CONFIDENTIAL

STATISTICAL ANALYSIS PLAN

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

Protocol CVT-CV-002

Author: Lisa Comarella
CROS NT S.r.l.

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Sponsor CVie Therapeutics Company Limited
Unit110-111, Bio-informatics center,
No2. Science Park West Avenue,
Hong Kong Science Park, Shatin, N.T. HK

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Author

Lisa Comarella

Lisa Comarella, Director, Biostatistics (CROS NT S.r.l.)

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Date

Approver(s)

G. Bianchi, Appointed Medical Expert (CVie Therapeutics Company Limited)

Date

Gianni Bianchi

30 October 2018

DISTRIBUTION LIST

Lisa Comarella, Director, Biostatistics (CROS NT S.r.l.)
Marta Zanus, Director, Project Management (CROS NT S.r.l.)
Giuseppe Bianchi, Appointed Medical Expert (CVie Therapeutics Company Limited)

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

A	Pick mitral flow velocity during atrial contraction
ADR	Adverse Drug Reaction
AE	Adverse Event
Ae	Amount excreted unchanged into urine
Ae%	Percent of the dose excreted unchanged into urine
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ATC	Anatomical Therapeutic Chemical
AUC	Area under the curve
AUC0-∞	Area under the concentration-time curve extrapolated to infinity
AUC0-t	Area under the curve over the time interval 0 to the time of last quantifiable concentration
BNP	Brain Natriuretic Peptide
CI	Confidence interval
Cl_R	Renal clearance
Cmax	Maximum concentration
CRF	Case Report Form
CS	Clinically Significant
cTnT	Cardiac Troponin T
DMC	Data Monitoring Committee
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
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
EMEA	European Medicines Agency
ICH	International Conference on Harmonisation
ITT	Intention-to-treat
LV	Left Ventricular
LVEF	Left Ventricular Ejection Fraction
λz	Terminal rate constant
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed Models for Repeated Measures
NCS	Not Clinically Significant
NR	No result
NT-proBNP	N-terminal prohormone brain natriuretic peptide
PK	Pharmacokinetics
PP	Per protocol
PR	Interval between P and R waves
PT	Preferred Term
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
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Systems
SD	Standard deviation
SOC	System Organ Class
SVI	Stroke Volume Index
t1/2	Terminal half-life
Tmax	Time to maximum concentration
TEAE	Treatment-emergent adverse event
URL	Upper Reference Limit
Vss	Volume of distribution at steady-state
VAS	Visual Analog Scale
Vz	Volume of distribution during the terminal phase
WHO-DD	World Health Organization Drug Dictionary

1.0 INTRODUCTION

This document outlines the statistical methods to be implemented in the analysis of the data of CVT-CV-002 Clinical Trial. The purpose of this plan is to provide general guidelines from which the analysis will proceed, containing a more technical and detailed elaboration of the principal features of the analysis described in the protocol. Any changes to the protocol or Case Report Form (CRF) may necessitate updates to the Statistical Analysis Plan (SAP). In case of deviations from this updated SAP, explanations will be provided in the clinical study report.

Relevant applicable CROS NT quality document will be followed also according to the contract with the Client.

1.1 CHANGES FROM PROTOCOL

The analysis of echocardiographic parameters is changed from the protocol:

- The analysis of echocardiographic parameters will be performed separately for each cohort, instead of performed on the combined cohorts.
- An analysis of variance (ANOVA) model with treatment as covariate is now the primary analysis, instead of a mixed model for repeated measures (MMRM) including treatment and additional covariates.
- Missing response data will not be imputed in the ANOVA analysis.

The justification for these changes are documented in the Memo to File prepared by Tri Tat, dated 24Oct2018, entitled: Change in analysis of echocardiographic parameters.

2.0 VERSION HISTORY

Version Number	Summary/Reason for changes	Date Issued to Client
Draft 1	Draft specification based on: • Study protocol (general protocol amendment no.1, 08 April 2013) • CRF (Version Final 2, 23 July 2013)	02 September 2013
Draft 2	The following change has been implemented after sponsor's revision: • The echocardiographic parameters to be considered in the global test have been defined.	15 October 2013
Final 1.0	The following change has been implemented after sponsor's revision: The analysis of echocardiographic parameters is changed to an analysis of variance using only treatment as covariate.	30 October 2018

3.0 STUDY OBJECTIVES AND ENDPOINTS

3.1 STUDY OBJECTIVES

3.1.1 Primary Objective

The primary objective of this study is to evaluate the efficacy of two different doses of istaroxime (0.5 and 1.0 µg/kg/min) in comparison with placebo on the E/Ea ratio in patients with Acute Decompensated Heart failure.

3.1.2 Secondary Objectives

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 cardiac 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 (PK) and metabolism of istaroxime shall also be studied.

3.2 STUDY ENDPOINTS

3.2.1 Primary Endpoint

- The primary efficacy endpoint is the change from baseline to 24 hours after infusion start (treatment period Day 1) in the E/Ea ratio assessed by tissue Doppler.

3.2.2 Secondary Endpoints

The secondary efficacy endpoints are the following:

- 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:
  - Left Ventricular Ejection Fraction (LVEF)
  - LV end systolic and end diastolic volumes
  - Stroke volume index (SVI), and CI
  - E, A and E/A ratio
  - Ea and TAPSE
- 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;
- 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 dyspnoea at baseline);
- Changes in Brain Natriuretic Peptide (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 heart failure 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.

The safety endpoints are:

- Incidence of Adverse Events (AEs);
- Change in vital signs;
- Change in 12-lead Electrocardiogram (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 (Estimated glomerular filtration rate (eGFR));
- 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% Upper Reference Limit (URL, as defined for the Roche hs test to be 14 ng/L) respectively;
- Mortality at Day 30.

The PK parameters are the following:

- Istaroxime plasma concentrations: C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, λ_z, $t_{½}$, Cl_T, MRT, V_{ss}, V_z;
- Istaroxime urine concentrations: A_e, A_e%, Cl_R;
- 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%.

4.0 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 (Day -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.

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.

4.1 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 (Gheorghiade M. et al. Haemodynamic, echocardiographic, and neurohormonal effects of Istaroxime, a novel intravenous inotropic and lusitropic agent: a randomized controlled trial in patients hospitalized with heart failure. J Am Coll Cardiol. 2008; 51(23): 2276-85), 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.

4.2 RANDOMIZATION

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 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.

5.0 ANALYSIS SETS

5.1 INTENT-TO-TREAT POPULATION (ITT)

All randomized patients who will receive at least one administration of the study medication and with at least one available evaluation of efficacy after the baseline will be included in the ITT population.
The ITT population is the primary population for the efficacy analysis.

5.2 PER-PROTOCOL POPULATION (PP)

All randomized patients from the ITT population without any major protocol deviation will be included in the PP population.
Exact definition of major protocol deviations will be discussed by the clinical team case by case during the blind review of the data.

5.3 SAFETY POPULATION

All randomized patients who will receive at least one administration of the study medication will be included in the safety population.

5.4 OTHER ANALYSIS SET

All randomized patients will be included in the randomized population. The randomized population will be considered for descriptive purposes.

5.5 TREATMENT MISALLOCATIONS

In case an error occurs in treatment allocation, the following rule will be followed: if a patient was randomized but received the incorrect treatment, he/she will be reported under his/her randomized treatment group for all analyses performed on the randomized and ITT populations, but he/she will be reported under the treatment actually received for all analyses performed on the safety. The patient will be excluded from the PP population.

5.6 PROTOCOL DEVIATIONS

All the protocol deviations will be discussed case by case before unblinding of the treatment code with the clinical team during the blind review of the data and described in the Data Review Report.

6.0 DEFINITIONS AND DATA CONVENTIONS

This section contains definitions and conventions that will be used for the analysis.

6.1 DEMOGRAPHIC AND SCREENING/BASELINE CHARACTERISTICS VARIABLES

General, demographic and baseline characteristics

Age

If the date of birth is not missing, age will be calculated using the following rules:

- If month of date of screening is greater than month of date of birth or (month of date of screening is equal to month of date of birth and day of date of screening is greater than or equal to day of date of birth), then:

  Age (years) = year (date of screening) – year (date of birth)

- Otherwise:

  Age (years) = year (date of screening) – year (date of birth) - 1

In order to determinate the age of patient, the following rules will be applied for the partial date of birth:

- if only the day is missing, the 15th day of the month will be assumed;
- if the day and the month are missing, June 30th will be assumed.

If the date of birth is missing, the age recorded in the CRF will be used.

Duration of smoking (years)

Duration of smoking will be calculated using the following formula:

- For ex-smoker:
  Duration of smoking (years) = (stop date – start date)/365.25
- For smoker:
  Duration of smoking (years) = (date of screening – start date)/365.25

In order to determinate the duration of smoking, the following rules will be applied for the partial dates of start/stop of smoking:

- for start date and stop date, the first day of the month will be assumed;
- if the month of start date or stop date is missing, January 1st will be assumed.

Past disease and concomitant disease

Past disease

A disease is considered as past disease if it is not ongoing at screening visit ("ongoing" box is not ticked).

Concomitant disease

A disease is considered as concomitant disease if it is ongoing at screening visit ("ongoing" box is ticked).

Previous and Concomitant medications
The following categories of medications will be identified:

- previous medication (start date < date of first randomized study medication intake and stop date ≤ date of first randomized study medication intake);
- medication maintained during the treatment period (start date < date of first randomized study medication intake and stop date > date of first randomized study medication intake or ongoing);
- concomitant medication (start date ≥ date of first randomized study medication intake).

In case of missing or incomplete dates not directly allowing allocation to any of the three categories of medications, a worst-case allocation will be done according to the available parts of the start/end dates. The medication will be allocated to the first category allowed by the available data, according to the following order:

- concomitant medication;
- medication maintained during the treatment period;
- previous medication.

Study medication intake
Date/time of first randomized study medication intake is the date/time of first study medication intake recorded at Day 1.

Date/time of last randomized study medication intake is the date/time of last study medication intake recorded in the Study Termination form.

6.2 EXTENT OF EXPOSURE

Extent of exposure will be calculated in hours as difference between end date/time of infusion and start date/time of infusion:

Extent of exposure (hours) = end date/time of infusion - start date/time of infusion

6.3 EFFICACY VARIABLES

$AUC_{0\text{-}48h}$ on changes in dyspnoea
$AUC_{0\text{-}48h}$ on changes in dyspnoea will be calculated using the following formula:

$$AUC_{0-48h} = \sum_{i=3h}^{48h} \frac{(t_i - t_{i-1})(\Delta VAS_i + \Delta VAS_{i-1})}{2}$$

with i = 3, 6, 12, 24, 48 hours.

In the above formula, t_i represents the scheduled timepoints in hours for VAS score measurements (see table below) and ΔVAS_i represents the change from pre-dose of the value of the VAS score at the timepoint *i*. The change from pre-dose at the timepoint $i = 0$ will be zero.

Time of evaluation (hours)					
T pre-dose	T3h	T6h	T12h	T24h	T48h
t_0	t_1	t_2	t_3	t_4	t_5
Time point for calculation					

The scheduled timepoint will be used for the calculation of $AUC_{0\text{-}48h}$.

In the calculation of $AUC_{0\text{-}48h}$, missing values will be replaced as follows:

- single, isolated missing values (different from pre-dose and last value) will be replaced by linear interpolation using the adjacent values;
- if the last value is missing, it will be replaced by the previous value;
- in case of pre-dose value is missing and/or two or more consecutive values are missing and/or three or more values are missing, no replacement will be performed and the entire curve will be considered as missing.

The $AUC_{0\text{-}48h}$ will be calculated after the replacement of missing values.

$AUC_{0\text{-}48h}$ will be calculated only for patients with VAS score at screening different from 100 or missing.

Length of hospitalization (days)
Length of hospitalization will be calculated using the following formula:

Length of hospitalization = 4 + Number of days of hospitalization after day 4

If the variable "Number of days of hospitalization after day 4" is missing, the length of hospitalisation will be 4 and the number of days of hospitalization after day 4 considered in the analysis will be zero.

6.4 SAFETY VARIABLES

Pre-treatment adverse events
An AE will be classified as pre-treatment AE if it starts before the date/time of first randomized study medication intake (AE onset date/time < date/time of first randomized study medication intake).

Treatment-emergent adverse event (TEAE)
An AE will be classified as a TEAE if it starts on or after the date/time of first randomized study medication intake (AE onset date/time ≥ date/time of first randomized study medication intake).
In case of missing or incomplete dates/times not allowing a direct allocation to any of the two categories of AEs, a worst-case allocation will be done according to the available parts of the onset dates/times and the end dates. The AE will be allocated to the first category allowed by the available data, according to the following order:

- TEAE
- Pre-treatment.

Serious adverse event (SAE)
A SAE is an AE judged as serious.

Adverse drug reaction (ADR)
An ADR is an AE judged as "Certainly related", "Possibly related", "Possibly unrelated", "Uncertain".

Adverse event leading to discontinuation
An AE leading to discontinuation is an AE with action taken equal to "Study drug withdrawn and/or patient withdrawn from the study".

Adverse event leading to death
An AE leading to discontinuation is an AE with outcome equal to "Fatal".

Count of adverse events
Two AEs with the same Preferred Term (PT) and classified in the same category (pre-treatment AE or TEAE) will be considered as two different events when calculating the "number of events" in the tables.

Incidence of cTnT elevation relative
Percent change from baseline in cTnT will be calculated using the following formula at all post-dose timepoints (3h, 6h, 12h, 24h, 48h, 72h):

% change from baseline = 100 * (cTnt at timepoint – cTnT at screening) / cTnT at screening

The following categories will be defined at each timepoint:

- For patients with normal cTnT value (< URL) at screening:
  - A patient will be classified as a "patient with a percent change from baseline in cTnT >50%" if the percent change from baseline in cTnT is greater than 50% (percent change from baseline in cTnT > 50%);
- For patients with abnormal cTnT value (≥ URL) at screening:

- A patient will be classified as a "patient with a percent change from baseline in cTnT >20%" if the percent change from baseline in cTnT is greater than 20% (percent change from baseline in cTnT > 20%);

6.5 BASELINE

The baseline used in the analysis for each variable (for which comparison with baseline is requested) is reported in the table below:

Endpoint	**Baseline**
Laboratory parameters, renal function	Screening
Echocardiographic parameters, VAS score for dyspnoea, vital signs	Pre-dose Day 1
BNP, cTnT	Mean (pre-dose 1, pre-dose 2) at Day 1

7.0 STATISTICAL METHODOLOGY

7.1 HANDLING OF MISSING DATA

The number of patients with missing data will be presented under the "Missing" category, if present. Missing values will be included in the denominator count when computing percentages.

When continuous data are being summarised, only the non-missing values will be evaluated for computing summary statistics.

Missing or incomplete data will be treated as described in sections 6.1, 6.3 and 6.4.

There will be no imputation of missing echocardiographic data.

Other critical missing data will be discussed prior to treatment unblinding, if any. Decisions will be taken during the blind review of the data and will be fully documented in the Data Review Report.

7.2 COVARIATES AND SUBGROUPS

Linear Mixed Models for Repeated Measures (MMRM) will include 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.

Analysis of Covariance (ANCOVA) models will include treatment, centre, gender, baseline cTnT (normal <URL, abnormal ≥URL) and atrial fibrillation (Yes/No) as fixed effects and baseline as covariate.

ANOVA models for echocardiographic parameters will include treatment only. For analysis of Cohort 1, treatment is defined as (0.5 µg/kg/min dose, placebo). For analysis of Cohort 2, treatment is defined as (1.0 µg/kg/min dose, placebo).

All baseline values considered in the statistical analyses have been defined in the paragraph 6.6.

7.3 INTERIM ANALYSIS AND SEQUENTIALITY OF ANALYSES

After the completion of cohort I, a safety interim analysis will be performed and the Data Monitoring Committee (DMC) members will evaluate the incidence of cTnT elevation. At interim analysis, the DMC may recommend to do not proceed with the next cohort if the incidence of cTnT 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 by an independent statistician to the DMC statistician. The DMC will be unblinded when performing the interim analysis.

During the interim analysis, DMC members will analyze and discuss the results in closed sessions. The DMC is required to keep confidential and undisclosed until study conclusion: i) these discussions, ii) the detailed minutes thereof, and iii) the interim analysis results.

For the primary endpoint, 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.

7.4 GENERAL METHODOLOGY

Descriptive statistics will be provided for all variables in the summary tables by treatment group according to the type of variable summarized.

Quantitative variables will be summarised by using n, mean, standard deviation (SD), 95% confidence interval (CI) of the mean, median and range (minimum and maximum). Categorical variables will be summarised by using frequency distributions and percentages.

Hypothesis testing will be carried out at the alpha = 0.05 level (two-sided) when comparing treatments. 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.

Statistical analyses will be carried-out pooling together the patients treated with placebo in the two groups with the exception of the echocardiographic parameters that will be analysed separately for each cohort.

All data collected in the CRF will be presented in the listings.

7.5 PATIENTS DISPOSITION

Disposition of patients will be presented by treatment group for the randomized population.

The number of patients included in each of the randomized, safety, ITT, PP will be summarised for each treatment group and for placebo subjects by cohort and overall.

Randomized patients who discontinued from the study prematurely will also be presented with a breakdown of the reasons for discontinuation by treatment group for the randomized population.
Major and minor protocol violations will also be summarised for each treatment group in the randomized population.

7.6 DEMOGRAPHIC AND SCREENING/BASELINE CHARACTERISTICS

The baseline demographic characteristics will be summarised by treatment group, and for placebo subjects by cohort and overall, by means of descriptive statistics.
The following characteristics will be provided for ITT population:

- Age (years)
- Gender (male/female)
- Race
- Smoking habits
- Alcohol consumption – Alcohol Breath test
- Viral serology
- Digoxin assay

Past disease and concomitant disease

Past disease and concomitant diseases will be coded using Medical Dictionary for regulatory activities (MedDRA) dictionary (version 16.0) and frequency distributions and percentages will be summarised by treatment group for the ITT population by System Organ Class (SOC) and PT.

Medications

Medications will be coded using World Health Organization Drug Dictionary (WHO DD), 2012Q1 (1st Quarter of 2012).
Previous medications, medications maintained during the treatment period and concomitant medications will be summarised by treatment group for the ITT population through frequency distributions and Anatomical Main Group (1st level of the Anatomical Therapeutic Chemical (ATC) classification), Chemical Subgroup (4th level of the ATC classification) and Preferred Name.
Subjects experiencing more than one medication classified in the same category (previous medications, medications maintained during the treatment period, concomitant medications) within the same anatomical main group, chemical subgroup and preferred name will be counted only once.

Echocardiographic parameters at baseline

Descriptive statistics will be presented for the following echocardiographic parameters at baseline: EDD, ESD, LV end diastolic volume, LV end systolic volume, LVEF, 2D global longitudinal strain, Left atrium diameter, Left atrium area, Left atrium volume, ERO, CI, SVI, E, A, E/A ratio, Sa, Ea, E/Ea ratio, PAPs,

TAPSE, Right ventricle Sa, Inferior vena cava diameter and Mitral regurgitation (None/Mild/Moderate/Severe).

No formal comparison between treatment groups on baseline characteristics will be done.

7.7 ANALYSIS OF EFFICACY

7.7.1 Primary efficacy endpoint

Analysis will be performed separately for each cohort on ITT and PP populations.

E/Ea ratio will be summarized at each timepoint by treatment group by means of descriptive statistics.
Comparisons between treatment groups will be done using an ANOVA with change from baseline in E/Ea at 24 hours as dependent variable, and treatment as covariate.
The number of patients and the number of patients considered in the model will be provided by treatment. P-values of the fixed effects and covariates will also be presented.
The adjusted means for treatments and 95% CIs will be presented. The difference between the adjusted means for Istaroxime groups versus Placebo will be calculated with the 95% CIs and p-values.
The primary comparison will be Istaroxime 0.5 µg/kg/min versus Placebo at 24 hours based on Cohort 1 subjects only. Highest dose of Istaroxime (1.0 µg/kg/min) versus Placebo will be tested as a secondary comparison based on Cohort 2 subjects only.
The same analysis will be repeated for change from baseline in E/Ea at 6 hours. All treatment comparisons at 6 hours will be secondary analyses.

E/Ea ratio will also be summarized separated by cohort at each timepoint by treatment group by means of descriptive statistics.

7.7.2 Secondary efficacy endpoints

For echocardiographic parameters, analysis will be performed separately for each cohort on ITT population.
For all other endpoints, analysis will be performed using the combined cohorts on ITT population.

<u>Echocardiographic parameters</u>
The following secondary endpoints:

- LVEF

- LV end systolic and end diastolic volumes
- SVI, and CI
- E, A, E/A ratio
- Sa (tissue doppler parameters)
- Ea and TAPSE

will be summarized at each timepoint by treatment group by means of descriptive statistics.
Comparison between treatment groups will be done using the ANOVA similar to the one used for the primary efficacy endpoint. Only change from baseline echocardiographic data at 6 hours and 24 hours values will be analyzed.

The global test using multivariate analysis of variance (MANOVA) considering multiple echocardiographic parameters will also be performed separately at 6 and 24 hours and by cohort for echocardiographic parameters grouped into 3 types of cardiac mechanics:

- Relaxation parameters such as: E/Ea, Ea
- Contraction parameters such as: Sa, TAPSE
- Overall cardiac pumping ability parameters such as: SVI, CI

The MANOVA p-value from Pallai's Trace will be reported. ((Olson (1976) suggests that Roy's largest root is too likely produce Type I errors and should be avoided, that Wilk's lambda and Hotelling's trace are sensitive to violations of equal covariances in smaller samples, and he recommends Pillai's trace for general use.)

Dyspnoea using VAS score

Analysis will be performed only for patients with VAS score at screening different from 100 or missing.
Dyspnoea using VAS score and changes from baseline will be summarized at each timepoint by treatment group by means of descriptive statistics.
Changes in dyspnoea using VAS score will be analyzed using a MMRM with change from baseline in dyspnoea using VAS score at 3, 6, 12, 24, 48 hours as dependent variable, treatment, centre, timepoint, gender, baseline cTnT (normal <URL, abnormal ≥URL), atrial fibrillation (Yes/No) and treatment*timepoint interaction and baseline and baseline*timepoint interaction as covariates. An unstructured covariance matrix will be considered and the Kenward-Roger adjustment will be used for the degrees of freedom. Only pre-dose, 3, 6, 12, 24, 48 hours values after infusion start will be considered in the MMRM.

$AUC_{0\text{-}48h}$ on change in dyspnoea by VAS score

Analysis will be performed only for patients with VAS score at screening different from 100 or missing.

$AUC_{0\text{-}48h}$ on change in dyspnoea by VAS score will be summarized by treatment group by means of descriptive statistics.
Comparison between treatment groups will be performed by means of an ANCOVA model with $AUC_{0\text{-}48h}$ on change in dyspnoea by VAS score as dependent variable, treatment, centre, gender, baseline cTnT (normal <URL, abnormal ≥URL) and atrial fibrillation (Yes/No) as fixed effects and baseline dyspnoea as covariate.
The number of patients and the number of patients considered in the model will be provided by treatment. P-values of the fixed effects and covariate will also be presented.
The adjusted means for treatments and the 95% CIs will be presented. The differences between the adjusted means for Istaroxime groups versus Placebo will be calculated with the 95% CIs and p-values.

BNP
BNP and change from baseline will be summarized at each timepoint by treatment group by means of descriptive statistics.
Comparison between treatment groups will be performed by means of an ANCOVA model with change from baseline in BNP at 24 hours as dependent variable, treatment, centre, gender, baseline cTnT (normal <URL, abnormal ≥URL) and atrial fibrillation (Yes/No) as fixed effects and baseline BNP as covariate
The number of patients and the number of patients considered in the model will be provided by treatment. P-values of the fixed effects and covariate will also be presented.
The adjusted means for treatments and the 95% CIs will be presented. The differences between the adjusted means for Istaroxime groups versus Placebo will be calculated with the 95% CIs and p-values.

Patients with hospital readmissions or emergency visits for cardiovascular reasons within Day 30
The number and percentage of patients with hospital readmissions or emergency visits for cardiovascular reasons within Day 30 will be summarized by treatment groups using descriptive statistics.

Patients with episodes of worsening heart failure during the hospitalization
The number and percentage of patients with episodes of worsening heart failure during the hospitalization will be summarized at Days 2, 3 and 4 by treatment groups using descriptive statistics.

Length of hospitalization
Length of hospitalization and the number days of hospitalization after day 4 will be summarized by treatment group using descriptive statistics.

7.8 ANALYSIS OF SAFETY

Analysis will be performed on Safety population.

Adverse events

Pre-treatment AEs and TEAEs will be presented separately. Pre-treatment AEs will be presented in the listings only.

The number of treatment-emergent AEs, SAEs, ADRs, AEs leading to discontinuation and AEs leading to death, and the number and the percentage of patients experiencing treatment-emergent AEs, SAEs, ADRs, AEs leading to discontinuation and AEs leading to death will be summarised by treatment group.

AEs will be coded using the MedDRA dictionary (version 16.0). The SOCs and PTs will be used for tabulation. The number of AEs and the number and the percentage of patients with at least one AE will be presented by SOC and PT for treatment-emergent AEs, SAEs, ADRs, AEs leading to discontinuation and AEs leading to death by treatment group.

Vital signs

Vital signs and change from pre-dose will be summarised by treatment group at each timepoint by means of descriptive statistics.

Biomarkers

NT-proBNP and cTNT and increase of cTnT will be summarised by treatment group at screening visit and at all timepoints during the study by means of descriptive statistics.

The number and percentage of patients with a percent change from pre-dose in cTnT >50% and >20% will be summarized at each post-dose timepoints by treatment group.

The number and percentage of patients with a normal cTnT value (<14 ng/L) at screening and with a percent change from pre-dose in cTnT >50% and the number and percentage of patients with an abnormal value of cTnT (≥14 ng/L) at screening and with a percent change from pre-dose in cTnT >20% will also be summarized at each post-dose timepoints by treatment group.

24 hours digital ECG Holter

Premature ventricular complexes, ventricular tachycardia, new onset atrial fibrillation and incidence of clinically or hemodynamically significant episodes of supraventricular or ventricular arrhythmias will be summarised by treatment group at screening and Days 1 and 2 by means of descriptive statistics.

ECG

ECG parameters derived from 12-lead ECG will be summarized by treatment group at screening and Days 1, 2, 3 and 4 by means of descriptive statistics.

Laboratory parameters

Shift tables presenting the number and the percentage of patients in each bivariate category (screening and Day 3) with regards to investigator's interpretation (normal, Not Clinically Significant (NCS), Clinically Significant (CS), No result (NR)) will be provided for all laboratory parameters (excluding potassium, eGFR, creatinine and creatinine clearance).

Renal function

Renal function will be evaluated by means of potassium, eGFR, creatinine and creatinine clearance parameters; for these parameters, the number and percentage of patients by investigator's interpretation (Normal, NCS, CS, NR) will be summarised by treatment group at screening and Days 2, 3, 4 and 30. In addition, the continuous values of eGFR will be summarized by means of descriptive statistics.

7.8.1 Extent of exposure

Extent of exposure will be summarized by treatment group by means of descriptive statistics.

7.9 OTHER PLANNED ANALYSIS

None

8.0 GENERAL CONSIDERATIONS

8.1 SOFTWARE TO BE USED

All statistical analyses and data processing will be performed using Statistical Analysis Systems (SAS®) Software (release 9.4) on a Windows 7 operating system.

8.2 PROGRAMS AND TABLES QUALITY CONTROL

The statistician-programmer of the tables, listings and figures will carefully review the programs and will verify that no error message is highlighted in the 'LOG' file.
Moreover, a second statistician-programmer will verify the internal consistency of each table and figure by checking the results using different SAS programs.

The following level of validation will be implemented:

- Tables:
  - Check the absence of errors and warning messages in the SAS LOG;
  - Check the 100% of data contained in the table through double programming;

- Check the layout of the table (including titles, footnotes) versus the one detailed in the mock-up/SAP.

- Listings:
  - Check the absence of errors and warning messages in the SAS LOG;
  - Check the correctness of the SAS code used for the production of the listings (e.g. the correct variables were used, the correct selections and formats were applied, etc);
  - Check the layout of the listing (including titles, footnotes) versus the one detailed in the mock-up/SAP.

9.0 PROGRAMMING SPECIFICATIONS

The following SAS® code will be used to calculate the age of patient:

age = floor((intck('month', date of birth, date of screening)-(day(date of screening)<day(date of birth)))/12)

The following SAS® code will be used to estimate the MMRM:

```
proc mixed data = dataset;
class tmt timepoint centre gender cTnT atr_fib patient;
model change = tmt timepoint tmt*timepoint centre cTnT atr_fib gender baseline baseline*timepoint / ddfm=kr;
repeated timepoint / subject=patient type=un;
lsmeans tmt tmt*timepoint / cl;
run;
```

where change represents the change from baseline to post-dose values of the variable, tmt the treatment group, timepoint the timepoint, centre the centre, gender the gender (male/female), cTnT the baseline cTnT (normal <URL, abnormal ≥URL), atr_fib the atrial fibrillation (Yes/No), baseline the baseline value of the variable and patient the Patient Number.
The variable tmt will be ordered in SAS® in the following order:

- Istaroxime 0.5 µg/kg/min
- Istaroxime 1.0 µg/kg/min
- Placebo

The following SAS® code will be used to estimate the ANCOVA model:

```
proc mixed data = dataset;
class tmt centre gender cTnT atr_fib;
model variable = tmt centre gender cTnT atr_fib predose;
```

```
lsmeans tmt / cl;
run;
```

where variable represents the efficacy variable (AUC on changes in dyspnoea by VAS score or changes in BNP), tmt the treatment group, centre the centre, gender the gender (male/female), cTnT the baseline cTnT (normal <URL, abnormal ≥URL), atr_fib the atrial fibrillation (Yes/No), predose the pre-dose of efficacy variable.
The variable tmt will be ordered in SAS® in the following order:

- Istaroxime 0.5 µg/kg/min
- Istaroxime 1.0 µg/kg/min
- Placebo

The following SAS code will be used for the Wilcoxon rank sum test for T_{max}:

```
proc npar1way wilcoxon hl alpha=0.1 data = dataset;
class tmt;
var Tmax;
run;
```

where tmt represents the treatment groups and Tmax the Tmax parameter value for each patient.

The following SAS code will be used for the ANOVA:

```
proc glm data = dataset;
class tmt;
model variable = tmt;
lsmeans tmt / cl;
run;
```

The following SAS code will be used for the MANOVA:

```
proc glm data = dataset;
class tmt;
model variable = tmt;
contrast 'active vs placebo' group 1 -1;
manova h=tmt;
lsmeans tmt / cl;
run;
```

The global test p-value from Pallai's Trace will be reported.

PROGRAMMING CONVENTIONS

All tables/figures/listings will be presented in landscape format.
The standard font size is 9 points Courier New for all tables. Listings will be presented with an 8 or 7 points Courier New.

Titles and footnotes will be left-aligned.
Each table/figure/listing will have 2 titles:

- The 1st title will be the table/figure/listing number with the description of the table/figure/listing;
- The 2nd title will be a description of the study population presented in the table/figure/listing.

Some tables will have a third title (before 2nd title) with a description of the statistical method used in those tables.

Tables identified by a "#" at the end of 1st title will also be presented in the Key First Results.

Any footnote added to explain the table/listing/figure contents will be presented in the following format:
Note 1: Percentages are calculated on the number of patients (N).
Note 2: A serious adverse event is an ....
Note 3: …….

The last two footnotes of each table/figure will be footers indicating:

- the reference listing of the data;
- the program name, the date and time of generation and the SAS® version used.

The last footnote of each listing will be a footer indicating the program name, the date and time of generation and the SAS® version used.

In the tables, listing and figures the treatment under comparison will be labelled as "Istaroxime 0.5 µg/kg/min", "Istaroxime 1.0 µg/kg/min" and "Placebo".

Except for the listing 16.2.1-2.1 (based on screening failure patients) all the listings will be based on the randomized population.
Unless otherwise stated, listings will be presented by randomized treatment group, and sorted by the patient number (5-digit).
In all the listings on safety variables, a column with a flag (§) for treatment misallocation will identify the treatment misallocations.
The derived variables will be identified in the listings with a flag (*).

In general, dates will be presented on listings in the format ddmmmyyyy (date9.) and time in the format hh:mm (time5.). In case of partial dates or times, missing information will be

replaced by dashes. Numeric variables will be listed generally with the same number of decimal places as in the actual data.

The following rules on decimal places will be considered in the listings for the derived variables (in the analyses approximations will not be performed):

- Age (years), length of hospitalization (days): 0 decimal place;
- Extent of exposure (hours), $AUC_{0\text{-}48h}$ on changes in dyspnoea: 1 decimal place;
- Change from pre-dose/baseline: same as the variable considered.

The following rules on decimal places will be considered for the results of the analyses (if the analyses are performed on derived variables, the level of precision of the actual data is derived from the previous list):

- Min, max: same as actual data;
- Mean and its confidence limits (unadjusted and adjusted), adjusted difference between means and its confidence limits, SD, median: actual data + 1 decimal;
- Percentage: 1 decimal place.

10.0 ARCHIVING

These deliverables will be archived in CROS NT study file. The paper TMT will be sent back to the Sponsor after completion of the study.

Deliverable	Electronic	Paper
Statistical Analysis Plan	X	X(*)
Randomization List	X	X(*)
Data Review Plan	X	X (*)
Data Review Listings	X	
Data Review Report	X	X(*)
Derived datasets	X	
Key First Results	X	
Tables, Listings and Figures	X	X(*) only for approval page
Tables, Listings, Figures validation		X(*)(^)
Statistical Report	X	X(*)

(*) Flag for original documents
(^) Only tables and figures

11.0 REFERENCES

- European Medicines Agency (EMEA), International Conference on Harmonisation (ICH) E3 Harmonised Guideline (1996) “Structure and Content of Clinical Study Reports”;
- EMEA, ICH E9 Harmonised Guideline (1998) “Statistical Principles for Clinical Trials”.

APPENDIX 1: MOCK-UPS OF TABLES, LISTINGS AND FIGURES

See document "AppendixI_CVT-CV-002_1.0_181030.docx".
Olson, C. L. (1976). On choosing a test statistic in multivariate analysis of variance. Psychological Bulletin, 83, 579-586.