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Clinical Study Report

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 Number: CVT-CV-002

EudraCT Number: 2013-000540-26

CFDA Drug Clinical Trial Approval document no.: 2015L00219

Name of test product:	Istaroxime (PST2744)
Indication:	Acute Decompensated Heart Failure (ADHF)
Study Design:	Phase II, Multi-center, double-blind, placebo-controlled, parallel-group study.
Sponsor:	CVie Therapeutics Company Limited, Unit110-111, Bio-informatics Center, No2. Science Park West Avenue, Hong Kong Science Park, Shatin, N.T. HK
Coordinating Investigators:	Prof. Jun Huang, MD, Jiangsu Province Hospital, Nanjing, China. Prof. Marco Metra, MD, Spedali Civili, Brescia, Italy.
Sponsor's Medical Approver:	Giuseppe Bianchi, MD, Chief Scientific Officer, Windtree Therapeutics, Inc.
Date of first patient first visit:	25 September 2013
Date of last patient last visit:	14 August 2018
Report date:	30 October 2023

This study was conducted in accordance with the ethical principles arising from the Helsinki Declaration, Guidelines on Good Clinical Practice (GCP) and applicable regulatory requirements.

2 SYNOPSIS

Name of Sponsor/Company: CVie Therapeutics Company Limited

Name of Finished Product: Istaroxime

Name of Active Ingredient: Istaroxime (PST2744)

Title of Study: 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

Investigators and Study Centers:

This a multi-center study; a total of 13 investigational sites participated in the study (11 China and 2 Italy). The names of the investigators, addresses of the study centers, and copies of the investigators' curricula vitae are provided in Appendix 16.1.4.

Publication (reference): Carubelli V, Zhang Y, Metra M, et al. Treatment with 24 hour istaroxime infusion in patients hospitalised for acute heart failure: a randomised, placebo-controlled trial. Eur J Heart Fail. 2020 Sep;22(9):1684-1693.

Studied Period (years):	**Phase of Development:**
(date of first enrollment): 25 September 2013 **(date of last completed):** 14 August 2018	Phase II

Objectives:

The primary objective of this study was 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 hospitalized with ADHF.

The secondary objectives of this study were to assess the safety, tolerability and efficacy of two different doses of istaroxime 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 Chinese and Italian/Caucasian patients with ADHF.

Pharmacokinetics (PK) and metabolism of istaroxime were also studied in all of the Italian patients and in a subset of Chinese patients.

Methodology:

This Phase II, Multi-center, double-blind, placebo-controlled, parallel-group study is intended to investigate the safety, tolerability and efficacy of i.v. istaroxime 0.5 and 1.0 µg/kg/min administered for 24 hours.

Number of Patients (planned and analyzed):

	Overall (N=122)		
	Istaroxime 0.5 µg/kg/min	**Istaroxime 1.0 µg/kg/min**	**Placebo**
Randomized population [1]	41	42	39
Safety population [2]	41	40	39
Intent-to-treat population [3]	41	40	39
Per protocol population [4]	39	38	38

Diagnosis and Main Criteria for Inclusion:

Inclusion Criteria;

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 hypoperfusion such as confusion;
5. Left ventricular (LV) Ejection fraction (EF) ≤ 40 % measured by 2D-Echocardiography;

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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).

Test Product, Dose and Mode of Administration, Batch Number:

Istaroxime (0.5 µg/kg/min): 2 vials of lyophilized istaroxime (10 mg) + 50 mg of lactose and 2 vials of matching placebo

Istaroxime (1.0 µg/kg/min): 4 vials of lyophilized istaroxime (10 mg) + 50 mg of lactose

Placebo: 4 vials of matching placebo (50 mg of lactose)

Duration of Treatment:

24 hours

Criteria for Evaluation:

Efficacy:

- E/Ea Ratio (Tissue Doppler)
- PGA
- BSA

Safety:

- Treatment-emergent adverse events (TEAEs)
- Vital signs
- Physical examination
- Clinical laboratory tests (liver, kidney, hematology, chemistry, urinalysis and pregnancy test for females)
- ECGs

Pharmacokinetics:

- Drug concentration levels

Statistical Methods:

The primary efficacy endpoint for this study is the change from baseline to 24 hours after infusion start (treatment period Day 1) in the E/Ea ratio assessed by tissue Doppler. The population for both efficacy and safety analysis consists of all patients who received at least one dose of study medication or placebo. The primary comparison was 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 was tested as a secondary comparison based on Cohort 2 subjects only. Hypothesis testing is carried out at the alpha = 0.05 level (two-sided) when comparing treatments pooling together the patients treated with placebo in the two groups. Descriptive statistics are provided for all variables in the summary tables by treatment group according to the type of variable summarized.

SUMMARY – CONCLUSIONS

STUDY POPULATION

Of the 144 unique patients screened, 122 passed screening although a further 2 were withdrawn due to violations of the inclusion/exclusion criteria prior to being treated in the study. Of the 120 treated patients, 108 patients completed the study to Day 30 follow up.

EFFICACY RESULTS:

Istaroxime was statistically superior to placebo in the primary comparison of istaroxime 0.5 µg/kg/min versus placebo at 24 hours based on Cohort 1 subjects only and in the secondary comparison of istaroxime 1.0 µg/kg/min versus placebo based on Cohort 2 subjects only

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ANOVA Change: Baseline to 24h				
N patients in the model	37	17	33	19
Adjusted mean	-4.55	-1.55	-3.16	-1.08
95% CI	-6.05 to -3.04	-3.77 to 0.67	-4.09 to -2.24	-2.30 to 0.13
Mean treatment difference	-3.00		-2.08	
95% CI	-5.68 to -0.32		-3.61 to -0.55	
p-value	**0.029**		**0.009**	

E/Ea ratio change from baseline to 6h showed statistical superiority of istaroxime 1.0 µg/kg/min versus placebo based on Cohort 2 subjects only.

For the individual echocardiographic parameters, the treatment difference in change from baseline to 24 hours was statistically significant for both doses and cohorts for E/A and Sa, for SVI at 0.5 µg/kg/min and for the combined cohort comparison for the 1.0 µg/kg/min dose. A statistically significant treatment difference was also seen for Ea at 0.5 µg/kg/min across both cohorts, and in both E and A for the 1.0 µg/kg/min dose in the combined cohort analysis. At 6 hours significant treatment differences were seen for SVI, E and Sa (1.0 µg/kg/min dose), and at 48 hours for SVI (0.5 µg/kg/min dose) and CI (both doses).

In the global test of echocardiographic parameters, relaxation parameters (E/Ea, Ea) showed a statistically significant difference between istaroxime 1.0 µg/kg/min and placebo at both 6 and 24 hours. Contraction parameters (Sa, TAPSE) showed a statistically significant difference between istaroxime 1.0 µg/kg/min and placebo at both 6 hours and between istaroxime 0.5 µg/kg/min and placebo at 24 hours. Overall cardiac pumping ability (SVI, CI) showed a statistically significant difference between istaroxime 1.0 µg/kg/min and placebo at both 6 hours and between istaroxime 0.5 µg/kg/min and placebo at 24 hours.

Dyspnea improved (increased VAS score) from baseline in all treatment groups with a statistically significantly higher improvement at 6 hours with both istaroxime doses (istaroxime 0.5 µg/kg/min – placebo: +4.1619, 95% CI: 0.1855 to 8.1383, p=0.040 and istaroxime 1.0 µg/kg/min – placebo +5.3152, 95% CI: 1.3904 to 9.2399, p=0.008) in the MMRM. The MMRM on VAS and the ANCOVA on dyspnea AUC0-48h did not show any significant effect of treatment overall.

BNP values were available for only 8 patients and no significant findings were observed. There were also no notable changes or treatment differences in length of hospitalization, hospital readmission or emergency visits and episodes of worsening of heart failure.

Pharmacokinetics

- Istaroxime (PST2744) infused to Chinese and Italian patients at the 2 ascending dose levels of 0.5 and 1 µg/kg/min for 24 h, showed rapidly increasing systemic concentrations just after the start of the infusion and an extremely rapid decline at the end of the infusion with a half-life shorter than 2 h. The short half-life of istaroxime is due to a very high systemic clearance (>100 L), but in spite of a large volume of distribution (> 100 L). The low recovery of unchanged istaroxime in urine (less than 10% of the infused dose) suggests an extensive metabolism and also that the drug may be removed also via non-renal mechanisms.
- The metabolites PST2915 and PST3093 have longer terminal half-lives which allow them to accumulate to a greater extent than their parent compound, while PST2922 on average showed a terminal half life as short as the parent compound. All 3 metabolites share with the parent compound notably large volumes of distribution and considerably rapid systemic clearances. The most important metabolite in terms of rate and extent of systemic exposure is PST3093, though PST2915 was the metabolite previously shown to have pharmacological activity as the parent compound. The excretion of all 3 metabolites lets envisage an extra-renal elimination for all the 3 as already observed for the parent compound.
- The kinetics of istaroxime as well as that of all 3 metabolites proved not to be linear in the investigated dose interval. No significant difference in t_{max} between the tested doses was detected for any analyte.

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SAFETY RESULTS:

TEAEs were reported by 31 (75.6%) patients in the istaroxime 0.5 µg/kg/min group, 33 (82.5%) patients in the istaroxime 1.0 µg/kg/min group and 23 (59.0%) patients in the placebo group. The most commonly reported TEAEs in any group were infusion site pain, most commonly in both istaroxime treatment groups, nausea and vomiting, which were most commonly in the istaroxime 1.0 µg/kg/min group, and hypotension, which was most commonly in the placebo group.

MedDRA System Organ Class	Istaroxime 0.5 µg/kg/min	Istaroxime 1.0 µg/kg/min	Placebo
Any	31 (71.6)	33 (82.5)	23 (59.0)
Cardiac Disorders	8 (19.5)	9 (22.5)	8 (20.5)
Gastrointestinal disorders	7	17	5 (12.8)
General disorders and admin. site conditions	17	18	5 (12.8)
Hepatobiliary disorders	1 (2.4)	0	2 (5.1)
Infections and infestations	4 (9.8)	5 (12.5)	0
Investigations	9 (22.0)	10 (25.0)	12 (30.8)
Metabolism and Nutritional disorders	2 (4.9)	4 (10.0)	4 (10.3)
Nervous system disorders	3 (7.3)	6 (15.0)	1 (2.5)
Vascular disorders	7 (17.1)	3 (7.5)	7 (17.9)

There were 12 serious adverse events reported in 10 patients. None of the non-fatal SAEs reported were considered related to IMP, and all resolved, one with sequelae. The most commonly reported SAE was worsening of heart failure, consistent with the diagnosis of subjects within the study.

No clinically significant mean changes from Baseline, and no differences were noted between treatment groups, were noted for any clinical laboratories, vital signs, weight, Body Mass Index, physical examination, or ECG parameters.

CONCLUSION:

Istaroxime was statistically superior to placebo at both doses, with a favorable safety profile. The beneficial effects on the E/Ea ratio were associated with a reduction in heart rate and increases in SBP, no changes in troponin release and improvement in eGFR.

Date of the Report: 30 October 2023