Criteria	**Inclusion criteria** Patients who fulfill the following inclusion criteria at screening will be considered for the study: 1. Signed informed consent; 2. Male or female patients 18-85 years (inclusive); 3. Admission for a recurrent ADHF episode with dyspnea at rest or minimal exertion and need of intravenous diuretic therapy (⩾20 mg iv. furosemide); 4. Systolic blood pressure between 90 and 125 mmHg (limits included) without signs or symptoms of hypoperfusion including cardiogenic shock, cold extremities and peripheral vasoconstriction, oliguria/anuria, signs of cerebral hypo perfusion such as confusion; 5. Left ventricular (LV) Ejection fraction (EF) ≤ 40 % measured by 2D-Echocardiography 6. E/Ea ratio >10 7. BNP ≥ 350pg/mL or NT-pro-BNP ≥1400 pg/mL 8. Adequate echocardiography window (defined as visualization of at least 13/16 segment of the left ventricle); **Exclusion Criteria** Any of the following criteria established at screening would render a patient ineligible for the study: 1. Pregnant or breast-feeding women (women of child bearing potential must have the results of a negative pregnancy test recorded prior to study drug administration)

	2. Current (within 6 hours prior to screening), during the screening or planned (if the patients was treated with dopamine or nitrates iv infusion) (through the completion of study drug infusion) treatment or within 12 hours prior to screening if the patient was treated with with other iv. vasodilators or other inotropic agents and vasopressors 3. Current or need of mechanical support (intra-aortic balloon pump, endotracheal intubation, mechanical ventilation, or any ventricular assist device), 4. Ongoing treatment with oral digoxin. Patient treated with digoxin cannot be randomized. (However, if digoxin treatment has been stopped during the last week before signing informed consent form and the digoxin plasma level is < 0.5 ng / ml, patient may be randomized; if the patients have stopped digoxin for more than one week before signing informed consent form, it is not necessary to test the plasma level of digoxin.) 5. History of hypersensitivity to the study medication or any related medication 6. Diagnosis of cardiogenic shock within the past month; 7. Acute coronary syndrome or stroke within the past 3 months; 8. Coronary artery bypass graft or percutaneous coronary intervention within the past month or planned in the next month; 9. Primary hypertrophic or restrictive cardiomyopathy or systemic illness known to be associated with infiltrative heart disease; 10. Cor pulmonale or other causes of right-sided HF not related to left ventricular dysfunction; 11. Pericardial constriction or active pericarditis; 12. Atrial fibrillation with marked irregularities of heart rhythm (Inclusion criteria 8 can not be met); 13. Life threatening ventricular arrhythmia or ICD (implantable cardioverter defibrillator) shock within the past month; 14. CRT (cardiac resynchronization therapy), ICD or pacemaker implantation within the past month; 15. Valvular disease as primary cause of HF (except those corrected by surgery without obstruction in left ventricle output); 16. Heart rate >120 bpm or < 50 bpm 17. Acute respiratory distress syndrome or ongoing sepsis; 18. Fever >38° 19. History of bronchial asthma or porphyria; 20. Donation or loss of blood equal to or exceeding 500 mL, during the 8 weeks before administration of study medication; 21. Positive testing for HIV, Hepatitis B and/or Hepatitis C with abnormal liver functions; 22. Participation in another interventional study within the past 30 days; 23. The following laboratory exclusion criteria, verified based on results obtained within the last 24 hours of hospitalization: a. Serum creatinine > 3.0 mg/dl (> 265 µmol/L); b. Aspartate aminotransferase (ASAT) or alanine aminotransferase (ALAT) > 3 x upper limit of normal, c. Hemoglobin (Hb) < 10 g/dL, d. Platelet count < 100,000/µL, e. Serum potassium > 5.3 mmol/L or < 3.8 mmol/L,

	(Note: 1. All the hematology and biochemistry results obtained within the past 24 hours before signing ICF can be used as baseline without retesting the patients. 2. For HIV、HBV、HCV、T3、T4、TSH, results obtained within one week before the patient signed informed consent form can be accepted.)
Study Drugs	Test drug: Istaroxime（10 mg per vial）
Mode of administration	Intravenous infusion via a syringe pump.
Treatment duration	Treatment by i.v. infusion will last 24 hours.
Dosing scheme	**Istaroxime 0.5 – 1.0 µg/kg/min since the beginning. A** continuous i.v. infusion for 24 hours not exceeding 144 mg for 24 hours of istaroxime for patients with body weight > 100 kg shall be carried out.
Sample Size	96 Chinese patients and 24 Italian patients
Study Procedures	✧ **Screening period (between Hours -24 to -1)** Within a maximum of 24 hours before administration of study medication (istaroxime), a 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 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. ✧ **Treatment period (Day 1)** 1) Confirm eligibility; 2) Randomization of patients (after eligibility has been confirmed) 3) Insertion of multiple lumen intravenous catheter (or administration via peripheral vein) 4) Start istaroxime or placebo infusion (date and time of infusion start must be recorded in the CRF) 5) cTnT (at pre-dose: two samples, then at 3 and 6, 12, 24, 48 and 72 hours after start of infusion) 6) NT pro-BNP at baseline and at the end of 24 hours infusion 7) Blood samples collection for metabolites and PK (at pre-dose, 0.5-3-6-12-24 after start of infusion and at 0.25, 0.5, 1, 4 12, 24, 48 hours after the end of infusion) in a subset of Italian patients and in a subset of Chinese patients pharmacokinetics and metabolism of istaroxime shall also be studied. 8) Vital signs (including arterial oxygen saturation and dyspnoea at pre-dose,3, 6, 12 and 24 after the start of infusion) 9) 12-lead ECG profile (pre-dose, 3, 6, 12 hours and between 23 hours and 23 hours 55 minutes after infusion, totally 5 times, each time 3 measurements) 10) Stop Day -1 Holter 11) Start 24-hour Holter ECG (Day 1 recording; to be started immediately before initiation of the study drug infusion)

	12) Echocardiography at baseline and 6 and 24 hours after infusion start 13) 24-hours urine collections for measurement of istaroxime and its metabolites and urinary creatinine for the calculation of the creatinine clearance; 14) Blood collection for K+ and eGFR between 23 hours and 30 minutes and 23 hours and 55 minutes since infusion start; 15) Concomitant medication monitoring (including chronic medication; dose, date and time must be recorded on CRF) 16) Adverse events monitoring ✧ **Post-treatment period (Day 2 to Day 4)** **Evaluations at 24 hours (day 2) from randomization include:** 1) Vital signs (including arterial oxygen saturation and dyspnea); 2) 12-lead ECG (triple ECGs) (between 23 hours and 23 hours 55 minutes since infusion start); 3) Stop 24-hour Holter ECG; 4) Start 24-hour Holter ECG (Day 2 recording); 5) Stop istaroxime infusion (date and time of infusion end must be recorded in the CRF); 6) Serum potassium level and 24-hour urine collection for measurement of istaroxime metabolites and urinary creatinine for calculation of the creatinine clearance; 7) Serum creatinine clearance and calculation of eGFR; 8) cTnT (50 % or 20 % relative increase over the basal cTnT levels, respectively for patients with cTnT basal levels < or > of the 99 % URL (upper reference levels, as defined for the Roche hs test, in patients with normal renal function, eGFR ⩾85 ml/min); in patients with eGFR below this value, the renal function variations must be considered in evaluating the significance of the cTnT changes); 9) NT pro-BNP; 10)Metabolites; 11) Echocardiography; 12) Concomitant medication monitoring (including chronic medication must be recorded in the CRF); 13) Adverse Events monitoring. **Evaluations at 48 hours (day 3) include:** 1) Vital signs (including arterial oxygen saturation and dyspnea); 2) 12-Lead ECG (single ECGs); 3) Stop 24-hour Holter ECG; 4) Standard hematology; 5) Standard blood chemistry; 6) Serum potassium level; 7) 24-h urine collection for measurement of istaroxime metabolites and urinary creatinine for the calculation of creatinine clearance ; 8) Calculation of eGFR; 9) NT-proBNP;

	10) cTnT; 11) Blood samples for istaroxime metabolites; 12) Echocardiography 13) Adverse events monitoring; 14) Concomitant medication monitoring (including chronic medication must be recorded in the CRF); **Evaluations at 72 hours (day 4) include:** 1) cTnT and NTproBNP (at 72 hours after start of infusion) 2) Vital signs (including arterial oxygen saturation and dyspnoea) 3) Physical examination (HF signs included) 4) 12-lead ECG 5) Adverse events monitoring 6) Concomitant medication monitoring (including chronic medication) 7) Istaroxime metabolites 8) Serum potassium and creatinine levels for calculation of eGFR 9) Creatinine clearance ✧ **Follow-up period and visit (Day 5 to Day 30)** During the follow-up period the Investigator/designee will make every effort to establish patient outcomes. **Evaluations on Day 30 (follow-up visit) include:** 1) Vital signs (including arterial oxygen saturation and dyspnoea); 2) 12-lead ECG in triplicate; 3) Calculation of eGFR; 4) Standard hematology; 5) Standard blood chemistry; 6) NT-proBNP 7) cTnT; 8) Urine pregnancy test (β-HCG) for females of childbearing potential 9) Urinalysis; 10) Physical examination (HF signs included); 11) Adverse events monitoring; 12) Concomitant medication monitoring (including chronic medication)
Efficacy Parameters	**Efficacy endpoints** 1. **Primary efficacy end-point:** • Change from baseline to 24 hours after infusion start (treatment period Day 1) in the E/Ea ratio assessed by tissue Doppler. **2. Secondary efficacy end-points:** • Change from baseline to 24 hours in the treatment period Day1 (addressing the differences between the changes at 6 and 24 hours from baseline) of the following Echo-Doppler parameters: - LV Ejection fraction (EF)

	- LV end systolic and end diastolic volumes - Stroke volume index (SVI) - E, A and E/A ratio • Difference between the changes at 6 and 24 hours from baseline of the Tissue Doppler parameter E/Ea • Others Tissue Doppler parameters such as Sa, Da and Aa • Changes in dyspnoea assessed at 3, 6, 12, 24, 48 hours after infusion start by Visual Analog Scale (VAS) (including only patients presenting dyspnoea at baseline); • Area under the curve (AUC) on changes in dyspnoea assessed at 3, 6, 12, 24, 48 hours after infusion start by VAS (including only patients presenting dyspnea at baseline); • Changes in BNP from baseline at 24 hours; • Proportion of patients with hospital readmissions or emergency visits for cardiovascular reasons by Day 30; • Proportion of patients with episodes of worsening HF defined by the need to increase the dose or reinitiate i.v. therapy with diuretics and/ or other inotropic agents during the hospitalization; • Length of the hospitalization;
Safety Parameters	**Safety endpoints:** The following safety endpoints will be assessed during treatment and the post-treatment/follow-up periods: • Incidence of adverse events; • Change in vital signs (including body temperature and dyspnoea); • Change in 12-lead ECG parameters; • Incidence of clinically or hemodynamically significant episodes of supraventricular or ventricular arrhythmias detected by continuous ECG dynamic monitoring; • Change in laboratory parameters (hematology, blood chemistry and urinalysis); • Change in renal function; • Change in in cTnT; • Incidence of cTnT elevation (>50% or > 20% relative increase over the basal cTnT levels at baseline, for patients with cTnT levels at baseline < or ≥ of the 99% URL (upper reference levels, as defined for the Roche hs test, in patients with normal renal function, eGFR ≥85 ml/min); in patients with eGFR below this value, the renal function variations must be considered in evaluating the significance of the cTnT changes); • Mortality at Day 30;
PK parameters	Full plasma and urine PK profile: • The following PK metrics will be computed for E and Z isomers (when applicable) of istaroxime plasma concentrations using

	non-compartmental analysis: C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$, λ_z, $t_{½}$, Cl_T, MRT, V_{ss}, V_z; • the following PK metrics will be computed for E and Z isomers (when applicable) of istaroxime urine concentrations: A_e, A_e%, Cl_R; • In addition, the following PK metrics will be computed as above for plasma and urine concentrations of the E and Z isomers (when applicable) of istaroxime metabolites PST2915, PST2922, and PST3093: C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$, λ_z, $t_{½}$ and, if possible, A_e and $A_{e\%}$; This endpoint will be addressed in all the Italian patients and in a subset of Chinese patients.
Statisical Analysis	<u>Primary efficacy endpoint:</u> The primary efficacy endpoint (change from baseline in E/Ea ratio) will be analyzed using a linear mixed model for repeated measures including treatment, centre, timepoint, gender, baseline cTnT (normal <URL, abnormal ≥URL), atrial fibrillation (Yes/No) and treatment*timepoint interaction as fixed effects and baseline and baseline*timepoint interaction as covariates. The primary comparison will be 0.5 µg/kg/min dose of istaroxime versus placebo at 24 hours. Highest dose of istaroxime (1.0 µg/kg/min) versus placebo will be tested as a secondary comparison. Additional analyses separated by cohort will be implemented for sensitivity purpose. <u>Secondary efficacy endpoints</u> The following secondary endpoints: • Change from baseline to 24 hours (addressing the differences between the changes at 6 and 24 hours from baseline) of the following Echo-Doppler parameters: - LV Ejection fraction (EF) - LV end systolic and end diastolic volumes - Stroke volume index (SVI) - E, A and E/A ratio • Change from baseline to 24 hours in the E/Ea ratio assessed by tissue Doppler (difference between the changes at 6 and 24 hours from baseline) • Others Tissue Doppler parameters such as Sa, Da and Aa • Changes in dyspnoea using VAS score will be analysed using a mixed model for repeated measures similar to the one used for the primary efficacy endpoint. AUC on changes in dyspnoea by VAS and changes in BNP will be analyzed using an ANCOVA model with treatment, centre, gender, baseline cTnT (normal <URL, abnormal ≥URL) and atrial fibrillation (Yes/No) as fixed effects and baseline dyspnea as covariate. Number and proportion of patients with: • hospital readmissions or emergency visits for cardiovascular reasons within Day 30 • episodes of worsening HF defined by the need to increase the dose or reinitiate i.v. therapy with diuretics and/ or other inotropic agents during the hospitalization will be summarized by treatment groups using descriptive statistics.

	Length of hospitalization will be summarized by treatment group using descriptive statistics. <u>Safety endpoints</u> The number and the percentage of patients experiencing adverse events, adverse drug reactions, serious adverse events and adverse events leading to study withdrawal will be summarized by treatment group. Adverse events will also be summarized by treatment group by means of System Organ Class and Preferred Term using the MedDRA dictionary. Vital signs (including body temperature and dyspnoea), 12-lead ECG parameters, incidence of clinically or hemodynamically significant episodes of supraventricular or ventricular arrhythmias, laboratory parameters, renal function, cTNT, increase of cTNT and mortality will be summarized by treatment group using descriptive statistics.

1. INTRODUCTION

1.1 Indications

Congestive Heart Failure and Acute Decompensation

Congestive heart failure (CHF) is a complex pathophysiologic syndrome associated with significant morbidity and mortality. Figures from the USA and Europe suggest that respectively some 5 and 10 million adults have CHF. The incidence and prevalence continue to rise as the proportion of elderly in the population increases [1]. In patients with CHF, acute decompensation is a major cause of hospitalization and of cost associated with treatment. Acute decompensated heart failure (ADHF) is characterized by a rapid or gradual onset of symptoms and signs secondary to abnormal cardiac function. As said, it often results in unplanned hospitalization and a need for urgent therapy [2].

Hospitalizations for ADHF are increasing with more than 1 million per year in the USA and as many in Europe. In-hospital mortality ranges from 4 to 7% in large ADHF registries [2, 3]. Post-discharge mortality is approximately 10% during the next 1-2 months, and hospital readmission common, with 25 to 30% of patients being readmitted within the first 3 months after discharge [2- 4].

Signs and symptoms of heart failure (HF) result primarily from severe pulmonary congestion due to elevated left ventricular pressures, with or without low cardiac output. Cardiac dysfunction, renal impairment and neurohormonal activation play a significant role in the development of congestion by creating a vicious circle: systolic and/or diastolic dysfunctions lead to increased left ventricular diastolic pressure and blood volume resulting in increased pulmonary capillary wedge pressure (PCWP) [5]. Increased PCWP might further lead to increased pulmonary arterial pressure, increased right ventricular and atrial pressures, inducing pulmonary and/or systemic congestion with dyspnoea and edema. Activation of neurohormonal systems and renal dysfunction also contribute to the development of congestion through impaired sodium and water handling [5].

Approach to Treatment

In chronic congestive heart failure, treatment involves both non-pharmacological and pharmacological interventions. Stabilization and improvement of cardiac hemodynamics is an important goal of therapy and diuretics, ACE inhibitors, Beta Blockers and vasodilators are the most commonly utilized agents. Cardiac glycosides such as digoxin, which is mainly used as adjunctive therapy, have an extremely long history in the management of heart failure [6]. Little controversy exists as to the benefit of digoxin in patients with symptomatic left ventricular systolic dysfunction (LVEF $\leq$ 40%) and concomitant atrial fibrillation [7].

The therapeutic goals in patients hospitalized with ADHF are to reverse acute hemodynamic abnormalities, relieve symptoms, and preserve myocardial tissue and renal function without

inducing arrhythmia or hypotension [8]. Commonly-used agents in the treatment of ADHF with normal to high systolic blood pressure (SBP) include diuretics and vasodilators (e.g. nitroglycerin, nitroprusside, and nesiritide). Patients with low blood pressure (BP) may require intravenous (i.v.) inotropic support for their low output state, essentially adrenergic stimulating agents such as dobutamine or dopamine, or phosphodiesterase inhibitors such as milrinone. However, none of the currently available agents encompasses all the properties desired for an "ideal" agent for treatment of ADHF: diuretics may worsen renal function; vasodilators and nesiritide are associated with symptomatic hypotension; conventional inotropes induce myocardial cell calcium accumulation and may cause atrial and ventricular arrhythmias, myocardial ischemia due to increase of oxygen consumption, cell apoptosis and possibly increase post-discharge mortality [9, 10]. Thus, conventional inotropes such as dobutamine, dopamine or milrinone are indicated in the presence of peripheral hypoperfusion with or without congestion or in pulmonary edema refractory to diuretics and vasodilators [11].

Development of drugs that improve contractility without producing calcium overload, therefore represents an important goal in the treatment of ADHF.

1.2 Pharmaceutical and background information

Istaroxime

Background Information

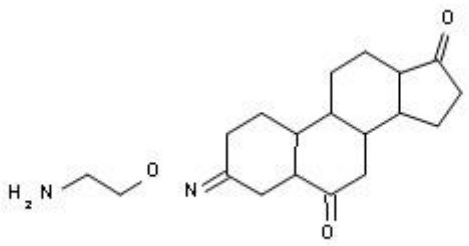
Istaroxime is a first-in-class luso-inotropic agent under development for the treatment of acute decompensated heart failure. It possesses a dual mode of action, combining inotropic (myocyte contraction) and lusitropic (myocyte relaxation) effects. Istaroxime is a potent positive inotropic agent that increases myocardiac contractility through inhibition of Na^+/K^+-ATPase, subsequently augmenting cytoplasmic calcium levels. In addition, istaroxime exerts positive lusitropic effects through activation of sarcoplasmic reticulum calcium ATPase isoform (SERCA2) promoting reuptake of cytoplasmic calcium with subsequent myocardial relaxation, thus decreasing left ventricular diastolic pressure and congestion. Based on its mechanism of action, preclinical studies and early clinical findings, it is anticipated that istaroxime at the doses proposed in this trial will be devoid of the deleterious effects of conventional inotropes. Istaroxime does not increase heart rate (HR), minimizes the increase in oxygen consumption, is less arrhythmogenic and does not cause hypotension [12, 13, 14].

Chemical Structure, Formula and Molecular Weight

Istaroxime is an original derivative of androstanedione, a lipophilic steroid based compound, chemically unrelated to cardiac glycosides or to phosophodiesterase inhibitors.

IUPAC name:	Androstane-3,6,17-trione (E,Z)-3-[*O*-(2-aminoethyl)]oxime hydrochloride
Other names:	(E,Z)-3-[O-(2-aminoethyl)oxime]-5α-androstane-3,6,17-trione monohydrochloride (E,Z)-3-(2-Aminoethoxyimino)androstane-6,17-dione hydrochloride
Molecular weight:	396.95
Molecular formula:	C_{21} H_{33} ClN_2 O_3

Molecular structure:



Stereoisomerism: The active substance is a 1:1 mixture of E and Z isomers at the oximic C(3)=N double bound.

International Non-proprietary Name, Brand Name and Code Name

International non-proprietary name:

Istaroxime

Brand name:	Not yet designated
Code names:	PST2744

Mechanism of Action

Istaroxime is a dual-action Na^+/K^+-ATPase inhibitor/sarcoplasmic reticulum calcium pump (SERCA2) activator that improves myocardial efficiency by modulating calcium cycling in cardiac myocytes. Na+/K+-ATPase inhibition augments cytoplasmic calcium levels and subsequently promotes myocardial contractility, enhancing inotropy. SERCA2

activation promotes the reuptake of cytoplasmic calcium with subsequent myocardial relaxation and improved filling of the heart. As a result of the combination of these two mechanisms, istaroxime improves cardiac function [13, 14, 15]. Compared to inotropes such as dobutamine or milrinone, istaroxime, at therapeutic doses, does not induce myocardial "calcium intoxication", does not increase HR, oxygen consumption, and proarrhythmic effect. Moreover, being devoid of vasodilating effects, istaroxime does not induce arterial hypotension [14].

Brief Summary of Non-clinical Pharmacology

Hemodynamic *in vivo* studies showed enhanced cardiac systolic and diastolic function in anesthetized guinea pigs, pigs and dogs both with and without HF. In guinea pigs with 3-month aortic banding, echocardiographic results showed that i.v. istaroxime infusion significantly increased both contraction and relaxation indices [16]. In pigs without HF, the effects of istaroxime on contractility and HR were evaluated in comparison with dobutamine as the reference inotropic agent: istaroxime showed a positive inotropic effect on cardiac output and stroke volume similar to dobutamine, however without increasing HR or oxygen consumption as seen with the latter [17].

Pharmacological studies carried out in the dog model of advanced HF, escalating doses of i.v. istaroxime from 0.5 µg/kg/min to 5.0 µg/kg/min were administered, each dose being maintained for one hour. Istaroxime substantially improved systolic and diastolic left ventricular function [18]. Furthermore, istaroxime was administered i.v. for 24 hours at 1, 3, and 4 µg/kg/min, compared to saline 50 mL/24h infusion in conscious male Beagle dogs with HF after repeated coronary microembolization [19]. Istaroxime induced a dose-dependent increase in cardiac inotropy ($+dP/dt_{max}$), which peaked after 6-8 hours and remained constant throughout the experiment. Cardiac lusitropy ($-dP/dt_{max}$) improved although not statistically significantly during infusion rates of 1 and 3 µg/kg/min. Administration of istaroxime at 3 µg/kg/min, induced vomiting after 4-5 hours of continuous infusion. At 4 µg/kg/min, vomiting episodes increased and persisted for the first 10-12 hours of infusion. The maximum tolerated dose of istaroxime in dogs was 3 µg/kg/min for 24 hours.

Noteworthy, unlike classic cyclic adenosine monophosphate-dependent positive inotropic agents such as milrinone or β-adrenergic compounds like dobutamine, istaroxime improves both inotropic and lusitropic states with much less increase of oxygen consumption. These improvements were not associated with any apparent increase in positive chronotropic and proarrhythmic activity.

The toxicological profile of 24 hour infusion of istaroxime was investigated in rats and dogs under GLP conditions. The maximum tolerated dose (MTD) was 10 µg/kg/min in rats and 3 µg/kg/min in dogs.

In conclusion, istaroxime is a powerful luso-inotropic agent that, in animal models of HF in

doses up to 4 µg/kg/min, does not increase the risk of arrhythmia. From the pharmacology data, istaroxime appears to be a promising and safe new drug for improving cardiac performance in HF.

More detailed pre-clinical information can be found in the Investigator's Drug Brochure [20].

Brief Summary of Human Pharmacology

Istaroxime efficacy was first evaluated in two phase I-II safety and tolerability studies performed in patients with chronic HF and left ventricular dysfunction. In the first study, istaroxime was administered as 1-hour infusions of ascending doses from 0.005 to a maximum of 5.0 µg/kg/min in an escalating dose, placebo-controlled, double-blind design in 6 patients for each dose. No increases in HR or arrhythmias were detected at any of the doses administered including the highest dose (5.0 µg/kg/min). Reported AEs were gastrointestinal symptoms and injection site pain starting at the dose of 3.33 µg/kg/min. Hemodynamic measurements within 2 hours prior to dosing and during the 1-hour infusion were carried out using bio-impedance methodology. Istaroxime infusions up to a dose of 1.0 µg/kg/min did not appear to have an effect on any of the measured hemodynamic parameters. At the subsequent dose levels of 1.67, 3.33, and 5.0 µg/kg/min, dose-dependent increases from baseline were observed at the end of the infusion period in cardiac index, acceleration index, velocity index, and left cardiac work index, with no clinically significant decrease from baseline in systemic vascular resistance (SVR) [20].

In a second double-blind study, patients received 1-hour istaroxime infusions at doses escalating from 0.005µg/kg/min to 3.0µg/kg/min. Swan Ganz catheterization was used to measure hemodynamic parameters. No clinically relevant inotropic and lusitropic effects were observed at the lowest doses of 0.005, 0.0167 and 0.5 µg/kg/min. At the 1.0, 2.0, and 3.0 µg/kg/min infusions clinically relevant lusitropic and inotropic effects were observed. The echocardiographic sub-study results, performed in a subset of patients, confirmed a strong and consistent improvement in left ventricle systolic function with a significant decrease in left ventricle systolic volume and increases in ejection fraction (EF) and stroke volume (SV). In addition the patients evaluated had severe left ventricle diastolic impairment at baseline, and demonstrated a significant improvement in diastolic function as measured by E/E' (ratio of transmitral peak early filling velocity to early diastolic velocity of the mitral annulus) assessed 10 minutes prior to completion of the istaroxime infusion. Cardiac tolerability was good, without any significant arrhythmia even at the highest dose of 3.0µg/kg/min. Nausea and vomiting were observed at the dose of 3.0 µg/kg/min.

A further phase I placebo-controlled dose escalating study was performed to evaluate the safety and tolerability of istaroxime 6-hour infusion in healthy male volunteers [20]. Four different escalating istaroxime doses (0.67, 1.33, 1.67, and 2.0 µg/kg/min) were infused i.v. for 6 hours. Each dose, except for the 2.0µg/kg/min dose administered in 1 cohort only, was infused in two different cohorts of 4 subjects (3 subjects receiving istaroxime and 1 receiving

placebo in each cohort). No clinically relevant abnormalities were observed on vital signs and ECGs. While the 0.67 and 1.33 µg/kg/min doses were well tolerated, the highest dose group (2.0 µg/kg/min) could not be completed due to non-serious adverse events (AEs), mainly gastrointestinal disorders (nausea and vomiting) and pain at the injection site. The 1.67 µg/kg/min dose was relatively well tolerated by 2 of the 6 subjects. In this treatment group, the infusion was terminated prior to 6 hours in 4 subjects due to severe AEs (nausea and vomiting). All AEs resolved shortly after cessation of the infusion.

Finally, to determine the minimum effective dose, a phase IIa, double-blind, randomized, placebo-controlled, dose escalating (0.5-1.0-1.5 µg/kg/min) efficacy study (HORIZON-HF) was conducted assessing the hemodynamic effects of istaroxime in 120 patients hospitalized with worsening HF and reduced left ventricle systolic function [14]. 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. A trend towards an increase in cardiac output was also observed in the treated groups. A significant increase in cardiac index (CI) was observed only in patients with a CI<2.5 at baseline. Statistically significant difference between istaroxime and placebo were found for LV end systolic volume at 1.0 µg/kg/min and for LV end diastolic volume at 1.5 µg/kg/min. Doppler echocardiography and tissue Doppler imaging showed changes in the E/Ea parameter that resulted statistically significant at 0.5 µg/kg/min (-3.8, p= 0.03) and close to significance at 1.0 µg/kg/min (-1.12, p= 0.059). In conclusion, echocardiographic data showed istaroxime to improve diastolic and systolic volumes, as well as Doppler and tissue Doppler parameters of diastolic function. As far as safety concerns, this study showed an increase in gastrointestinal TEAEs in the highest dose group (1.5 µg/kg/min) in which 10% and 17% of patients experienced nausea and vomiting, respectively. No patients experienced serious or clinically relevant episodes of arrhythmia during the infusion and post-infusion period. A mild increase in the frequency of isolated ventricular extrasystoles was found in the 1.5 µg/kg/min group in 8 out of 30 patients. It is difficult to ascertain whether this increase was related to drug infusion or to spontaneous variability of patients, because of insufficient baseline recordings. One patient 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

Globally, istaroxime was well tolerated and safe up to 1.5 µg/kg/min in patients suffering from acute HF. Doses above 1.5 µg/kg/min were not completely tolerated due to

gastrointestinal disorders and infusion site pain.

In conclusion, in this study the lowest tested dose of 0.5 µg/kg/min of istaroxime infused for 6 hours produces a significant PWCP improvement over placebo, being the istaroxime safety profile satisfactory even at higher dose, in this setting.

Pharmacokinetic results showed that istaroxime levels increased proportionally in plasma reaching steady state concentration within the 1hour infusion. Estimated half-life for istaroxime was about 10 to 20 minutes at infusion rates higher than 1.66 µg/kg/min. For rates of infusion lower than 1.66 µg/kg/min, concentration dropped below the limit of quantification (10 ng/ml) at 5 min after stopping of the infusion.

The systemic exposure to istaroxime resulted to be linearly related to the administered dose [20]. A very high clearance was shown, resulting around 4500 ml/min for a 70 kg body weight man. The concentration of PST 2915, the main metabolite (6β-alcholol) of the parent compound, was also measured, showing that its concentration slowly increased during infusion period, reaching a maximum concentration within 10 min after the stop of the intravenous infusion of istaroxime [20]. The metabolite maximum concentration resulted to be about one half of the corresponding C_{max} of istaroxime. Half-life of PST 2915 resulted to be around 3 to 4.5 hours [20]. PST 2915 resulted to be an active metabolite showing the same stimulatory activity on SERCA2a of istaroxime but about 10 time less inhibitory activity on Na-KATPase and potency in in vivo animal models than istaroxime [20].

For infusion longer than 1 hour, PST 2915 plasma concentrations increase with time, while those of istaroxime remain at steady state, being constant the rate of infusion.

To evaluate the risk of a prolongation of istaroxime intravenous infusion over six hours, pharmacokinetic, efficacy and tolerability clinical studies have to be carried out.

The above clinical evidence indicates that istaroxime could be a future treatment option for patients with ADHF and a low cardiac output state.

More detailed clinical information can be found in the Investigator's Drug Brochure [20].

2. STUDY OBJECTIVE

To assess the safety, tolerability and efficacy of two different doses of istaroxime (0.5 and 1.0 µg/kg/min) in comparison with placebo, including cardiovascular and renal tolerability, as well as changes in biological markers such as N-terminal prohormone brain natriuretic peptide (NT-proBNP) and troponin T (cTnT), in two cohorts, Chinese and Italian /Caucasian patients with Acute Decompensated Heart failure.

In all the Italian patients and in a subset of Chinese patients pharmacokinetics and metabolism of istaroxime shall also be studied.

3. STUDY DESIGN

This is a phase II, multicenter, randomized, double-blind, placebo-controlled, parallel group study.

96 Chinese patients and 24 Italian patients will be randomly assigned to one of two doses of istaroxime or placebo in a 2:1 ratio within two sequential cohorts of 60 patients each.
This 31-day study includes a screening period (Days -1), a treatment period (Day 1), a post-treatment period (Days 2-4), and a follow-up period (which includes one patient visit on Day 30).

When a patient is considered fully eligible (i.e. s/he fulfills all the inclusion criteria and does not meet any exclusion criteria as stipulated), the patient will be randomized.
Treatment details are presented below:

Cohort I: 0.5 µg/kg/min istaroxime or placebo **(40 patients randomized to istaroxime 0.5 µg/kg/min group and 20 patients randomized to placebo group).**
Istaroxime 0.5 µg/kg/min (30 µg/kg/h) continuous i.v. infusion for 24 hours **not exceeding 72 mg per 24 hours for patients with body weight > 100 kg**
Cohort II: 1.0 µg/kg/min istaroxime or placebo (40 patients randomized to istaroxime 1.0 µg/kg/min group and 20 patients randomized to placebo group).
Istaroxime 1.0 µg/kg/min (60 µg/kg/h) continuous i.v. infusion for 24 hours **not exceeding 144 mg per 24 hours for patients with body weight > 100 kg**

Escalation to the next dose (second cohort) will occur after completion of the first cohort and determined by the evaluation of the safety data performed by the Data Safety Monitoring Board.

4. STUDY POPULATION

A total of 96 Chinese and 24 Italian patients hospitalized with ADHF not requiring inotropic therapy at inclusion into the study (according to the opinion of the Investigator) and not presenting signs and/or symptoms of severe low output state or peripheral hypo perfusion will be randomized.

To be included into the study, patients have to fulfill eligibility criteria both at their admission to the hospital (screening criteria) and just prior to randomization (randomization criteria).

4.1 Inclusion criteria

Patients who fulfill the following inclusion criteria **at screening** will be considered for the study:

1. Signed informed consent;
2. Male or female patients 18-85 years (inclusive);

3.Admission for a recurrent ADHF episode with dyspnea at rest or minimal exertion and need of intravenous diuretic therapy (≥ 40 mg iv. furosemide);

4. Systolic blood pressure between 90 and 125 mmHg (limits included) without signs or symptoms of hypoperfusion including cardiogenic shock, cold extremities and peripheral vasoconstriction, oliguria/anuria, signs of cerebral hypo-perfusion such as confusion;

5. Left ventricular (LV) Ejection fraction (EF) ≤ 40 % measured by 2D-Echocardiography

6. E/Ea ratio > 10

7. BNP ≥ 350pg/mL or NT-pro-BNP ≥1400 pg//mL;

8. Adequate echocardiography window (defined as visualization of at least 13/16 segment of the left ventricle);

4.2 Exclusion criteria

Any of the following criteria established **at screening** would render a patient ineligible for the study:

1. Pregnant or breast-feeding women (women of child bearing potential must have the results of a negative pregnancy test recorded prior to study drug administration)

2. Current (within 6 hours prior to screening), during the screening or planned (if the patients was treated with dopamine or nitrates iv infusion) (through the completion of study drug infusion) treatment or within 12 hours prior to screening if the patient was treated with other iv. vasodilators or other inotropic agents and vasopressors,

3) Current or need of mechanical support (intra-aortic balloon pump, endotracheal intubation, mechanical ventilation, or any ventricular assist device),

4. Ongoing treatment with oral digoxin. Patient treated with digoxin cannot be randomized. (However, if digoxin treatment has been stopped during the last week before signing informed consent form and the digoxin plasma level is < 0.5 ng / ml, patient may be randomized; if the patients have stopped digoxin for more than one week before signing informed consent form, it is not necessary to test the plasma level of digoxin.)

5. History of hypersensitivity to the study medication or any related medication;

6. Diagnosis of cardiogenic shock within the past month;

7. Acute coronary syndrome or stroke within the past 3 months;

8. Coronary artery bypass graft or percutaneous coronary intervention within the past month or planned in the next month;

9. Primary hypertrophic or restrictive cardiomyopathy or systemic illness known to be associated with infiltrative heart disease;

10. Cor pulmonale or other causes of right-sided HF not related to left ventricular dysfunction;

11. Pericardial constriction or active pericarditis;

12. Atrial fibrillation with marked irregularities of heart rhythm (Inclusion criteria 8 can not be met);

13. Life threatening ventricular arrhythmia or ICD (implantable cardioverter defibrillator) shock within the past month;

14. Presence of a CRT (cardiac resynchronization therapy), ICD or pacemaker devices implanted within the past month;

15. Valvular disease as the primary cause of HF (except those corrected by surgery without obstruction in left ventricle output);

16. Heart rate > 120 bpm or < 50 bpm

17. Acute respiratory distress syndrome or ongoing sepsis;

18. Fever >38°

19. History of bronchial asthma or porphyria;

20. Donation or loss of blood equal to or exceeding 500 mL, during the 8 weeks before administration of study medication;

21. Positive testing for HIV, Hepatitis B and/or Hepatitis C with abnormal liver functions;

22. Participation in another interventional study within the past 30 days;

23. The following laboratory exclusion criteria, verified based on results obtained within the last 24 hours of hospitalization:

a. Serum creatinine > 3.0 mg/dl (> 265 μmol/L);

b. Aspartate aminotransferase (ASAT) or alanine aminotransferase (ALAT) > 3 x upper limit of normal,

c. Hemoglobin (Hb) < 10 g/dL,

d. Platelet count < 100,000/μL,

e. Serum potassium > 5.3 mmol/L or < 3.8 mmol/L,

(Note:

1. All the hematology and biochemistry results obtained within the past 24 hours before signing ICF can be used as baseline without retesting the patients.

2. For HIV、HBV、HCV、T3、T4、TSH, results obtained within one week before the patient signed informed consent form can be accepted.)

5. STUDY PRODUCTS AND DOSE REGIMEN

5.1 TEST DRUG

5.1.1 ISTAROXIME

Name: Istaroxime

Composition: Istaroxime 10 mg, lactose 50 mg, and if needed sodium hydroxide as pH adjuvant.

Dosage form: Powder for solution for infusion.

Presentation: 10 mg istaroxime glass vial to be reconstituted with 5 ml saline for injection, according to instructions in leaflet.

Storage Condition: Istaroxime should be stored at room temperature below or equal to 25°C (77°F) in a secure location. Do not freeze.

Source: provided by CVie Therapeutics Company Limited.

The certificate of analyses will be provided together with the product.

5.1.2 PLACEBO

Composition: Lactose 50 mg, and if needed sodium hydroxide as pH adjuvant.

Dosage form: Powder for solution for infusion.

Presentation: Glass vial to be reconstituted with 5 ml saline for injection, according to instructions in leaflet.

Storage condition: Placebo should be stored at room temperature below or equal to 25°C (77°F) in a secure location. Do not freeze

Source: provided by CVie Therapeutics Company Limited.

The certificate of analyses will be provided together with the product.

5.2 Drug Blind Code

The computer generated randomization list stratified by centre and atrial fibrillation (Yes/No) will be prepared via a computerised method. Starting from the lowest number provided, in each center and and atrial fibrillation, at Day 1, patients will be sequentially assigned to the next randomization number, following the order in which they present themselves for the study.
The investigator will keep the code break envelopes in a locked, secure storage facility.
A code break envelope can only be opened in an emergency situation (mainly SUSAR occurrence) where the investigator considers it essential to know which treatment the subject was taking. If possible, it is recommended that the investigator contacts the Sponsor before opening an envelope. The monitor shall be promptly notified when a treatment code envelope is opened. The investigator shall provide a certified explanation of why the treatment code was opened in the Case Report Form for the subject or directly on the opened treatment code envelope.

IMPORTANT: The randomization codes will only be disclosed to the laboratory in charge of the bioanalytical assay (plasma and urine PK assessment) of istaroxime and its metabolites, in order to prevent analyses of samples from patients who have received placebo. This laboratory will re-code identifiers of patients, allowing only to differentiate but not to identify the patients, in order to maintain blinding integrity for the other assessments of the study, until database lock. PK results will be maintained separate from the electronic CRF data until database lock at study completion.

Emergency Un-blinding: 1) Un-blinding condition: any emergencies of unknown origin that occur to the subject during the trial, should find out the medication type, or the subject asks to quit and wants to know the medication type; 2) Un-blinding register: the principle investigator opens and reads the emergency letter of corresponding number, recording the reading date and reason in detail, and signs his/her name; 3) Un-blinding disposal: once the emergency letter is

read, the corresponding case is regarded as drop-out.

IMPORTANT: An emergency code break by the Investigator may be authorized only in case of an unexpected serious adverse event (SUSAR), suspected to be related to an investigational product and requiring, according to the Investigator, the knowledge of the study treatment administered. In such a case, the following information must be documented in the e-CRF: reason for code break, date, name, qualification, patient initials and birth date (as recorded in the eCRF) and electronic signature of the person who requested the code break. At completion of the study, all emergency code breaks tracked by the e-CRF will be checked for verification of the blinding integrity.

5.3 Drug packaging

The test drugs and placebo are packaged in the glass vials of identical size.
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.

Dummy: Packaging dummy technique will be adopted as follows:
The investigational drug and placebo will be provided as kits containing 4 study boxes. Each study box for each patient will be used for 6 hours iv. infusion, for a total of 24 hours infusion for the 4 boxes. Each study box for the 6 hour infusion will contain 4 glass vials of 7 ml.
According to the treatment (test drug-istaroxime or placebo) and to the dose (test drug-istaroxime: 0.5 or 1.0 mcg/kg/min) the 6-hour box will contain:

1. Istaroxime (0.5 mcg/kg/min):
    - 2 glass vials of 7 ml containing lyophilized powder of test drug (10 mg) + 50 mg of lactose
    - 2 glass vials of 7 ml containing lyophilized powder of placebo (50 mg of lactose)

2. Istaroxime (1.0 mcg/kg/min):
    - 4 glass vials of 7 ml containing lyophilized powder of test drug (10 mg) + 50 mg of lactose

3. Placebo
    - 4 glass vials of 7 ml containing lyophilized powder of placebo of istaroxime (50 mg of lactose)

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

Packaging: External packing and inner packing, inner packing is 4 glass vials (see above) while external packing is a white box. Outside the box and vials are pasted by study special label (refer the contents to the label drafts only for Chinese sites).

1). **External packing box label of** test drugs and control drug (OK)

Istaroxime injection for ADHF clinical study

Approve No.：2015L00219

Drug No.：_____ Drug batch.：_____

Study No.：_____ EUDRACT No.: _____

Strength：10 mg /vial，Lyophilized powder Expiry date：_____

Quantity：infusion for 24h, contain 4 small boxes, each with 4 vials

Storage condition：below 25℃(77°F) room temperature，don't freeze

Dose regimen：adjust infusion rate according to body weight, prepare drug every 6h

Storage temperature: XXX°C.

Sponsor: **CVie Therapeutics Company Limited**

To be used only for clinical trials. Keep out of children's reach**. Please return the remaining. drugs in timely**

2). **Inner packing box label of** test drugs and control drug

Istaroxime injection for ADHF clinical study

Approve No.：2015L00219

Drug No.：_____ Drug batch.：_____

Study No.：_____ EUDRACT No.: _____

Strength：10 mg /vial，Lyophilized powder Expiry date：_____

Quantity：infusion for 6h, 4 vials per box

Storage condition：below 25℃(77°F) room temperature，don't freeze

Dose regimen：adjust infusion rate according to body weight, prepare drug every 6h

Sponsor: **CVie Therapeutics Company Limited**

To be used only for clinical trials. Keep out of children's reach**. Please return the remaining. drugs in timely**

3). **Vial label of** test drugs and control drug

Istaroxime injection for ADHF clinical study

Approve No.：2015L00219

Drug No.：_____ Drug batch.：_____

Study No.：_____ EUDRACT No.: _____

Strength：10 mg /vial，Lyophilized powder Expiry date：_____

Storage condition：below 25℃(77°F) room temperature，don't freeze

Dose regimen：adjust infusion rate according to body weight, prepare drug every 6h

Sponsor: **CVie Therapeutics Company Limited**

To be used only for clinical trials. Keep out of children's reach**. Please return the remaining. drugs in timely**

5.4 Emergency Envelope/Emergency Unblinding Card

Emergency envelope is prepared for SAE happening, one opens the envelope to rescue in time, and avoid overall un-blinding. "A randomized, double-blind clinical study of Istaroxime", and the randomization number are printed on the envelopes. The emergency envelope draft is as follow:

Double-blind clinical study emergency

Un-blinding record Drug Randomization No.:

Study project: A randomized, double-blind clinical study of Istaroxime

Sponsor: CVie Therapeutics Company Limited

Clinical Study Responsible Sites: XX

The subject of this Randomization No. is randomly divided into:

□ **Test group:** Istaroxime 0.5 mcg/kg

□ **Test group:** Istaroxime 1.0 mcg/kg

□ **Control group:** Placebo

Reason for emergency unblinding: _____

Study center: _____

Signature of investigator: _____ Date: _____

Signature of principal investigator : _____ Date: _____

Note: the case should withdraw from the study after emergency un-blinding

5.5 Drug Distribution

Registration: All drugs of each site will be stored in one place and managed by a specially-assigned person. The distribution and reclaiming should be registered.

Sequence: the investigator obtain the random no. and drug no. of the subject according to the visiting sequence of the qualified subjects in each study center, and the drug administration office dispense the study drugs of corresponding drug no.

Distribution: The subjects are randomized to the test group (istaroxime) and control group (placebo) in a ratio of 2:1. All study drugs are accompanied by emergency envelopes of corresponding numbers and the emergency envelopes are kept by the principal investigator in attending site.
The drugs should be given to the subjects according to their drug numbers, and shouldn't be chosen. The random numbers should remain unchanged during the study. Each subject will be provided with a random number and sufficient study drugs. The investigator should fill in the Drug Distribution Registration Form exactly in time.

Storage: The study drugs are stored, administrated and distributed by each site. The drugs should be stored hermetically in the shade.

Remaining Drugs: If the drugs remain because patients do not take them on time, or quit midway, or the treatment changes, the drugs should be reclaimed and recorded in detail.

5.6 Dosage Regimen

Istaroxime, in powder form, will be provided in glass vials. NaCl 0.9% bags for preparation of the 4 x 50ml infusion over 24 hours, will be provided by the Center. The infusion pumps, sets, filters and connections will be provided by the research center.

Preparation of the solution for infusion will be performed by a physician or a pharmacist of the Pharmaceutical Services Division, in the presence of a suitably qualified witness according to the randomization code. A record for the preparation of the infusion will be kept for each patient according to the research center's standard operating procedure and will be approved by the Sponsor before first dosing. Stability data available support the infusion of the mixed preparation over 6 hours.

The istaroxime vials will be handled according to the manufacturer's specifications to ensure that the products are appropriately mixed prior to infusion.

In order to calculate the amount of istaroxime given to each patient in each cohort, the body weight will be determined to one decimal place. The body weight recorded on Day 1 at pre-dose will be used for calculation of doses of istaroxime for each cohort.

5.6.1 Method of administration and compliance

Istaroxime and placebo will be provided as kits containing 4 study drug boxes. Each study drug box will contain 4 glass vials (see section 5.3). The infusion solution will be administered via a syringe pump. The exact istaroxime dose to be administered will be calculated according to patient body weight and will be modulated by the infusion rate (see Appendix A: infusion rates)

On the treatment day, a solution for infusion will be prepared every 6 hours (with 4 vials) and will be administered to the patient via the syringe pump.

The procedure for preparation of a 6-hour infusion is the following:

1. Check that the *Study Drug Box #* displayed on the kit is the one that has been allocated to the patient;

2. Select one of the 4 study drug boxes;

3. Remove the flip offs from the containers contained in the box;

4. Reconstitute each of the 4 vials with 5 mL of saline, taken from the NaCl 0.9% bag (supplied by the local center) that will be utilized for the preparation of the infusion solution, according to instructions in leaflet;

5. Draw completely the clear solution from the 4 reconstituted vials into a single 50 mL syringe;

6. Remove 30 ml of saline from the NaCl 0.9% bag with the same syringe used for the reconstitution of the 4 vials in order to obtain a total volume of 50 ml (20 ml of the 4 vials+30 ml of saline from the NaCl 0.9 % bag)

7. Mix the whole content of the syringe

8. Invert the syringe carefully 4 times and connect the syringe to the infusion line;

9. Prime the infusion line to the most proximal point nearest to the catheter;

10. Start the infusion pump at the pre-determined dosing time for the patients; Calculate the catheter volume according to the indications reported in the Section 14 of Operation Manual

11. Modulate the infusion rate according to patient weight (see Appendix A: Infusion rates). **Syringe infusion time is 6 hours**.

    - The infusion syringe must be clearly labeled with the investigational product name, the study number, and the patient number.

    - A pre-printed label is available inside the Study Drug Box. In case of use, the *Patient Screening or Randomization* # has to be added by the person preparing the investigational product.

Under close supervision, the investigational product will be continuously infused intravenously in a vein at high blood flow.

In case of clotting of a line, the infusion tube has to be disconnected from the PAC and immediately connected with the infusion line available on the introducer to continue investigational product infusion.

Treatment compliance will be verified by direct visual supervision at dosing by the Investigator or his/her designee. Drug accountability records will be kept as outlined in section 15.1.

In case of a damaged study drug box, a broken vial, or for any need for replacement of one of the 4 study drug boxes within the kit, the Investigator must discard the entire study drug box, and obtain a replacement box using the study drug box number retrieved from the replacement list.

5.7 Concomitant Medication

All oral treatments, are permitted during the study with the exception of positive inotropic agents**.**
Dosage of oral HF medications must remain stable for at least 6 hours before study entry, during the entire treatment period (24-hour infusion), until 72 hours (on Day 4) after the start

of infusion on Day 1. Concomitant medications should be administered in the pre-randomization phase and after at least 6 hours from the initiation of study drug infusion, if possible.
Intravenous diuretics administered during the screening period to stabilize the patient's condition will be permitted during the treatment period at stable doses, unless changes are required due to clinically relevant variations of the patient's condition.

Any introduction of new drugs or increase of the dosage of oral or i.v. medications for the treatment of heart failure, during treatment and till the 72 hours after the start of infusion (on day 4), will be considered as "rescue medication".

Patient supplementation of magnesium or potassium, prior to randomization, will not be considered as a change of the standardized therapy but just as a supplementation to maintain serum electrolytes within the normal ranges.

Administration of prior medications *(i.e. drug treatments administered during the last month)* and concomitant medications/therapies *(i.e. medications ongoing at admission time and with a start date between admission to the research center and Day 4 or discharge)* must be reported on the appropriate medication pages of the CRF, including dosage information, dates and time of administration and reasons for use. The international non-proprietary name and the trade name in brackets should be used for concomitant medications. The total daily dose or the stat dose, if appropriate, should also be reported. Rescue therapies will be recorded on the specific rescue medication page, recording the same information as for concomitant therapies, plus the time of administration of each single dose.

In case an inotrope should be necessary as rescue medication during the investigational product infusion period, the investigational product infusion should be terminated.

6. CLINICAL ENDPOINTS

6.1 Efficacy Endpoints

1. Primary efficacy end-points:

- Change from baseline to 24 hours after infusion start (treatment period Day 1) in the E/Ea ratio assessed by tissue Doppler.

2. Secondary efficacy end-points:

- Change from baseline to 24 hours in the treatment period Day1 (addressing the differences between the changes at 6 and 24 hours from baseline) of the following Echo-Doppler parameters:
  - LV Ejection Fraction (EF)
  - LV end systolic and end diastolic volumes
  - Stroke volume index (SVI)

- E, A and E/A ratio

- Change from baseline to 24 hours in the E/Ea ratio assessed by tissue Doppler (difference between the changes at 6 and 24 hours from baseline)
- Others Tissue Doppler parameters such as Sa, Da and Aa
- Changes in dyspnea assessed at 3, 6, 12, 24, 48 hours after infusion start by Visual Analog Scale (VAS) (including only patients presenting dyspnea at baseline);
- Area under the curve (AUC) on changes in dyspnea assessed at 3, 6, 12, 24, 48 hours after infusion start by VAS (including only patients presenting dyspnea at baseline);
- Changes in BNP from baseline at 24 hours;
- Proportion of patients with hospital readmissions or emergency visits for cardiovascular reasons by Day 30;
- Proportion of patients with episodes of worsening HF defined by the need to increase the dose or reinitiate i.v. therapy with diuretics and/ or other inotropic agents during the hospitalization;
- Length of hospitalization.

6.2 Safety Endpoints

The following safety endpoints will be assessed during treatment and the post-treatment/follow-up periods:

- Incidence of adverse events;
- Change in vital signs (including body temperature and dyspnoea);
- Change in 12-lead ECG parameters;
- Incidence of clinically or hemodynamically significant episodes of supraventricular or ventricular arrhythmias detected by continuous ECG dynamic monitoring;
- Change in laboratory parameters (hematology, blood chemistry and urinalysis);
- Change in renal function;
- Change in cTnT;
- Incidence of cTnT elevation relative (>50% or >20% relative increase over the cTnT levels at baseline, for patients with cTnT basal levels at baseline < or ≥ of the 99% URL (upper reference levels, as defined for the Roche hs test in patients with normal renal function, eGFR ≥ 85 ml/min); in patients with eGFR below this value, the renal function variations must be considered in evaluating the significance of the cTnT changes);

- Mortality at Day 30.

6.3 PK Parameters

After infusion, the following PK parameters will be measured and/or calculated for plasma E and Z isomers (when applicable) of istaroxime and its metabolites PST2915, PST2922, and PST3093, using the validated software WinNonLin® 5.2 or higher (Pharsight Corporation):

C_{max}:	Maximum plasma concentration achieved after single dose
t_{max}:	Time to achieve C_{max}
AUC_{0-t}:	Area under the concentration curve from administration to the last observed concentration time t, calculated after single dose with the linear trapezoidal method
$AUC_{0-\infty}$:	Area under the concentration/time curve extrapolated to infinity, calculated, if feasible, as $AUC_{0-t} + C_t/\lambda_z$, where C_t is the last measurable drug concentration
λ_z:	Terminal elimination rate constant, calculated after single dose, if feasible, by log-linear regression using at least 3 points
$t_{1/2}$:	Half-life, calculated, if feasible, as $\ln 2/\lambda_z$
MRT:	Mean Residence time, calculated, if feasible, as $AUMC_{0-\infty}$ / $AUC_{0-\infty}$ - $t_i/2$, where $AUMC_{0-\infty}$ is the area under the moment curve extrapolated to infinity and t_i is the infusion duration
Cl_T:	Total Body Clearance, calculated, if feasible, as Dose / $AUC_{0-\infty}$
V_z:	Volume of distribution of the terminal phase calculated, if feasible as Dose / ($AUC_{0-\infty}$ x λ_z)
V_{ss}:	Volume of distribution at steady state calculated, if feasible as (Dose x $AUMC_{0-\infty}$) / $AUC_{0-\infty}^2$

The quality of log-linear regression (and, consequently, the reliability of the extrapolated PK parameters) should be demonstrated by a correlation coefficient $R^2 > 0.8$.

After infusion, the following PK parameters will be measured and/or calculated for urine E and Z isomers (when applicable) of istaroxime and its metabolites PST2915, PST2922, and PST3093, using the validated software WinNonLin® 5.2 or higher (Pharsight Corporation):

A_e:	Total amount of analyte excreted in urine

A_e%: Percentage of analyte excreted in urine

Cl_R: Renal Clearance calculated, if feasible as A_e / $AUC_{0-\infty}$ (for istaroxime only)

These endpoints will be addressed in all the Italian patients and in a subset of Chinese patients.

Data below the lower quantification limit (LQL) will be considered as 0 in the calculations and presented as BLQL in listings and tables. As a consequence of BLQL (i.e. 0) values, calculated geometric means (if requested) could be null. For this reason, in the presence of any null value, the geometric mean will be reported as not calculated (NC).

7. STUDY PROCEDURES

✧ Screening period (between Hours -24 to -1)

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. Prior to conducting any study specific procedures, the investigator or his/her designee will explain the study fully to the patient 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.

1) **Obtaining of ICF;**
2) Evaluation of screening inclusion and exclusion criteria;
3) Stratification: Atrial fibrillation YES/NO;
4) Medical history, demographic/anthropometric data;
5) Alcohol and tobacco consumption patterns;
6) Medication history (previous and concomitant), including a list of the patient's previous and current intake of vitamins, over-the-counter drugs and herbal products for the last 3 months. Chronic oral treatment with digoxin and other medications (e.g. diuretic and/or vasodilator) will also be recorded;
7) Vital signs (including body temperature and dyspnea);
8) 12-lead ECG; (Data obtained within 3 hours before the patient signed informed consent form can be accepted.)
9) Holter ECG;

10) Standard blood chemistry and Standard haematology;
11) TSH, T3 and T4;
12) Estimated glomerular filtration rate (eGFR);
13) Digoxin assay;
14) Urinalysis;
15) Alcohol breath test (at random, if deemed necessary by the Investigator/designee);
16) Serum pregnancy test (beta human chorionic gonadotropin [β-hCG]) for females of childbearing potential;
17) Echocardiography;
18) Serum creatinine clearance;
19) Human Immunodeficiency Virus, Hepatitis B and Hepatitis C tests;
20) Adverse events monitoring (after obtaining informed consent);
21) BNP, NT-proBNP and cTnT;

✧ **Treatment period (Day 1)**

1) Confirm eligibility;
2) Randomization of patients (after eligibility has been confirmed);
3) Insertion of multiple lumen intravenous catheter (or administration via peripheral vein);
4) Start istaroxime or placebo infusion (date and time of infusion start must be recorded in the CRF);
5) cTnT (at pre-dose in two samples, 3, 6, 12, 24, 48 and 72 hours after start of infusion);
6) NT pro-BNP at baseline and at the end of 24 hours infusion
7) Blood sample collection for metabolites and PK (at pre-dose, 0.5, 3, 6, 12, 24 hours after start of infusion and 0.25, 0.5, 1, 4, 12, 24, 48 hours after the end of infusion) in a subset of Italian patients and in a subset of Chinese patients pharmacokinetics and metabolism of istaroxime shall also be studied;
8) Vital signs (including arterial oxygen saturation and dyspnoea at pre-dose, 3, 6, 12 and 24 hours after the start of infusion);
9) 12-lead ECG profile (pre-dose, 3, 6, 12 hours and between 23 hours and 23 hours 55 minutes after infusion, totally 5 times, each time 3 measurements);
10) Stop 24-hour Holter ECG (Day -1 recording; Holter ECG monitor stops automatically 24 hours after the recording has been started;
11) Start 24-hour Holter ECG (Day 1 recording; to be started immediately before initiation of the study drug infusion);
12) Echocardiography at baseline and 6 and 24 hours after infusion start;
13) 24-hours urine collections for measurement of istaroxime and its metabolites and urinary

creatinine for the calculation of the creatinine clearance

14) Blood collection for K+ and eGFR between 23 hours and 30 minutes and 23 hours and 55 minutes since infusion start
15) Concomitant medication monitoring (including chronic medication; dose, date and time must be recorded in the CRF);
16) Adverse events monitoring.

✧ **Post-treatment period (Day 2 to Day 4)**

Evaluations on Day 2 (end of the 24 hour infusion) include:

1) Vital signs (including arterial oxygen saturation and dyspnoea);
2) 12-lead ECG (triple ECGs) (between 23 hours and 23 hours 55 minutes since infusion start);
3) Stop 24-hour Holter ECG (Day 1 recording; to be stopped immediately after the end of study drug infusion;
4) Start 24-hour Holter ECG (Day 2 recording);
5) Stop istaroxime or placebo infusion (date and time of infusion end must be recorded in the CRF);
6) Serum potassium levels and 24-hour urine collection for measurement of istaroxime metabolites and urinary creatinine for calculation of the creatinine clearance;
7) Serum creatine clearance and calculation of eGFR;
8) cTnT (50% or 20% relative increase over the basal cTnT levels, respectively for patients with cTnT basal levels < or ≥ of the 99% URL (upper reference levels, as defined for the Roche hs test, in patients with normal renal function, eGFR≥85 ml/min); in patients with eGFR below this value, the renal function variations must be considered in evaluating the significance of the cTnT changes);
9) NT pro-BNP
10) Metabolites
11) Echocardiography;
12) Concomitant medication monitoring (including chronic medication must be recorded in the CRF);
13) Adverse Events monitoring

Evaluations on Day 3 include:

1) Vital signs (including arterial oxygen saturation and dyspnea)
2) 12-Lead ECG (single ECGs)
3) Stop 24-hour Holter ECG
4) Standard hematology;
5) Standard blood chemistry;
6) Serum Potassium levels;

7) 24-h urine collection for measurement of istaroxime metabolites and urinary creatinine for the calculation of creatinine clearance;
8) Calculation of eGFR;
9) NT-proBNP
10) cTnT;
11) Metabolites;
12) Echocardiography;
13) Adverse events monitoring;
14) Concomitant medication monitoring (including chronic medication must be recorded in the CRF)

Evaluations on Day 4 include:

1) cTnT and NT-proBNP (at 72 hours after start of infusion)
2) Vital signs (including arterial oxygen saturation and dyspnoea)
3) Physical examination (HF signs included)
4) 12-lead ECG
5) Adverse events monitoring
6) Concomitant medication monitoring (including chronic medication)
7) Metabolites (at 72 hours after start of infusion)
8) Serum potassium and creatinine levels for calculation of eGFR
9) Creatinine clearance

✧ **Follow-up period and visit (Day 5 to Day 30)**

During the follow-up period the Investigator/designee will make every effort to establish patient outcomes.

Evaluations on Day 30 (follow-up visit) include:

1) Vital signs (including arterial oxygen saturation and dyspnoea);
2) 12-lead ECG in triplicate;
3) Calculation of eGFR;
4) Standard hematology;
5) Standard blood chemistry;
6) NT-proBNP ;
7) cTnT;
8) Urine pregnancy test (β-HCG) for females of childbearing potential;
9) Urinalysis;

10) Physical examination (HF signs included);

11) Adverse events monitoring;

12) Concomitant medication monitoring (including chronic medication).

8. EFFICACY ASSESSMENTS

Change from baseline to 24 hours after infusion start in the E/Ea ratio assessed by tissue Doppler will be assessed as primary end-point. Other echocardiographic parameters (LVEF, LV end diastolic and end systolic volumes, SVI, E/A ratio, other Tissue Doppler parameters such as Sa, Da and Aa) will be assessed as secondary end-points. These parameters will be assessed also at 48 hours as further exploratory analyses.

Dyspnoea will be assessed at baseline using a 100 mm VAS scale. Further assessments will be repeated at 3, 6, 12, 24 and 48 hours after the start of drug infusion only in patients presenting dyspnoea at baseline.
NT-proBNP will be measured by the local laboratory only for evaluation and screening inclusion. While at baseline, at 24 hours after infusion start (day 2), on Day 3, at day 4 or at discharge) and at day 30, NT-proBNP will be measured by the central laboratory
Episodes of worsening HF, defined by the need to increase the dose or reinitiate i.v. therapy with diuretics and/ or other inotropic agents during the hospitalization, will be recorded.

9. SAFETY ASSESSMENTS

1. AEs will be monitored throughout the study (i.e. from the time of signature of informed consent to treatment end + 30 days);
2. Physical examination (HF signs included) at screening, baseline, on study Day 4 or at discharge, and on study Day 30 during the follow up visit;
3. Vital signs (HR, BP, respiratory rate, body temperature and dyspnoea)) will be measured at screening, (concomitantly to hemodynamic measurements) during the pre-treatment period, at 3, 6, 12, and 24 hours after infusion start, and then on study Day 3 and Day 4 or at discharge, and on study Day 30 during the follow up visit;
4. 12-lead ECG (HR, RR and PR intervals, QRS duration, QTc, rhythm, ectopy, ischemic changes) will be recorded and locally analyzed at screening and on study Day1, Day2, Day 3 and Day 4 or at discharge, and on study Day 30 during the follow up visit;
5. Continuous Holter monitoring will take place during screening, treatment (Day 1), and Day 2.
6. Blood samples will be collected and sent to the local laboratory during screening, 48 hours after infusion starts and on Day 30 to measure the following safety parameters:

a) Hematology: red blood cell (RBC) count, Hb, hematocrit (Hct), white blood cell (WBC) count, differential WBC count, platelet count;

b) Blood chemistry: electrolytes (Na^+, K^+, Ca^{++}, Cl^-), albumin, total protein, glucose, creatinine, eGFR, BUN (blood urea nitrogen), alkaline phosphatase, ALAT, ASAT, total and conjugated bilirubin, gamma glutamyl transferase (γ-GT), total cholesterol, triglycerides;

7. Blood samples for creatinine will be collected by the local laboratory at each time after infusion start
8. cTnT or cTnI will be measured locally during screening. While cTnT only will be measured by the central laboratory , at pre-dose in two samples, at 3, 6, 12, 24, 48, 72 hours after infusion start or at discharge and at 30days by using the hs test from Roche cobas. Incidence of cTnT elevation (>50% or > 20% relative increase over the basal cTnT levels at baseline, for patients with cTnT levels at baseline < or ≥ of the 99% URL (upper reference levels, as defined for the Roche hs test, in patients with normal renal function, eGFR ≥85 ml/min); in patients with eGFR below this value, the renal function variations must be considered in evaluating the significance of the cTnT changes).

10. REPORTING OF ADVERSE EVENTS

10.1 Definition of Adverse Event (AE)

Adverse event (AE) is an adverse medical event that occurs following informed consent signature, the event is not necessarily related to the treatment. Therefore, adverse events may be any discomfort and unconscious signs (such as abnormal laboratory examination results), symptoms or illnesses.

- Any adverse event, whether serious or not, whether related to the treatment or not, should be recorded on the CRF.
- Investigator should follow up the patients that dropped out because of any adverse event until recovery, and record the date of AE occurring and recovering.
- If during adverse event follow-up, the adverse event progresses to an "SAE", or if a subject experiences a new SAE whose relationship to the study drug(s) cannot be ruled out, the investigator must immediately report the information to the sponsor.

Even if the subject does not return to normal or to his or her previous state, the follow-up can be considered finished when the following procedures have been taken:

- The investigator judges that the follow-up of the subject concerned is no longer necessary based on the progress made during the follow-up,
- The reason for such a judgment is entered as a comment on the case report form for the follow-up.

10.2 Definition of Serious Adverse Event (SAE)

'Serious adverse event' is an event that induces hospitalization, prolonging the length of hospitalization, disability, affecting work ability, imperiling life or death, and congenital malformation.

(1) SAE is one of the following situations:

- That causes death
- Teratogenesis, carcinogenesis or birth defect
- That is dangerous to life and could induce permanent or remarkable disability
- That induces permanent injury in organ function
- That induces hospitalization or prolongs the length of hospitalization

(2) Hospitalization

According to the clinical study reports, the adverse events leading to hospitalization or prolongs the length of hospitalization will be regarded as serious adverse event (SAE).

Including: hospitalized in a healthcare facility for the first time (even less than 24 hours) and the hospital transferred to the emergency/intensive care ward.

The hospitalization does not include the following institutions:

- rehabilitation institutions;
- asylum;
- occasional care service ;
- nursing institution;
- sanatorium;
- routine hospitalization;

(3) Hospitalization or prolonging the length of hospitalization without emergency clinical adverse event does not belong to SAE. For example:

- hospitalization because of previous disease, which is unrelated to adverse event and with no worsening (e.g. examinations for the continuous abnormalities in laboratory tests prior to treatment);
- for social reasons (such as homelessness);
- for management reasons (such as routine health examinations per year or for convenience of drug administration);
- hospitalization assigned or permitted by the protocol during the trial;
- voluntary hospitalization unrelated to AE (such as selective plastic surgery);
- Scheduled treatment or surgery for the whole protocol and/or subject's benefit, which is recorded at baseline;
- Just for infusion of blood products.

10.3 Relativity assessment of AE and Study Drug

Relativity between AE and test drugs is assessed by investigator according to the following:

1) Certainly related
Evidence of having taken test drugs; time sequence of AE happening and taking the drugs is credible; more rational to explain for AE with test drugs than other reasons; positive withdrawal reaction; positive repetition medication test; AE pattern accords with the past understanding of such drug or this kind of drugs.

2) Possibly related

Evidence of having taken test drugs; time sequence of AE happening and taking the drugs is credible; AE may be induced by the test drugs, or other reasons; positive withdrawal reaction.

3) Possibly unrelated
Evidence of having taken test drugs; AE is more likely to be induced by other reasons; negative or equivocal withdrawal reaction; negative or equivocal repetition medication test.

4) Certainly unrelated
The patient has not taken test drugs; or time sequence of AE happening and taking the drugs is incredible; or AE is induced by other notable reasons.

5) Uncertain

10.4 Severity assessment of Adverse Event

The strength or the serious degree of the adverse events can be divided into3 levels:

Mild: mild discomfort, no significant impact on daily life (work and study);

Moderate: moderate discomfort and impact on daily life, patients self-conscious symptom obvious, but can stand, no need to stop medicine

Severe: severe discomfort, significant impact on daily life, need for staying in bed, patients self-conscious symptom obvious is remarkable, and cannot stand, so need to stop medicine

10.5 Reporting of SAE

Any 'serious adverse event' happens during the trial (from the time of signature of informed consent to visit Day 30):

Whether it is related to the drugs, the investigator should fill in 'Serious Adverse Event Reporting Form and within 24 hours after learning it, informs, by fax or e-mail, CFDA, local drug supervising departments, NHFPC, the sponsor- CVie Therapeutics Company Limited, the site's principal investigator and the ethics committee.

<table>
<tr><th>Department</th><th>Name</th><th colspan="2">Contact Information</th></tr>
<tr><td>CFDA</td><td>China Food and Drug Administration</td><td>Tel:
010-88330724</td><td>Fax:
010-88363228</td></tr>
<tr><td>NHFPC</td><td>Health Care Authority - National Health and Family Planning Commission</td><td>Tel:
010-68792201</td><td>Fax:
010-68792734</td></tr>
<tr><td>Local Food and Drug Administration</td><td></td><td></td><td></td></tr>
<tr><td rowspan="3">Sponsor: CVie Therapeutics Company Limited</td><td>Eric Tseng</td><td colspan="2">Email: erictseng@cvie.com</td></tr>
<tr><td rowspan="2">Giuseppe Bianchi</td><td colspan="2">Tel: +39-02745569; +39-3488545560</td></tr>
<tr><td colspan="2">Email:
giuseppebianchi1935@cvie.com</td></tr>
</table>

10.6 Reporting of Pregnancy

If a woman becomes pregnant during the study period until Day 30, the investigator should report the information to the sponsor as if it is an SAE. The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated fertility date, pregnancy result and neonatal data etc., should be included in this information.

The investigator will follow the medical status of the mother, as well as the fetus, as if the pregnancy is an SAE and will report the outcome to the sponsor.

When the outcome of the pregnancy falls under the criteria for SAEs [spontaneous abortion, induced abortion, stillbirth, death of newborn, congenital anomaly (including anomaly in a mis-carried fetus)], the investigator should respond in accordance with the report procedure for SAEs. Additional information regarding the outcome of a pregnancy (which is categorized as an SAE) is mentioned below.

- "Spontaneous abortion" includes abortion and missed abortion.
- Death of an infant within 1 month after birth should be reported as an SAE regardless of its relationship with the study drug.
- If an infant dies more than 1 month after the birth, it should be reported if a relationship between the death and intrauterine exposure to the study drug is judged as "possible" by the investigator.
- In the case of a delivery of a living newborn, the "normality" of the infant is evaluated at the birth.

"Normality" of the miscarried fetus is evaluated by visual examination unless test results which indicate a congenital anomaly are obtained prior to miscarriage.

11. ADVERSE DRUG REACTION (ADR)

11.1 Definition of ADR

Harm reaction which unrelated to the purpose of treatment with the use of given medications at a normal dosage.

11.2 Reporting and monitoring of ADR

Reporting and monitor ADR is the process of finding, reporting, evaluating and controlling the adverse drug reaction.

12. PHARMACOKINETIC ASSESSMENT

Plasma concentrations of istaroxime and its metabolites PST2915, PST2922, and PST3093 will

be measured by liquid chromatography coupled to mass spectrometry (LC/MS/MS). When applicable, E and Z isomers will be measured separately.

In a subset of patients, a blood sample will be collected during the study period. In addition, a full PK profile will be established in a subset of Italian patients and in a subset of Chinese patients (to have 16 patients with istaroxime 0.5 µg/kg/min and 8 patients with istaroxime 1.0 µg/kg/min 24 total patients).
Blood will be sampled at the following time-points: 0 hour (pre-dose); 0.5, 3, 6, 12, and 24 hours after infusion start; 0.25, 0.5, 1, 4, 12, 24 hours after infusion end.

In the same subset of patients, concentrations of istaroxime and its metabolites will be measured by LC/MS/MS in urine collected from pre-dose to 48 hours after infusion end (3 collections will be scheduled: the first from pre-dose to 24 hours after investigational product infusion start, the second at 48 hours after infusion start, and the third at 72 hours after infusion start).

13. DATA MANAGEMENT

13.1 Filling the CRFs and its handover

Data Management will identify and implement the most effective data acquisition and management strategy for the clinical trial protocol and deliver datasets which support the protocol objectives. Subject data will be entered into an e-CRFs in a validated data system.

13.2 Data entry and amendment

The data manager will prepare a Data Management Plan (DMP) to describe the procedures and processes of data collection and data coding (including data entry, data validation, data clarification process and database closure). For data coding (e.g., AEs, medical and surgical history, concomitant diseases, prior and concomitant medication), internationally recognized and accepted dictionaries will be used. The coding of the medical history, concomitant diseases and adverse events will be performed using MedDRA and the coding of concomitant medication by using WHO drug dictionary (WHODD). The clinical data will be reviewed for legibility, completeness, and logical consistency. Automated validation programs identify missing data, out-of-range data, and other data inconsistencies. Requests for data clarification will be forwarded to the investigative site for resolution. All plausibility checks will be defined in the Data Validation section of the DMP to be approved by the sponsor. Clinical data management will be performed in accordance with applicable CRO standards and data cleaning procedures with the objective of removing errors and inconsistencies in the data which would otherwise

impact on the analysis and reporting objectives.

13.3 Locking the Database

At the completion of the data management activities and when the clinical data are clean, the clinical database will be locked by the Data Manager and the clinical data released to the statistician for the analysis. With database lock no further changes are allowed to the clinical data.

14 STATISTICAL ANALYSIS

14.1 Datasets

Four populations will be defined:

1) Intention-To-Treat population (ITT): all randomized patients who will take at least one dose of the study medication and with at least one available evaluation of efficacy after the baseline.

2) Per-protocol population (PP): all randomized patients from the ITT population without any major protocol deviation; exact definition of major protocol deviations will be discussed by the clinical team case by case during the blind review of the data.

3) Safety population: all randomized patients who will take at least one dose of the study medication.

4) Pharmacokinetic population (PK): all randomized patients selected for the PK analysis (32 total patients).

The primary efficacy endpoint will be analysed both in the ITT and in the PP populations; the secondary efficacy variables will be analysed in the ITT population. The PK analysis will be performed in the Pharmacokinetic population.
Analysis of safety variables will be performed in the safety population. In case of deviation between as-randomised treatment and treatment actually received, the treatment actually received will be used in the analysis of safety and PK variables (i.e. an as-treated analysis will be performed).

14.2 Statistic Plan

All statistical analyses will be performed using Statistical Analysis Systems (SAS®) Software (release 9.2) on a Windows 7 pro operating system.

Sample size

No formal sample size calculation has been calculated since there is no preliminary study to evaluate these endpoints at 24 hours after the infusion start.
In a previous study [14], there was a statistically significant decrease of the primary efficacy endpoint with istaroxime 0.5 µg/kg/min respect to placebo at 6 hours (twenty-nine patients in both groups). Since this study will investigate the primary efficacy endpoint at 24 hours and it is supposed that the profile at 24 hours should be similar to the one at 6 hours, it is expected that forty patients per group will be sufficient to evaluate a potential difference between istaroxime groups and placebo group on the primary efficacy endpoint at 24 hours.

Descriptive statistics

Descriptive statistics will be provided for each variable in summary tables by treatment group. Quantitative variables will be summarized by using n (sample size), mean, 95% confidence interval (CI) of the mean, median, standard deviation, minimum and maximum. Categorical variables will be summarized by using frequency count and percent distribution.

Principles of statistical analysis

For quantitative efficacy and safety variables, analysis within treatment groups will be presented. Mean changes from baseline and their 95% CIs will be calculated.
For all inferential analyses, p-value will be rounded to three decimal places. Statistical significance will be declared if the rounded p-value will be less than or equal to 0.05.

Subject's accountability

Disposition of patients, patient status and patients excluded from populations will be summarized by treatment group.

Description of the population-description of baseline characteristics

Descriptive statistics will be presented at baseline for demographic and baseline characteristics for the ITT population.

Primary efficacy endpoint

The primary efficacy endpoint (change from baseline in E/Ea ratio) will be analyzed using a linear mixed model for repeated measures including treatment, centre, timepoint, gender, baseline cTnT (normal <URL, abnormal ≥URL), atrial fibrillation (Yes/No) and treatment*timepoint interaction as fixed effects and baseline and baseline*timepoint interaction as covariates.
The primary comparison will be 0.5 µg/kg/min dose of istaroxime versus placebo at 24 hours. Highest dose of istaroxime (1.0 µg/kg/min) versus placebo will be tested as a secondary comparison.
Additional analyses separated by cohort will be implemented for sensitivity purpose.

Secondary efficacy endpoints

The following secondary endpoints:

- Change from baseline to 24 hours in the treatment period Day 1 (addressing the differences between the changes at 6 and 24 hours from baseline) of the following Echo-Doppler parameters:
  - LV Ejection fraction (EF)
  - LV end systolic and end diastolic volumes
  - Stroke volume index (SVI)
  - E, A and E/A ratio
- Change from baseline to 24 hours in the E/Ea ratio assessed by tissue Doppler (difference between the changes at 6 and 24 hours from baseline)
- Others Tissue Doppler parameters such as Sa, Da and Aa
- Changes in dyspnoea using VAS score will be analysed using a mixed model for repeated measures similar to the one used for the primary efficacy endpoint.

A global test considering multiple echocardiographic parameters at 6 and 24 hours will also be performed.

AUC on changes in dyspnea by VAS and changes in BNP will be analyzed using an ANCOVA model with treatment, centre, gender, baseline cTnT (normal <URL, abnormal ≥URL) and atrial fibrillation (Yes/No) as fixed effects and baseline dyspnea as covariate.

Number and proportion of patients with:

- hospital readmissions or emergency visits for cardiovascular reasons within Day 30
- episodes of worsening HF defined by the need to increase the dose or reinitiate i.v. therapy with diuretics and/ or other inotropic agents during the hospitalization

will be summarized by treatment groups using descriptive statistics.
Length of hospitalization will be summarized by treatment group using descriptive statistics.

Safety endpoints

The number and the percentage of patients experiencing adverse events, adverse drug reactions, serious adverse events and adverse events leading to study withdrawal will be summarized by treatment group. Adverse events will also be summarized by treatment group by means of System Organ Class and Preferred Term using the MedDRA dictionary.
Vital signs (including body temperature and dyspnoea), 12-lead ECG parameters, incidence of clinically or hemodynamically significant episodes of supraventricular or ventricular arrhythmias, laboratory parameters, renal function, cTNT, increase of cTNT and mortality will be summarized by treatment group using descriptive statistics.

Interim Analysis

A safety interim analysis on cTNT changes is planned after the completion of Cohort 1.

PK analysis of istaroxime and its metabolites

PK parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ (if feasible) and A_e for istaroxime and its metabolites will be analysed using analysis of variance (ANOVA). The data will be dose-normalised and transformed prior to analysis using a neperian logarithmic transformation.

The statistical analysis will take into account gender, dose, centre, baseline cTnT (normal <URL, abnormal ≥URL) as fixed factors. The statistical method for testing linearity is based upon the 90% confidence interval for the ratio of the population geometric means (Dose1/Dose2), for the parameters under consideration. This method is equivalent to the corresponding two one-sided test procedure with the null hypothesis of no linearity at the 5% significance level.

Acceptance criterion for linearity will be a 90% confidence interval of the ratio of geometric means within the range 75.00 – 133.33%.

t_{max} will be analyzed using a non-parametric model providing an estimate and 90% CI to the median difference of dose pairs.

15. QUALITY CONTROL AND QUALITY ASSURANCE

During the trial， monitor will be appointed by Sponsor to visit the study centers regularly, in order to ensure rigorous performance of all contents in the protocol and accuracy of study materials.

Investigators must attend unified training to unify the record manner and the judgment standard.

- the clinical trial process should be strictly conducted in a blind state.
- Investigators should fill in the CRF accurately, detailed, and seriously according to the requirements, to insure that contents of the CRF are true and credible;
- Judgment standard of laboratory examination abnormality should be subject to the normal range of the centers;
- All observation results and findings of the trial should be verified to ensure the reliability of the data and that each conclusion comes from source data. Corresponding data management should be taken during the trial and data processing period;
- For possible drop-out, active measures should be taken to keep the dropout rate within 20%.

15.1 Drug Accountability

The Investigator must ensure the maintenance of accurate records of the amounts and dates investigational products were received from the Sponsor, dispensed and unused supplies returned or destroyed. All clinical supplies must be accounted for at the termination of the study and a written explanation provided for discrepancies. All unused supplies must be returned promptly to the Sponsor or destroyed properly at the study site; written authorization from the Sponsor must be obtained prior to destruction. The Investigator will maintain a record of the amount and dates when unused supplies were returned to the Sponsor or destroyed locally. When the above tasks are delegated to a Pharmacist, the latter is under the supervision of the Investigator, who remains ultimately responsible for the maintenance of accurate drug dispensing records.

16. MONITORING AND STUDY COMMITTEES

16.1 Monitoring

CVie Therapeutics Company Limited, Hong Kong, is the study Sponsor. CVie Therapeutics will delegate CROs (CROS NT, Verona and CRONOS Srl, Ravenna) for the operational aspects of the study (set up, e-CRF and data management) and for monitoring of the Italian centers. Local operational representatives will be determined in each participating country before the launch of the study.

At mutually convenient times during the study, the Investigator will allow CVie Therapeutics or its representative to review all completed e-CRFs and related source documentation, i.e. patients' office, hospital, clinic and laboratory records. At timely intervals, and at the closing of the study, all investigational products will be accounted for, and dispensing records will be made available to CVie Therapeutics or its representative.

In case CRFs require support information, CVie Therapeutics may request copies of patient records or other source documents from the Investigator. All necessary steps will then be taken to protect patient identity. Adherence to local and national laws governing protection of patient identity will be ensured.

16.2 Steering Committee

The main tasks of the Steering Committee will be to provide input to the Sponsor on protocol content, follow study progress, and answer technical or medical questions on the study. This Committee will meet periodically (even by teleconference) during the planning, implementation and conduct of the Study and will be under the direction of the two Committee Responsible, one for each Country (China and Italy):

Prof. Jun Huang - Chairmen and Responsible for China

The First Hospital Affiliated to Nanjing Medical University

Prof. Marco Metra - Co-Chairmen and Responsible for Italy
Head of the U.O. of Cardiology, Applied and Experimental Medicine
Brescia University – Spedali Civili Brescia- Italy

The Steering Committee Members will be:

For China
Prof. Jian Zhang
Chairman of the Association of Heart Failure in China, Fuwai Hospital CAMS&PUMC - China

Prof. LIU Lisheng
Chinese Academy of Medical Sciences - Fu Wai Hospital and Cardiovascular Institute – China

For Italy:
Prof. Gianfranco Parati
Internal Medicine Bicocca University, Milan – Head of the U.O. of Cardiology S. Luca Hospital – Centro Auxlogico Italiano – Milan, Italy

16.3 Data Safety Monitoring Board

An independent DSMB will be established prior to study initiation. The committee will consist of at least two physicians (cardiologists) and a statistician who are not otherwise associated with the study, and who are experienced in HF multicenter trials.
The main tasks of the Board will be to provide recommendations to the Sponsor concerning safety aspects and study continuation/discontinuation within a cohort and from one cohort to the next one. If a cohort is stopped, the randomisation will be ended and the study treatment will be prematurely discontinued in patients on treatment. Escalation to the next dose level will not take place.
After the completion of cohort I, a safety interim analysis will be performed and the DSMB members will evaluate the incidence of troponin (cTnT) elevation. At interim analysis, the DSMB may recommend to stop the cohort if the incidence of troponin elevation is found to be statistically significantly higher on the istaroxime dose than on placebo, or if this difference is deemed to be clinically relevant. Consequently, the clinical study will be prematurely terminated.
Randomization data and datasets needed to conduct the interim analysis will be given to the DSMB statistician. The DSMB will be unblinded when performing the interim analysis.
During the interim analysis, DSMB members will analyze and discuss the results in closed

sessions. The DSMB is required to keep confidential and undisclosed until study conclusion: i) these discussions, ii) the detailed minutes thereof, and iii) the interim analysis results. At study conclusion, after database lock and unblinding, the DSMB will make the minutes and interim analysis results available as part of the study documentation.

Members of the DSMB will be:

Prof. Aldo Maggioni – Chairman of the DSMB
Head of Centro Ricerche Associazione Nazionale Medici Cardiologi (ANMCO)
Firenze – Italy

Prof. Luigi Tavazzi – Member of the DSMB as Physician
Scientific Director GVM Care and Research Unit Maria Cecilia Hospital
Ravenna- Italy

Prof. Renato Urso – Member of the DSMB as Statistician
Unità of Pharmacology "Giorgio Segre" – Siena University
Siena - Italy

17. ETHICS

Prior to the starting of the trial, the study protocol, informed consent form and other materials for the patients should be submitted to the ethics committee of the responsible study center for approval. Sponsor must obtain the signed and approval document from the ethics committee before starting the study. Any amendments of the protocol and ICF should be approved by the EC. ICF should be signed again if necessary.

Investigator should obtain patient's informed consent and the signed ICF before implementation. Ensure patients' rights and keep confidential to patients' information throughout the study.

18. RESPONSIBILITY AND PUBLICATION

Investigator should keep confidential all information provided by sponsor. Other staff participating in the study and ethics committee should keep confidential the information as well. Without the written permission, the information cannot be provided to others.

All data and results of the clinical study are possessed by sponsor and investigator. Investigator agrees not to submit the results of this study for publication or presentation before the sponsor review the accuracy and no leakage of confidential information in the manuscript.

19. ANTICIPATED PROGRESS AND COMPLETION DATE OF THE STUDY

Procedure	Time	Working Time
Final version of study protocol, ICF and CRF		
Approval document of ethics committee		
Initial meeting and articles delivery of each center		
The first subject into screening		
The last subject enrolled		
The last subject finishing efficacy observation		
The collection of CRF		
Statistics analysis report		
Clinical report		
Summing-up meeting		
Anticipated completion date		

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21. DECLARATION SIGNATURE PAGE

The clinical study of the safety and efficacy of Istaroxime in Treatment of Acute Decompensated Heart Failure - A multicenter, randomized, double-blind, placebo controlled, parallel group clinical study.

Declaration of investigator:

I have read the protocol carefully. I will conduct this clinical trial according to the Declaration of Helsinki, as well as to the moral, ethical and scientific principles required by GCP. I agree to conduct this clinical study in accordance with GCP, the protocol design and requirements, and fulfill the investigator's responsibility. I have known the procedures and time requirements of reporting SAE, and I will record and report the SAE and give medical treatment to the subject in time. I ensure that all data will be recorded in CRF accurately, completely and timely. I will support and coordinate with the monitor. And I will accept the monitoring, auditing or inspection to ensure the quality of the clinical trial.

Responsible study center:

Principal Investigator (signature): _____ Date: _____

Study center:

Principal Investigator (signature): _____ Date: _____

Statistics institute:

Project Leader (signature): _____ Date: _____

The clinical study of the safety and efficacy of Istaroxime in Treatment of Acute Decompensated Heart Failure - A multicenter, randomized, double-blind, placebo controlled, parallel group clinical study.

Declaration of Sponsor:

As the sponsor of the clinical trial, we will carry out the responsibility of sponsor defined in GCP. We are responsible for initiation, application, organization, financial support and management of the clinical trial. We will assign qualified monitors and auditors for quality control of the study. According to relevant laws and regulations, we will give active treatment and afford corresponding cost to the patients with AE related to the study drugs, and give advisable financial compensation to those with SAE related to the study drugs. And we will provide the investigators the legal and financial guarantee.

Sponsor: CVie Therapeutics Company Limited

Project Leader (signature): _____ Date: _____

Head of the company (signature): _____ Date: _____

22. APPENDICES

22.1 Appendix A: Istaroxime Infusion Rate

Reconstitute 4 vials with 5 mL of saline each every 6 hours, draw into one single 50 mL syringe, remove 30 ml of saline from the NaCl 0.9% bag with the same syringe used for the reconstitution of the 4 vials in order to obtain a total volume of 50 ml (20 ml of the 4 vials+30 ml of saline the NaCl 0.9% bag) and infuse at the following rate (according to the precision of the electrical syringe of about 0.1 mL per hour).

PATIENT WEIGHT (kg)	INFUSION RATE (ml/h)	PATIENT WEIGHT (kg)	INFUSION RATE (ml/h)	PATIENT WEIGHT (kg)	INFUSION RATE (ml/h)
35	2.6	57	4.3	79	5.9
36	2.7	58	4.3	80	6.0
37	2.8	59	4.4	81	6.0
38	2.8	60	4.5	82	6.1
39	2.9	61	4.6	83	6.2
40	3.0	62	4.7	84	6.3
41	3.0	63	4.7	85	6.3
42	3.2	64	4.8	86	6.4
43	3.2	65	4.9	87	6.5
44	3.3	66	5.0	88	6.6
45	3.3	67	5.1	89	6.7
46	3.5	68	5.1	90	6.7
47	3.5	69	5.2	91	6.8
48	3.6	70	5.2	92	6.9
49	3.6	71	5.3	93	7.0
50	3.7	72	5.4	94	7.0
51	3.8	73	5.5	95	7.1
52	3.9	74	5.5	96	7.2
53	4.0	75	5.6	97	7.2
54	4.0	76	5.7	98	7.3
55	4.1	77	5.8	99	7.4
56	4.2	78	5.8	>100	7.5