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RESEARCH ARTICLE

Treatment with 24 hour istaroxime infusion in patients hospitalised for acute heart failure: a randomised, placebo-controlled trial

Valentina Carubelli[1]*†, Yuhui Zhang[2†], Marco Metra[1], Carlo Lombardi[1], G. Michael Felker[3], Gerasimos Filippatos[4,5], Christopher M. O'Connor[6], John R. Teerlink[7,8], Phillip Simmons[9], Robert Segal[9], Gabriella Malfatto[10], Maria Teresa La Rovere[11], Dianfu Li[12], Xiumin Han[13], Zuyi Yuan[14], Yali Yao[15], Benjamin Li[16], Lit Fui Lau[17], Giuseppe Bianchi[9], and Jian Zhang[2]*, on behalf of the Istaroxime ADHF Trial Group

[1]Cardiology, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University and Civil Hospital of Brescia, Brescia, Italy; [2]Heart Failure Center, Fuwai Hospital, National Center for Cardiovascular Diseases, Beijing, China; [3]Duke University School of Medicine and Duke Clinical Research Institute, Durham, NC, USA; [4]National and Kapodistrian University of Athens, Athens, Greece; [5]Medical School, University of Cyprus, Nicosia, Cyprus; [6]Inova Heart and Vascular Institute, Falls Church, VA, USA; [7]Section of Cardiology, San Francisco Veterans Affairs Medical Center, San Francisco, CA, USA; [8]School of Medicine, University of California, San Francisco, CA, USA; [9]Windtree Therapeutics, Inc., Warrington, PA, USA; [10]IRCCS Istituto Auxologico Italiano, Department of Cardiovascular, Neural and Metabolic Sciences, S. Luca Hospital, Milan, Italy; [11]Istituti Clinici Scientifici Maugeri IRCCS, Department of Cardiology, Institute of Montescano, Pavia, Italy; [12]Jiangsu Provincial People's Hospital, First Affiliated Hospital of Nanjing Medical University, Nanjing, China; [13]The General Hospital of Shenyang Military Region of Chinese People's Liberation Arm, Shenyang, China; [14]The First Affiliated Hospital of Xi'An Jiaotong University, Xi'an, China; [15]The First Hospital of Lanzhou University, Lanzhou, China; [16]Lee's Pharmaceutical Limited, Taipei, Taiwan; and [17]CVie Therapeutics Limited, Taipei, Taiwan

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Aim

Istaroxime is a first-in-class agent which acts through inhibition of the sarcolemmal Na^+/K^+ pump and activation of the SERCA2a pump. This study assessed the effects of a 24 h infusion of istaroxime in patients hospitalised for acute heart failure (AHF).

Methods and results

We included patients hospitalised for AHF with left ventricular ejection fraction ≤40% and E/e' > 10. Patients were randomised to a 24 h intravenous infusion of placebo or istaroxime at doses of 0.5 μg/kg/min (cohort 1: placebo $n = 19$; istaroxime $n = 41$) or 1.0 μg/kg/min (cohort 2: placebo $n = 20$, istaroxime $n = 40$). The primary endpoint of change in E/e' ratio from baseline to 24 h decreased with istaroxime vs. placebo (cohort 1: -4.55 ± 4.75 istaroxime 0.5 μg/kg/min vs. -1.55 ± 4.11 placebo, $P = 0.029$; cohort 2: -3.16 ± 2.59 istaroxime 1.0 μg/kg/min vs. -1.08 ± 2.72 placebo, $P = 0.009$). Both istaroxime doses significantly increased stroke volume index and decreased heart rate. Systolic blood pressure increased with istaroxime, achieving significance with the high dose. Self-reported dyspnoea and N-terminal pro-brain natriuretic peptide improved in all groups without significant differences between istaroxime and placebo. No significant differences in cardiac troponin absolute values or clinically relevant arrhythmias were observed during or after istaroxime infusion. Serious cardiac adverse events (including arrhythmias and hypotension) did not differ between placebo and istaroxime groups. The most common adverse events were injection site reactions and gastrointestinal events, the latter primarily with istaroxime 1.0 μg/kg/min.

*Corresponding authors. Valentina Carubelli, Division of Cardiology, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University and Civil Hospital of Brescia, Piazzale Spedali Civili 1, 25123 Brescia, Italy. Fax: +39 0303996177, Email: valentina.carubelli@gmail.com
Jian Zhang, State Key Laboratory of Cardiovascular Disease, Heart Failure Center, Heart Failure Care Units (HFCU), Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, 167 Bei Li Tu Road, Beijing 100037, China. Email: fwzhangjian62@126.com

†These authors contributed equally to this study.

Conclusions In patients hospitalised for AHF with reduced ejection fraction, a 24 h infusion of istaroxime improved parameters of diastolic and systolic cardiac function without major cardiac adverse effects.

Keywords Acute heart failure • Istaroxime • SERCA2a • Therapy • Outcomes

Introduction

Despite the overwhelming epidemiological, clinical, and economic burden, the natural history of acute heart failure (AHF) has remained unchanged over recent decades.[1–3] Within the AHF population, patients presenting with low systolic blood pressure (SBP) are a high-risk group with unfavourable outcomes.[4] No evidence-based, safe treatment has emerged for this subgroup and current therapy is still based on traditional inotropic agents, which are associated with untoward effects, including tachycardia, arrhythmias, myocardial ischaemia, hypotension, and increased mortality.[5–9] Many of these adverse cardiac events are mediated by an increase in cytosolic calcium concentrations, worsened by the depression of sarcoplasmic reticulum calcium adenosine triphosphatase isoform 2a (SERCA2a) activity that occurs in heart failure. Hypotension is caused by concomitant vascular effects of many inotropic agents and may further deteriorate tissue hypoperfusion.[5,6,9]

Istaroxime is a first-in-class agent with a dual mechanism of action involving the inhibition of the sarcolemmal Na^+/K^+ pump and the activation of the SERCA2a pump activity (online supplementary *Figure S1*). This is mediated by the displacement of phospholamban from SERCA2a causing enhanced calcium reuptake by the sarcoplasmic reticulum, independently of intracellular cyclic AMP concentrations.[10–13] Reduced cytoplasmic free calcium levels during diastole improve relaxation and result in increased calcium release during systole, enhancing cardiac contractility.[14] In a phase II trial in patients with worsening heart failure and reduced left ventricular systolic function (HORIZON-HF), a 6 h istaroxime infusion resulted in improvements of both left ventricular diastolic and systolic function, with a slight increase in SBP and reduction in heart rate (HR).[15,16] These effects support a potential therapeutic role for istaroxime in the treatment of patients with AHF and reduced left ventricular ejection fraction (LVEF) since improvements in cardiac function without tachycardia or hypotension differentiate this agent from traditional inotropes.[12,17,18] We designed a clinical trial to assess the effects of a prolonged istaroxime infusion of 24 h in patients hospitalised for AHF with reduced LVEF and low SBP.

Methods

This study was a phase II, multicentre, randomised, double-blind, placebo-controlled, parallel group investigation, conducted at two sites in Italy and eight sites in China (online supplementary *Appendix S1*). All patients provided written informed consent. The study was approved by country regulatory authorities and by local institutional review boards. The trial is registered in the European Clinical Trials Database (EudraCT 2013−000540-26) and ClinicalTrials.gov (NCT02617446).

Study population

Patients were randomised 2:1 to istaroxime or placebo 24 h infusion and were enrolled in two sequential cohorts, the first with an istaroxime dose of 0.5 μg/kg/min and the second with an istaroxime dose of 1.0 μg/kg/min. At the end of cohort 1 enrolment, an independent Data Safety and Monitoring Board conducted an interim safety analysis of unblinded data before proceeding to cohort 2 enrolment.

Inclusion criteria were: age ≥ 18 years, an ongoing hospitalisation for AHF with dyspnoea at rest or minimal exertion and need for intravenous diuretic therapy (> 40 mg intravenous furosemide), SBP between 90 and 125 mmHg without signs or symptoms of peripheral hypoperfusion; LVEF ≤ 40%; intermediate to high E/e' ratio by tissue Doppler echocardiography (> 10); brain natriuretic peptide (BNP) or N-terminal pro BNP (NT-proBNP) plasma levels ≥ 350 pg/mL or ≥ 1400 pg/mL, respectively, and presence of adequate echocardiographic windows.

The main exclusion criteria were: concomitant or planned treatment with intravenous vasodilators, inotropes or vasopressors; treatment with oral digoxin (patients treated with digoxin within the last week could be randomised if the plasma concentration at screening was < 0.5 ng/mL); acute coronary syndrome or stroke within the past 3 months; coronary artery bypass graft or percutaneous coronary intervention within the past month or planned in the next month; resting HR > 120 bpm or < 50 bpm; life-threatening ventricular arrhythmia or implantable carrdioverter-defibrillator shock within the past month; fever > 38°C; serum creatinine > 3.0 mg/dL or estimated glomerular filtration rate (eGFR) < 30 mL/min/m^2; serum potassium > 5.3 mmol/L or < 3.8 mmol/L; severe hepatic dysfunction.

Clinical endpoints

The primary efficacy endpoint was the change from baseline to 24 h after the start of the infusion in the E/e' ratio assessed by tissue Doppler echocardiography. Secondary endpoints included the change from baseline to 24 h of other echocardiography parameters: LVEF, left ventricular end-systolic and end-diastolic volumes, stroke volume index, E, A, E/A ratio, and S′; changes in dyspnoea by visual analogue scale (VAS) and in NT-proBNP from baseline to 24 h; proportion of patients with hospital readmissions or emergency visits for cardiovascular reasons by day 30; proportion of patients with episodes of in-hospital worsening heart failure to day 4, and length of stay. In-hospital worsening heart failure was defined by the need to increase the dose or reinitiate intravenous therapy with diuretics and/or other inotropic agents to day 4.[19]

Safety endpoints were assessed throughout the study and included: incidence of adverse events (AEs); change in vital signs and in 12-lead electrocardiographic (ECG) parameters; incidence of clinically or haemodynamically significant episodes of supraventricular or ventricular arrhythmias detected by continuous ECG monitoring; standard laboratory parameters; renal function; cardiac troponin T (cTnT); incidence of cTnT elevations, and mortality at day 30. Elevations in cTnT

were considered significant if they were > 50% or > 20% from baseline values, for patients with cTnT below or above the 99% upper reference limit at baseline, respectively.

Study procedures

The study design included a 24 h screening period, a 24 h study drug infusion phase (day 1), a post-treatment phase (days 2–4), and a follow-up visit at day 30 (online supplementary *Figure S2*). Patients could be enrolled at any time after admission. The use of oral medications was permitted during the study with the exception of digoxin. Intravenous diuretics were permitted during the treatment period at stable doses, unless changes were required due to clinically relevant variations of the patient's condition. If treatment with an inotrope was clinically necessary during the infusion period, the investigational product was discontinued.

Echocardiography was performed according to international standards[20] at screening, baseline (just before starting study drug infusion), 6, 24 h (just before infusion end), and 48 h after infusion start. Echocardiographic measurements at baseline, 6, 24, and 48 h were evaluated in a blinded fashion by a CoreLab located at the University and Civil Hospital of Brescia, Italy. E/e' ratio was calculated as the ratio between the peak early diastolic mitral flow velocity (E wave) and the mean of the annular lateral (e' lateral) and septal (e' septal) velocities, according to standard guidelines.[20] Intraobserver and interobserver variability in E/e' measurement was tested on the 15% of the recordings and is shown in online supplementary *Figure S3*.

Self-reported dyspnoea was assessed using a 100 mm VAS, with zero representing the worst level of dyspnoea and 10 the best breathing condition.

Twenty-four hour ECG monitoring was performed using a three-lead device (Mortara Industries, Milwaukee, WI, USA) during the day of screening, the infusion period, and the day after (the latter was optional). Analysis was conducted by a blinded CoreLab (ICS Maugeri, Italy).

Serial measurements of NT-proBNP throughout the study were analysed by a central laboratory (Elecsys® proBNP II, Roche®, Basel, Switzerland). Samples for cTnT were taken at baseline, 3, 6, 12, 24, 48, and 72 h and on day 30, and were analysed by a core laboratory (Elecsys® high-sensitivity assay, Roche®, Basel, Switzerland). AEs were monitored throughout the duration of the study.

Statistical analysis

The study was designed as a mechanistic and safety study to assess the effects of a 24 h istaroxime infusion in patients hospitalised for AHF. Based on the changes observed in the HORIZON-HF trial,[15] we assumed that a sample size of at least 20 patients in the placebo group in each cohort with 2:1 randomisation to istaroxime would provide sufficient power to assess the effect of istaroxime on the main haemodynamic parameters.

Analyses were conducted using a pre-specified modified intention-to-treat (mITT) protocol, including all randomised patients in whom study drug infusion had been initiated and with at least one available evaluation of efficacy after the baseline. Biomarker and eGFR values were analysed using medians and inner/outer quartiles, and active treatment was compared to placebo using the Wilcoxon rank-sum test. Dyspnoea scores by VAS were analysed using mixed model regression with treatment, centre, timepoint, gender, baseline cTnT, presence of atrial fibrillation, treatment by timepoint interaction, baseline value, and baseline value by timepoint interaction in the model.

Echocardiographic parameters were compared between treatment groups using linear regression with treatment in the model. Categorical variables were summarised as frequency and per cent and compared between treatment groups using the Cochran–Mantel–Haenszel test, controlling for study site. AEs were recorded for all enrolled patients and were summarized as AEs, serious AEs (SAEs), AEs leading to discontinuation, and SAEs leading to death. All analyses were conducted using the SAS® System for Windows™, versions 9.1.3 and 9.4.

Results

Study population

Patient disposition is summarised in *Figure 1*. Enrolment took place at two Italian centres, from October 2013 to March 2015, with the inclusion of 24 patients, and in eight Chinese centres, from November 2016 to July 2018, with the inclusion of 96 patients. Among 144 patients screened, 21 were screening failures and one patient withdrew consent. Additionally, two patients from cohort 2 were excluded from the mITT analysis as pre-specified in the protocol because they withdrew within 2 h before any efficacy assessments were performed due to violation of eligibility criteria. The mITT population included 60 patients in each cohort; 19 on placebo (12 Asian and 7 Caucasian) and 41 on istaroxime 0.5 μg/kg/min in cohort 1 (24 Asian and 17 Caucasian), and 20 on placebo and 40 on istaroxime 1.0 μg/kg/min in cohort 2 (all Asian patients). In the per-protocol analysis, one patient on placebo in cohort 2, two patients on istaroxime 0.5 μg/kg/min, and four patients on istaroxime 1.0 μg/kg/min were excluded due to violations of exclusion criteria ($n = 2$), major Good Clinical Practice violation (Holter was started prior to informed consent form signature), and four patients used prohibited medications (digoxin, dopamine, and levosimendan).

Baseline characteristics

Baseline characteristics are shown in *Table 1*. Besides the higher prevalence of Asian patients in cohort 2 (100% vs. 60% in cohort 1), there were no major differences within each cohort.

Primary endpoint

The primary endpoint (change in the E/e' ratio from baseline to 24 h) was significantly reduced with both istaroxime doses compared with placebo within each individual cohort. The magnitude of the reduction from baseline was similar with both doses (cohort 1: -4.55 ± 4.75 istaroxime 0.5 μg/kg/min vs. -1.55 ± 4.11 placebo, $P = 0.029$; cohort 2: -3.16 ± 2.59 istaroxime 1.0 μg/kg/min vs. -1.08 ± 2.72 placebo, $P = 0.009$) (*Table 2*, online supplementary *Table S1*, and *Figure 2A*). In the per-protocol population, results were identical for cohort 1, whereas the E/e' change was -3.19 ± 2.63 in the istaroxime 1.0 μg/kg/min group vs. -1.08 ± 2.72 in the placebo cohort 2 group ($P = 0.009$).

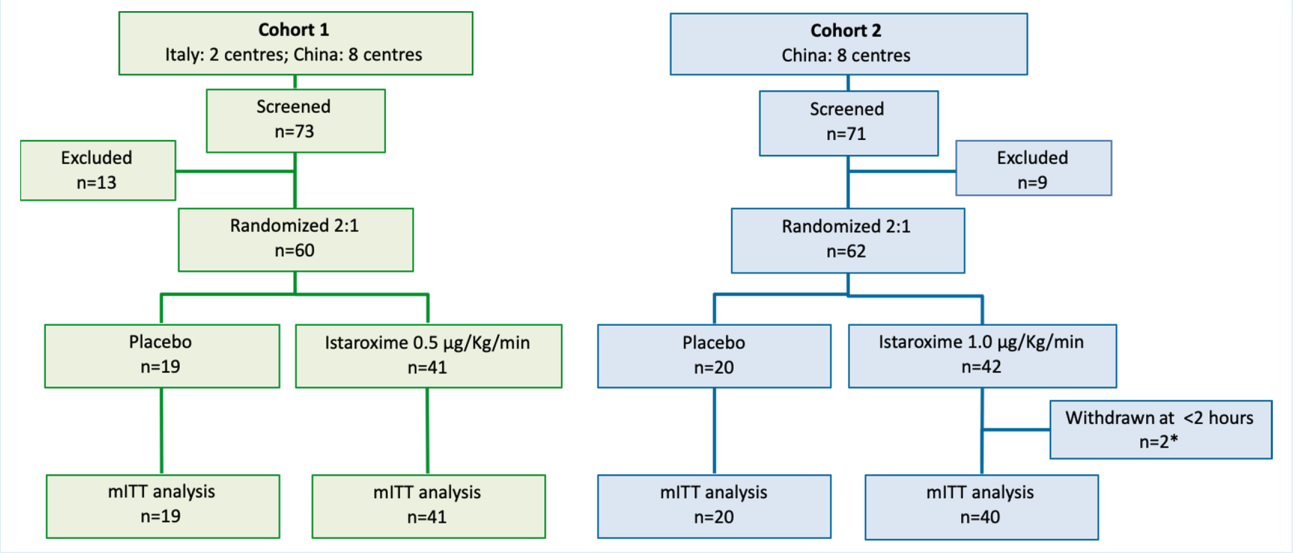

Figure 1 Consort plot. mITT, modified intention-to-treat. *Exclusion of patients from mITT analysis was pre-specified in the study protocol.

Secondary endpoints

Both istaroxime doses increased stroke volume index from baseline to 24 h (*Table 2*, online supplementary *Table S1*, and *Figure 2B*). Changes in LVEF and left ventricular volumes did not reach statistical significance compared to placebo. Treatment with istaroxime was associated with improvement of other echocardiographic parameters such as E/A ratio, left atrial dimensions and inferior vena cava diameter (*Table 2* and online supplementary *Table S1*). HR decreased from baseline by about 3 bpm with istaroxime 0.5 µg/kg/min and by 8–9 bpm with istaroxime 1.0 µg/kg/min with significant changes vs. placebo at 3 to 24 h in the high-dose group. SBP increased from baseline by about 3 mmHg with istaroxime 0.5 µg/kg/min and by 6–8 mmHg with istaroxime 1.0 µg/kg/min reaching statistical significance compared to placebo at 3 to 12 h in istaroxime 1.0 µg/kg/min group (*Figure 2C,2D* and online supplementary *Table S1*). Individual changes from baseline to 24 h in the main echocardiographic parameters and vital signs are reported in online supplementary *Figure S4*.

None of the other secondary endpoints showed significant differences between istaroxime and placebo (online supplementary *Table S2*). Overall diuresis during the 24 h infusion was numerically higher in patients treated with istaroxime compared to placebo (*Table 3*).

Safety endpoints

No significant changes in cardiac troponin absolute values were observed during and after istaroxime infusion (*Figure 3* and online supplementary *Tables S3* and *S4*). Renal function measured by eGFR generally increased with both istaroxime doses over 24 h in contrast to placebo where a decrease was observed (online supplementary *Table S3*). No other clinically meaningful changes in laboratory parameters were observed comparing istaroxime vs. placebo. In particular, worsening of hepatic function or signs of hepatic drug toxicity were not reported during the study (data not shown). Data from 24 h Holter monitoring showed no differences in clinically significant arrhythmias between istaroxime and placebo groups (online supplementary *Table S5*).

The summary of AEs is reported in *Table 4*. The rate of SAEs did not show meaningful differences in the three arms. One patient died in the istaroxime 1.0 µg/kg/min group within the 30-day study period (cardiogenic shock); one additional cardiac death was recorded after day 30. Analysis of treatment emergent adverse drug reactions showed that the number of patients experiencing cardiovascular AEs was numerically higher in the placebo group. There was a substantially higher rate of patients experiencing gastrointestinal events (mainly abdominal pain, nausea, and vomiting) in the istaroxime 1.0 µg/kg/min group (study drug was discontinued in one patient due to nausea and in one patient due to vomiting concomitant to renal artery embolism). The rate of injection site reactions was high in all patients treated with istaroxime and short intravenous catheters, requiring the change of peripheral line and/or use of peripheral long line or a central line (study drug was discontinued only in one patient treated with istaroxime 1.0 µg/kg/min).

Discussion

Our results show that a 24 h infusion of istaroxime at 0.5 or 1.0 µg/kg/min in patients with AHF and reduced LVEF improves left ventricular diastolic and systolic function without an increase in major cardiac AEs. The primary endpoint of our study (E/e' ratio change from baseline to 24 h) was significantly reduced by both doses of istaroxime compared to placebo. Other parameters related to left ventricular diastolic function (i.e. E/A wave ratio and left atrial dimensions) were also reduced with istaroxime. Among

Table 1 Baseline characteristics

Variable	Placebo cohort 1 ($n = 19$)	Istaroxime 0.5 μg/kg/min ($n = 41$)	Placebo cohort 2 ($n = 20$)	Istaroxime 1.0 μg/kg/min ($n = 40$)
Age (years)	57.9 ± 16.5	59.7 ± 15.5	55.5 ± 16.3	52.3 ± 13.0
Male sex	16 (84.2)	34 (82.9)	18 (90.0)	34 (85.0)
Race				
Caucasian	7 (37.0)	17 (41.5)	–	–
Asian	12 (63.0)	24 (58.5)	20 (100)	40 (100)
BMI (kg/m^2)	25.2 ± 3.4	25.4 ± 4.0	23.7 ± 4.2	23.3 ± 3.8
Atrial fibrillation at screening	5 (26.3)	8 (19.5)	6 (30.0)	11 (27.5)
Systolic blood pressure (mmHg)	104.6 ± 8.3	105.0 ± 11.6	107.9 ± 9.9	106.0 ± 10.4
Heart rate (bpm)	76.7 ± 17.4	71.8 ± 13.3	79.2 ± 13.4	77.9 ± 11.0
QRS duration (ms)	135.6 ± 41.6	132.9 ± 32.1	120.4 ± 22.6	126.9 ± 26.5
Dyspnoea VAS score	69.7 ± 18.3	73.6 ± 19.5	71.8 ± 24.3	71.5 ± 21.0
Ischaemic aetiology	10 (52.6)	16 (39.0)	6 (30.0)	13 (32.5)
Medical history				
Diabetes	5 (26.3)	14 (34.1)	4 (20.0)	6 (15.0)
Arterial hypertension	11 (57.9)	17 (41.5)	7 (35.0)	14 (35.0)
Chronic kidney disease	5 (26.3)	12 (29.3)	6 (30.0)	10 (23.8)
Laboratory				
Glomerular filtration rate (mL/min/m^2)	85.2 [41.0–95.1]	74.6 [51.2–88.6]	73.0 [54.1–96.4]	78.1 [58.3–91.3]
NT-proBNP (pg/mL)	4215 [2184–6093]	3437 [1924–6214]	3830 [1957–9356]	2818 [1441–5110]
cTnT (ng/mL)	27.0 [17–42]	23.0 [16–37]	19.0 [14–36]	24.8 [14–38]
Medications[a]				
ACEi	10 (52.6)	25 (61.0)	13 (65.0)	27 (67.5)
ARB	6 (31.6)	11 (26.8)	5 (25.0)	6 (15.0)
Beta-blockers	16 (84.2)	32 (78.1)	15 (75.0)	30 (75.0)
MRA	18 (94.7)	37 (90.2)	20 (100)	39 (97.5)
Diuretic	19 (100)	41 (100)	20 (100)	40 (100)
Digitalis glycoside[b]	3 (15.8)	11 (26.8)	9 (45.0)	12 (30.0)
Echocardiography				
LV end-diastolic volume index (mL/m^2)	127.8 ± 39.9	126.1 ± 32.6	123.2 ± 49.4	118.7 ± 36.2
LV end-systolic volume index (mL/m^2)	97.0 ± 37.1	92.2 ± 28.1	90.5 ± 40.6	85.6 ± 32.1
LV ejection fraction (%)	25.4 ± 7.3	27.4 ± 6.7	27.0 ± 7.8	28.7 ± 7.2
S′ (cm/s)	3.50 ± 0.78	3.66 ± 1.07	3.73 ± 1.08	3.94 ± 1.09
Stroke volume index (mL/beat/m^2)	24.29 ± 7.93	25.88 ± 7.10	19.0 ± 6.7	17.11 ± 5.69
E/e' ratio	19.45 ± 5.82	20.44 ± 7.87	14.62 ± 3.58	15.14 ± 5.48
E/A ratio	2.62 ± 1.17	2.54 ± 1.09	2.34 ± 1.13	2.08 ± 0.92
Left atrial area (cm^2)	27.8 ± 4.14	28.5 ± 5.36	24.7 ± 6.03	25.9 ± 7.05
Left atrial volume index (mL/m^2)	55.6 ± 12.1	58.3 ± 16.6	50.1 ± 17.2	52.3 ± 21.0
Mitral regurgitation, severe	9 (47)	17 (44)	7 (37)	19 (49)
IVC diameter (mm)	19.9 ± 3.6	21.2 ± 4.6	18.7 ± 5.2	19.5 ± 5.3

Data are shown as mean ± standard deviation, *n* (%), or median [interquartile range].
ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; cTnT, cardiac troponin T; IVC, inferior vena cava; LV, left ventricular; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal brain natriuretic peptide; VAS, visual analogue scale.
[a]Medications are those taken prior to the study and may have been maintained through study treatment.
[b]Refers to patients treated before randomisation. Digitalis glycosides were not allowed during the study.
All intracohort comparisons are not significant.

the parameters related with left ventricular systolic function, stroke volume index increased with istaroxime compared to placebo, though the increase in LVEF did not reach statistical significance. These effects were accompanied by a decrease in HR and an increase in SBP, which reached statistical significance with the 1.0 μg/kg/min dose. These latter changes occurred early after the start of the infusion, with significant differences already evident at 3 h. This is consistent with the pharmacokinetic profile of istaroxime, which has a very rapid onset of action and a similar fast washout after infusion termination, making this drug suitable for the use in high-risk patients and potentially in critically ill patients. As observed in this study, possible rebound effects should be taken into account, since a mild increase in HR was present at 48 h in the high-dose group. This would probably require a down-titration phase before withdrawal in patients receiving high dose in future studies.

Table 2 Echocardiographic parameters change at 24 h

Variable	Placebo cohort 1 ($n = 19$)	Istaroxime 0.5 µg/kg/min ($n = 41$)	*P*-value	Placebo cohort 2 ($n = 20$)	Istaroxime 1.0 µg/kg/min ($n = 40$)	*P*-value
LV end-diastolic volume index (mL/m^2)	$n = 19$ 0.53 ± 6.94	$n = 38$ -0.20 ± 5.45	0.668	$n = 18$ -4.34 ± 9.88	$n = 33$ -2.08 ± 9.05	0.414
LV end-systolic volume index (mL/m^2)	$n = 19$ -0.97 ± 5.26	$n = 38$ -3.78 ± 6.76	0.118	$n = 18$ -6.53 ± 9.83	$n = 33$ -6.23 ± 9.23	0.914
LV ejection fraction (%)	$n = 19$ 1.26 ± 2.54	$n = 38$ 3.03 ± 4.04	0.089	$n = 18$ 2.83 ± 4.20	$n = 33$ 3.82 ± 4.14	0.423
Stroke volume index (mL/beat/m^2)	$n = 17$ 1.65 ± 2.76	$n = 34$ 5.33 ± 6.67	**0.034**	$n = 19$ 3.18 ± 4.02	$n = 34$ 5.49 ± 4.98	0.090
E/e′ ratio	$n = 17$ -1.55 ± 4.11	$n = 37$ -4.55 ± 4.75	**0.029**	$n = 19$ -1.08 ± 2.72	$n = 33$ -3.16 ± 2.59	**0.009**
E/A ratio	$n = 9$ 0.13 ± 0.98	$n = 16$ -0.64 ± 0.79	**0.042**	$n = 12$ 0.18 ± 0.80	$n = 22$ -0.65 ± 1.10	**0.029**
Left atrial area (cm^2)	$n = 19$ -0.36 ± 1.33	$n = 35$ -1.70 ± 2.46	**0.032**	$n = 18$ -0.22 ± 2.32	$n = 33$ -2.21 ± 3.18	**0.024**
Left atrial volume index (mL/m^2)	$n = 19$ -0.46 ± 3.86	$n = 35$ -4.32 ± 7.28	**0.037**	$n = 18$ -2.65 ± 7.46	$n = 33$ -7.32 ± 10.0	0.091
S′ (cm/s)	$n = 18$ 0.11 ± 0.85	$n = 39$ 0.68 ± 0.91	**0.029**	$n = 19$ 0.16 ± 1.18	$n = 34$ 0.81 ± 1.01	**0.039**

Data are shown as mean ± standard deviation.
LV, left ventricular.

The overall diuresis during the infusion period was higher in patients treated with istaroxime, consistently with the change in E/e' ratio. A favourable effect on renal function was observed with istaroxime, with an increase in eGFR with the high dose compared to placebo at 24 h. Absolute troponin levels did not differ between the istaroxime and placebo groups, and no major untoward effects, including no increase in arrhythmias, were observed. Despite being frequent, especially with high-dose istaroxime, gastrointestinal symptoms required stopping of infusion only in one patient due to nausea and in a second patient due to vomiting which occurred concomitantly to a SAE (renal artery embolism attributed to a pre-existing ventricular thrombus). The proportion of patients experiencing injection site reactions was high, requiring the change of the peripheral line or a shift to a peripheral long line or a central line. However, only one patient (treated with istaroxime 1.0 µg/kg/min) discontinued the study drug due to this AE. These findings support the hypothesis that despite frequent side effects, the overall tolerability of istaroxime was acceptable.

Our results confirm and extend to a 24 h infusion the findings of HORIZON-HF, where a 6 h istaroxime infusion was tested.[15,16] Different from HORIZON-HF where patients underwent right heart catheterisation, we used a non-invasive assessment based on Doppler echocardiography for practical purposes. The assessment of the effects of a 24 h infusion was mandatory for the clinical development of this drug, as a short-term 6 h administration is unlikely to be sufficient for symptom relief and haemodynamic stabilisation in clinical practice. Our results demonstrated persistent and adjunctive favourable haemodynamic effects of istaroxime at 24 h and also provide more data on drug safety.

Treatment of patients hospitalised for AHF with low or borderline SBP remains a major unmet need. Low SBP identifies patients who are at an extremely high risk of death.[4,21,22] No safe and effective therapy has emerged for these patients, and inotropes have been associated with major cardiovascular AEs and possibly increased mortality.[1–3,6,8,21,23] The mechanism of action and the haemodynamic effects of istaroxime provide the potential for improving clinical outcomes in these patients. Istaroxime has favourable effects on left ventricular diastolic and systolic function without increasing free cytoplasmic calcium levels, the possible mechanism of the arrhythmogenic and toxic effects of digoxin, catecholamines, and phosphodiesterase inhibitors on cardiac myocytes.[6,8,14] A sustained increase in SBP was observed with both doses of istaroxime, reaching significance with the higher dose. Together with improvement in renal function, these effects suggest better organ perfusion, making the drug potentially attractive for the treatment of patients with hypotension and low cardiac output. These haemodynamic effects appear to be obtained with no increase in HR, whereas tachycardia caused by traditional inotropic agents is a major determinant of increased myocardial oxygen consumption and ischaemia.[6,21] In addition, no clinical events related to myocardial ischaemia with istaroxime, compared to placebo, were observed in our study.

Limitations

The major limitation of this study relates to enrolment discrepancies between the study populations and sites of the two cohorts, with cohort 2 enrolling exclusively Asian patients. We observed

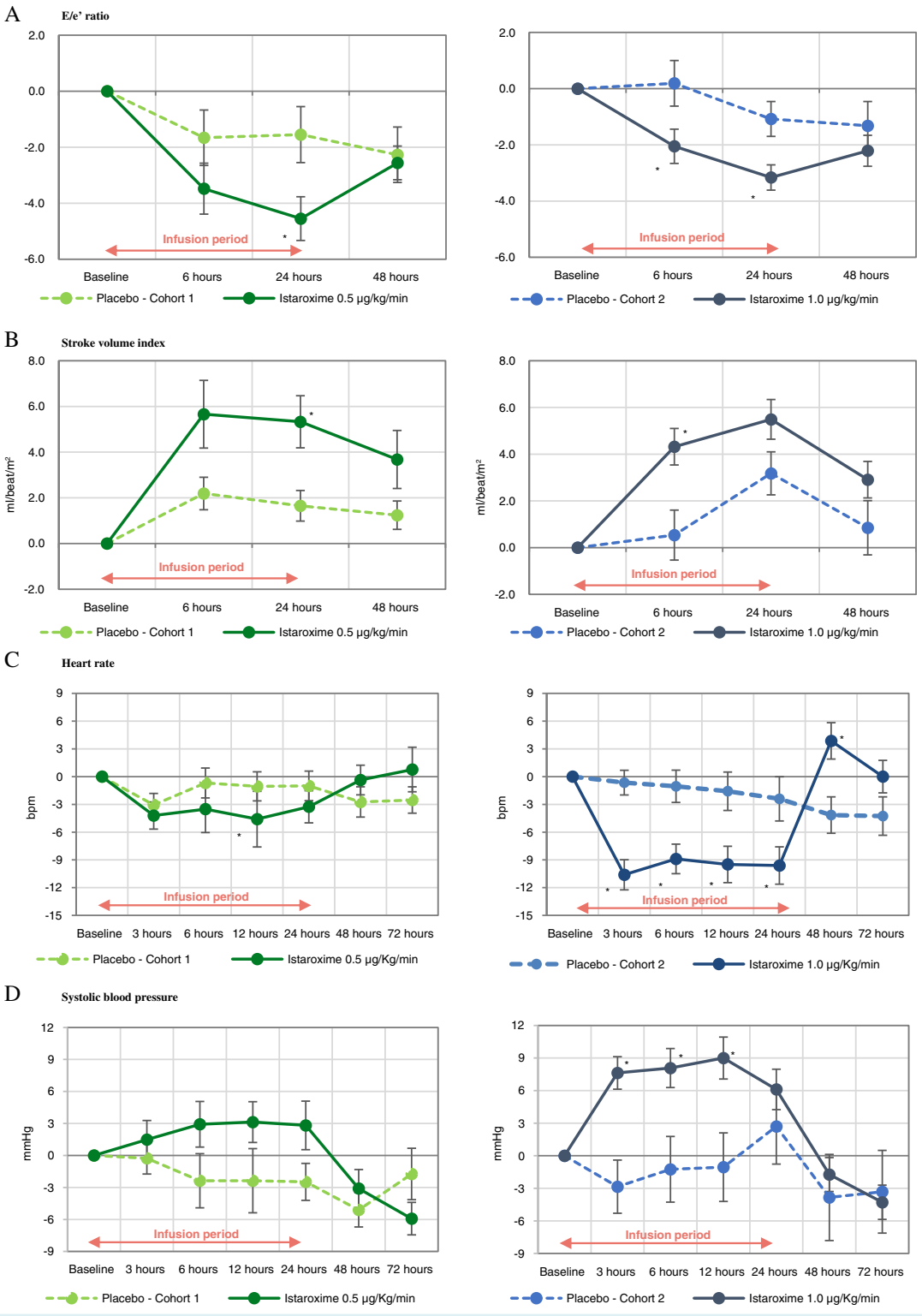

Figure 2 Changes in echocardiographic and haemodynamic parameters: (*A*) E/e' ratio, (*B*) stroke volume index, (*C*) heart rate, (*D*) systolic blood pressure. Data are shown as mean change from baseline. *$P<0.05$.

Table 3 Diuresis over 24 h during and after study drug infusion

	Placebo cohort 1 (*n* = 19)	Istaroxime 0.5 µg/kg/min (*n* = 41)	Placebo cohort 2 (*n* = 20)	Istaroxime 1.0 µg/kg/min (*n* = 40)
0–24 h (mL)	*n* = 14	*n* = 30	*n* = 20	*n* = 38
	1850 [1050–2530]	2700 [2000–3000]	1515 [1130–1965]	1975 [1530–2750]
24–48 h (mL)	*n* = 14	*n* = 29	*n* = 19	*n* = 33
	2300 [1100–2850]	1800 [1200–2300]	1820 [1200–2000]	1260 [1010–1450]
48–72 h (mL)	*n* = 13	*n* = 30	*n* = 19	*n* = 33
	2000 [1500–2600]	1650 [1000–2000]	1810 [1230–2290]	1350 [1000–1750]

Data are shown as median [interquartile range].

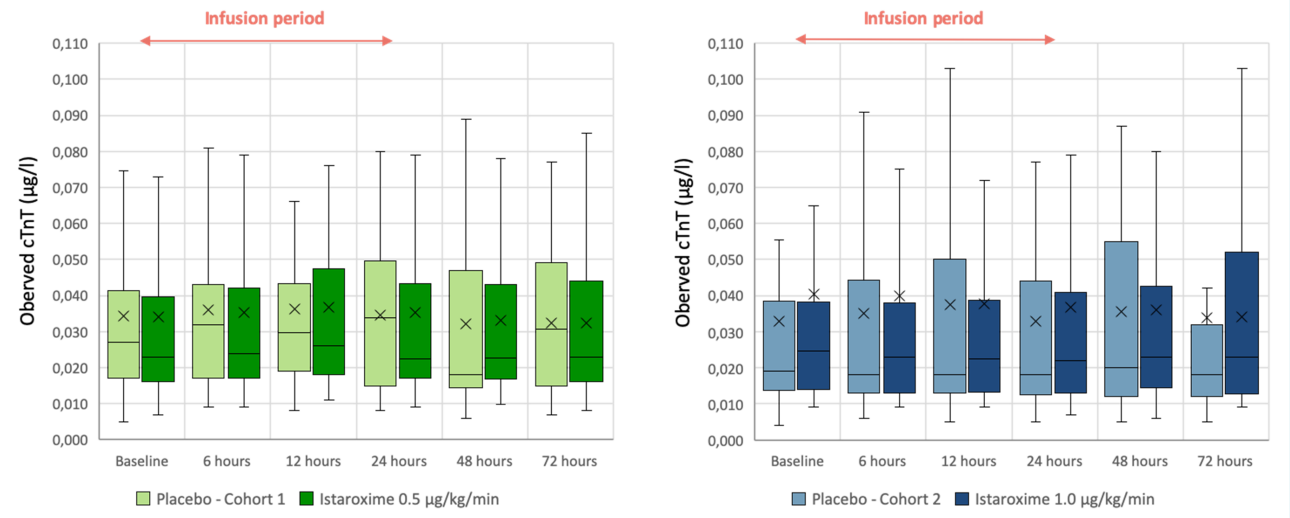

Figure 3 Box and whisker plot for cardiac troponin T (cTnT).

Table 4 Adverse events

Event	Pooled placebo (*n* = 39)	Istaroxime 0.5 µg/kg/min (*n* = 41)	Istaroxime 1.0 µg/kg/min (*n* = 40)
All adverse events	23 (59.0)	31 (75.6)	33 (82.5)
Adverse events leading to discontinuation	1 (2.6)	–	4 (10.0)
Serious adverse events	2 (5.1)	2 (4.9)	6 (15.0)
Cardiac death	–	–	1 (2.5)
Cardiogenic shock	–	–	1 (2.5)[d]
Cardiac failure	1 (2.6)	2 (4.9)	3 (7.5)
Renal embolism	–	–	1 (2.5)
Transient ischemic attack	1 (2.6)	–	–
Hyperventilation	1 (2.6)	–	–
Hypotension	1 (2.6)	–	–
Adverse drug reactions[a]	10 (25.6)	23 (56.1)	25 (62.5)
Cardiovascular[b]	9 (23.1)	4 (9.8)	7 (17.5)
Gastrointestinal[c]	2 (5.1)	4 (9.8)	14 (35.0)
Infusion site pain/inflammation	–	20 (48.8)	13 (32.5)

Data are shown as number of patients (%). Patients can have more than one event during the 30-day follow-up period.
[a]Adverse events related to study drug.
[b]Most common: arrhythmia, atrial fibrillation, cardiac failure, ventricular tachycardia.
[c]Most common: abdominal pain, nausea, vomiting, diarrhoea.
[d]Same patient who then died.

that patients enrolled in this trial were younger and had better renal function compared with other studies.[22] Of note, an interim analysis showed similar echocardiographic characteristics between different ethnic groups within cohort 1. Although variation in AHF patients' management cannot be excluded, these differences may have had a limited impact on the primary endpoint and our results are consistent with previous data from the HORIZON-HF trial. In addition, the efficacy and safety analyses were performed by blinded central laboratories with a robust and uniform methodology and our results showed similar effects of istaroxime in both cohorts. Istaroxime promoting SERCA2a activity has a direct effect on a pathophysiological pathway that may represent the common cardiac mechanism at the basis of heart failure progression, thus overcoming the heterogeneity in AHF populations, which is probably one of the most important causes of the failure of several investigational drugs in this area.

Our study was powered to detect a significant change in the E/e' ratio at 24 h with istaroxime vs. placebo. Estimation of the size of the study group was based on previous data,[15] and the study achieved its primary endpoint. However, other parameters such as LVEF and left ventricular volumes may have a larger variability with echocardiography compared to the invasive assessment, and/or are less affected by a 24 h infusion of the study drug. A larger study group would be required to ascertain istaroxime effects on these parameters. In addition, enrolling patients closer to admission will likely be required to show istaroxime effects on the patient symptoms and clinical course.

Conclusions

This randomised, double-blind, placebo-controlled, phase II trial shows that a 24 h infusion of istaroxime in patients hospitalised for AHF improves parameters of left ventricular systolic and diastolic function, with a decrease in HR and maintenance or increase of SBP at low and high dose, respectively. This study represents a proof-of-concept that SERCA2a stimulation is a novel and valid target for the treatment of patients with AHF. Despite most clinical trials in this clinical context failed to confirm preliminary results in larger trials, this drug may have potential benefits in a more selected population of patients with low blood pressure and low cardiac output, which represents a high-risk group with poor outcomes and no evidence of safe and effective treatments. Further research evaluating istaroxime effects on clinical symptoms, clinical course, and outcomes is warranted in such targeted population.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Committees, investigators and central laboratories.

Table S1. Hemodynamic and echocardiographic parameters at baseline and changes during and after study drug infusion.

Table S2. Secondary endpoints – changes from baseline during and after study drug infusion.

Table S3. Changes in cardiac troponin T and estimated glomerular filtration rate during and after study drug infusion.

Table S4. Troponin percent change from baseline analysis in the safety population.

Table S5. Holter monitoring parameters.

Figure S1. Istaroxime mechanism of action.

Figure S2. Study flow-chart.

Figure S3. Bland–Altman plots for intraobserver and interobserver variation in E/e' measurement.

Figure S4. Individual changes from baseline to 24 h in (*A*) E/e' ratio, (*B*) stroke volume index, (*C*) left atrial volume index, (*D*) heart rate, and (*E*) systolic blood pressure.

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Conflict of interest: V.C. received consulting honoraria from CVie Therapeutics Limited. M.M. reports personal consulting honoraria from Bayer, Novartis, Fresenius, Servier, and Windtree Therapeutics for participation to advisory board meetings and executive committees of clinical trials. G.M.F. has received research grants from NHLBI, American Heart Association, Amgen, Merck, Cytokinetics, and Roche Diagnostics; he has acted as a consultant to Windtree Therapeutics, Novartis, Amgen, BMS, Medtronic, Cardionomic, Relypsa, V-Wave, Myokardia, Innolife, EBR Systems, Arena, Abbott, Sphingotec, Roche Diagnostics, Alnylam, LivaNova, Rocket Pharma, and SC Pharma. G.F. participated in committees of trials and/or registries sponsored by Medtronic, Novartis, BI, Vifor. C.M.O'C. worked as consultant for Merck, Bayer, BMS, Magenta, Windtree Therapeutics, NHLBI, FDA. J.R.T. has received research grants and/or consulting fees from Abbott, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, BMS, Cytokinetics, Medtronic, Merck, Novartis, and Windtree Therapeutics, Inc. P.S., R.S., and G.B. are employees of Windtree Therapeutics, Inc. B.L. is employee of Lee's Pharmaceutical Limited, Taipei; CVie Therapeutics Limited as subsidiary. L.F.L is employee of CVie Therapeutics Limited. All the other authors have nothing to disclose.

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