2 SYNOPSIS

Sponsor/company **Orion Corporation** **Orion Pharma**	**Individual study table referring to a specific part of the dossier** **Volume:** **Page**	(for National Competent Authority use only)
Finished product: Not applicable		
Active ingredient: Levosimendan		
Study code: 3001088		
Study title: Effects of oral levosimendan on ambulatory electrocardiographic variables and cerebrovascular reactivity in patients with recent stroke or TIA. A randomised, double blind, placebo-controlled, dose finding, multicentre study with parallel group design		
Investigators and study centres: The study was conducted at 7 centres: 2 in Finland, 1 in Germany, 1 in Hungary and 3 in Sweden. The coordinating investigator of the study was Risto O. Roine at the Turku University Hospital in Finland. Appendix 16.1.4 contains a list of study centres and CVs of the investigators.		
Development phase: II	**Study period:** 20 Oct 2008 - 17 Sep 2009	
Objectives: The primary objective was to explore the safety of low doses of oral levosimendan in patients with recent history of ischemic cerebrovascular event (stroke or transient ischemic attack [TIA]). The main focus was on the evaluation of proarrhythmic potential of the different dose regimens. The secondary objectives were: • to evaluate the effect of oral levosimendan on cerebrovascular reactivity • to evaluate the effect of oral levosimendan on biomarkers • to evaluate the effect of oral levosimendan on thrombocyte aggregation • to study the pharmacokinetics (PK) of different doses of levosimendan.		
Methodology: This was a prospective, multicentre, phase II, randomised, double-blind, placebo-controlled 2-arm parallel group study. In the double-blind phase 5 escalating doses of oral levosimendan or placebo were given for maximum of 18 days in 5 treatment periods. The double-blind phase was preceded by a maximum of 18-day long single-blind treatment with placebo (placebo run-in). The study consisted of 9 visits (screening visit, 7 visits during the treatment periods and an end-of-study visit). Each subject was on the study treatment for 78-108 days, including the maximum of 18-day long treatment with placebo (placebo run-in). The duration of the study for each subject including the screening period and the end-of-study visit was approximately 17 weeks.		
Sample size: The planned number of subjects was approximately 30. A total of 21 subjects were included in the study. The subjects were planned to be randomised to levosimendan and placebo treatments in the ratio of 2:1, but because the recruitment was discontinued prematurely, the final ratio was 3:1. 16 subjects received levosimendan and 5 subjects received only placebo during the study. The number of subjects per centre was 1-6.		
Diagnosis and main criteria for inclusion: Male and female patients 50-80 years of age with ischaemic stroke or TIA within 1-9 months before the screening visit. Maintenance treatment with an angiotensin II receptor blocker (ARB) or angiotensin converting enzyme inhibitor, cholesterol lowering agent (preferably 3-hydroxy-3-methyl-glutaryl-Coenzyme A [HMG-CoA] reductase inhibitor) and antiaggregatory agent, started at least 1 month before the screening visit, written informed consent (IC) obtained. Main criteria for exclusion were: • Stroke or TIA due to cardiac embolism, vasculitis or arterial dissection.		

• Severe hemiparesis or dysphasia inhibiting the ability to fully comply with the study protocol requirements. • Haemodynamically significant uncorrected valve disease or hypertrophic cardiomyopathy or restrictive cardiomyopathy. • Acute myocardial infarction or any other acute coronary event within 1 month before the screening visit. • Cardiovascular surgery (e.g. carotid endarterectomy, coronary by-pass) or angioplasty or any other major surgery within 1 month before the screening visit. • Patients who were scheduled for coronary by-pass or angioplasty, or carotid endarterectomy or any major surgery during the planned study period. • History of life-threatening ventricular arrhythmia within 3 months before randomisation defined as an episode of resuscitated sudden death, ventricular fibrillation (VF) or sustained or haemodynamically destabilising ventricular tachycardia (VT). • History of Torsades de Pointes (TdP) or family history of long QT-syndrome. • Anticipated technical problems in transcranial doppler (TCD) assessments (e.g. poor insonation of the temporal bone window, patient's inability to hold breath for at least 30 seconds). • Heart rate (HR) < 50 or > 100 bpm in the 12-lead electrocardiogram (ECG) or as an average in the 24-h ambulatory Holter recording at screening. • Systolic blood pressure (SBP) < 100 mmHg or > 180 mmHg, or diastolic blood pressure (DBP) > 100 mmHg at screening. • VT (wide complex tachycardia > 100/min, ≥ 3 consecutive beats) in the 24-h ambulatory Holter recording at screening. • Episode of atrial fibrillation (AF) or atrial flutter lasting > 60 seconds in 24-h ambulatory Holter recording at screening. • Second or third degree atrioventricular (AV) block in the 12-lead ECG or in the 24-hambulatory Holter recording at screening. • Potassium (K) < 3.7 mmol/l or > 5.5 mmol/l at screening. • Creatinine > 170 µmol/l at screening or on dialysis. • Blood haemoglobin < 10 g/dl at screening. • Clinically significant hepatic impairment at the discretion of the investigator.
Investigational product, dose and mode of administration, batch numbers: Levosimendan 0.125 mg, 0.25 mg, 0.50 mg and 1.0 mg immediate-release capsules for oral administration. Doses administered were 0.125, 0.25, 0.5, 1 and 2 mg. Batch numbers were KA004L2 for 0.125 mg, IE006L2 for 0.25 mg, HM005L2 for 0.5 mg and HM006L2 for 1 mg capsules.
Duration of treatment: 5 escalating doses of oral levosimendan or placebo were given once daily for maximum of 18 days.
Reference product, dose and mode of administration, batch number: Placebo capsules for oral administration. Batch number was HM004L2.
Variables and methods of assessments: Primary safety variable: VES/h in 24-h ambulatory ECG (Holter). Other safety variables in the 24-h ambulatory ECG (Holter): • Prevailing rhythm (sinus, AF/flutter, other) • Mean HR as beats per minute (bpm) using the total number of QRS complexes during the recording • VT:

- sustained (wide complex tachycardia > 100/min for > 30 seconds):
  - number of episodes during the recording
- non-sustained (wide complex tachycardia > 100/min and ≥ 3 consecutive beats, but duration < 30 seconds)
  - number of episodes during the recording
- frequency during the fastest episode
- duration of the longest episode
- type of VT (monomorphic, polymorphic)
- Torsades de Pointes (TdP)

- Ventricular fibrillation
- Supraventricular extrasystoles (SVES):
  - SVES/h calculated as the total number of SVES divided by the duration (in hours) of the recording
- Supraventricular tachycardia (SVT) (≥ 3 consecutive beats, frequency > 100/min):
  - number of episodes during the recording
  - frequency during the fastest episode
  - duration of the longest episode
- AF:
  - total duration during the recording
  - number of episodes during the recording
- Atrial flutter:
  - total duration during the recording
  - number of episodes during the recording
- AV-blocks:
  - second degree (Wenkebach = Mobitz I)
  - second degree (Mobitz II)
  - third degree
- Longest RR interval:
- Other abnormalities
  - as specified by the analyst

Pharmacodynamic variables:

- Cerebrovascular reactivity to hypercapnia was assessed by means of breath holding index (BHI) using TCD ultrasonography.
- Biomarkers:
  - N-terminal-pro-B-type natriuretic peptide (NT-pro-BNP)
  - High sensitivity C-reactive protein (hs-CRP)
  - Thrombocyte aggregation

PK variables: the steady state concentrations of OR-1855 and OR-1896 were determined. The effect of acetylation status on the concentrations of these metabolites was also determined.

Other safety variables: Adverse events (AEs), 12-lead ECG, vital signs, laboratory safety assessments and physical examination.

Statistical methods:

Primary safety evaluation

The number of VES/h in the 24-h ambulatory Holter was the primary safety variable in this study. The Holter

recording of the placebo-run in period was used as a baseline from where all changes were calculated.

Ventricular arrhythmias

Number of VES/h was summarised as absolute values and change from baseline for each visit by randomisation group, using descriptive statistics. Repeated measures (RM) analysis of covariance (ANCOVA) model with randomisation group, stratification variables and centre as between factors, period as within and baseline value as covariate, was used to evaluate the differences between randomisation and treatment groups. Treatment group by period interaction was included in statistical model to evaluate dose response. Contrasts were used to evaluate the safety of each dose level. In statistical analysis log-transformed data were used to ensure normality.

Fastest VT, duration of the longest VT and number of different types of VTs (non-sustained and sustained VTs) were summarised and analysed using similar to VES/h.

Number and percentage of subjects with VTs, VFs and possible other ventricular abnormalities were tabulated for all treatment periods by randomisation group and grade. Comparisons between randomisation groups were performed using Chi-square test.

Other safety variables in the 24-h ambulatory ECG:

- Rhythm and conduction disturbances: number and percentage of subjects with AV-blocks was tabulated for all treatment periods by randomisation group and grade. Comparisons between the randomisation groups were performed using Chi-square test.
- Mean HR during total registration, minimum frequency and maximum frequency were evaluated. Data were summarised and analysed using methods similar to VES/h.
- Supraventricular arrhythmias: number and percentage of subjects with AF and runs of atrial or SVT were tabulated for all treatment periods by randomisation group. Comparisons between randomisation groups were performed using Chi-square test. Fastest frequency, longest duration of supraventricular arrhythmias and total number of SVT runs were summarised and analysed using similar to VES/h.

Pharmacodynamic evaluations:

- The BHI data from TCD ultrasonography was summarised for all treatment periods using descriptive statistics. Repeated measures analysis of variance (RM-ANOVA) model with randomisation group, stratification variables and centre as between factors and period as within factor, was used for primary comparison. Treatment group by period interaction was included in statistical model to evaluate dose response. Contrasts were used to make inferences for paired comparisons, i.e. levosimendan 0.125 mg vs. placebo, levosimendan 0.25 mg vs. placebo, levosimendan 0.5 mg vs. placebo, levosimendan 1 mg vs. placebo and levosimendan 2 mg vs. placebo.
- NT-pro-BNP concentration, hs-CRP and thrombocyte aggregation were summarised for all treatment periods using descriptive statistics. Assessment at day 12 was considered as baseline and median changes from baseline were reported for each of the treatment. Non-parametric Kruskal-Wallis test was used for comparisons against placebo.

No adjustment for multiplicity was made due to explorative nature of analysis, and all comparisons reaching two-sided 10% significance level were considered significant.

PK evaluation:

OR-1855 and OR-1896 steady state concentrations for all treatment periods were summarised using descriptive statistics. In addition, the concentrations were summarised by acetylator status within each treatment period.

Pharmacogenetic (PG) evaluations:

The results of any PG analysis will be reported in a separate report.

Evaluation and analysis of other safety and tolerability:

- AEs and serious adverse events (SAEs) reported during the study were classified by System Organ Classes (SOCs) and preferred terms using the Medical Dictionary for Regulatory Activities (MedDRA). The number and proportion (%) of subjects having each AE were given by treatment

period using onset period of the AE. The numbers and proportions were additionally broken down by severity and by the causality. The incidence rate of AEs and SAEs was compared between randomisation groups using Fisher's Exact test. Where there were multiple MedDRA terms describing very similar or apparently identical clinical scenarios these were combined in separate listings. SAEs and other significant AEs were evaluated case by case. Additionally, narrative descriptions are included in the study report for all SAEs and for certain other significant AEs. AEs occurring before and after the initiation of study treatments were reported separately.

- Clinical safety evaluations:
  - Mean SBP and DBP were summarised as absolute values and change from baseline for each visit by randomisation group, using descriptive statistics. RM ANCOVA model with treatment as between factor, period as within and baseline value as covariate was used to evaluate the differences between randomisation and treatment groups. Treatment group by period interaction was included in the statistical model to evaluate dose response.
  - From the 12-lead ECG recordings mean QT, QT corrected for HR (QTc), PR, QRS and RR intervals were summarised as absolute values and change from baseline for each visit by randomisation group using descriptive statistics. RM ANCOVA model with treatment as between factor, period as within and baseline value as covariate was used to evaluate differences between randomisation and treatment groups. Treatment group by period interaction was included in statistical model to evaluate dose response. In addition, categorical analyses using Chi-square test were performed for QTc interval. Categorical analyses involved absolute values at treatment period of QTc > 450 ms, > 480 ms or > 500 ms and increase from baseline of QTc ≥ 30 ms or ≥ 60 ms. Subjects with non-sinus rhythm could be excluded from analyses.
- Laboratory safety was evaluated between the randomisation groups. Variables were reported as absolute values and change from screening to end of study, using descriptive statistics. ANCOVA model was used to compare differences between randomisation groups, with the baseline value as the covariate and main effect for treatment. Sodium, potassium, creatinine and haematocrit were assessed for all treatment periods. For these safety laboratory variables additional analysis changes from baseline were compared between corresponding oral levosimendan dose and placebo using RM ANCOVA model with treatment as between factor, period as within and baseline value as covariate. Treatment group by period interaction were included in statistical model to evaluate dose response.
- Tolerability was evaluated e.g. by the occurrence of AEs, by abnormal findings in the safety laboratory or ECG variables (12-lead ECG and 24-h ambulatory Holter) and changes in vital signs. Of special interest were the AEs leading to study discontinuation. The eventual increases in VES/h, HR or other abnormalities, even if asymptomatic, were used in the assessment of tolerability.

Summary-Conclusions

Demography and other baseline characteristics: 21 subjects were included in the study, 16 in the levosimendan group and 5 in the placebo group. 17 subjects completed the study. The study population consisted of both male and female subjects, with mean age of 65 years. All subjects were Caucasian. The treatment groups were comparable at baseline.

Efficacy results:

The mean number of VES/h at baseline (period 1; placebo run-in) seemed to differ between the treatments: in the levosimendan group the mean number of VES/h (SD) was 21.8 (45.3) and in the placebo group 3.0 (3.8). In the placebo group the number of VES/h was fairly constant during the whole study period, the median varying between 0.8 and 3.7 at the different time points (the mean value varied correspondingly between 1.5 and 6.6). In the levosimendan group, no definite trend in the median values of the number of VES/h could be observed. The median varied between 1.5 and 3.2, the highest value being recorded at baseline. The corresponding mean values varied between 9 and 23 in the levosimendan group, with an unexpected increase at baseline (placebo run-in). The unexpected increase at baseline in the levosimendan group (distorting the data) was totally attributed to 2 subjects with an exceptionally large number of VES/h (135 and 138, respectively).

It is noteworthy that during the study period the maximum number of VES/h was consistently much higher in the levosimendan group compared with the placebo group (the maximum number varied between 43 and 167 in the levosimendan group, and between 5 and 22 in the placebo group at different time points).

To assess the safe dose levels of levosimendan, the baseline-adjusted number of VES/h at each dose level were compared to the corresponding values in the placebo group. All dose levels up to 1 mg seem safe, but 2 mg means an increased risk. However, this is to a large extent attributed to the fact that the estimate for the placebo for 2 mg is markedly lower than the other placebo estimates, which leads to a higher ratio with 2 mg. The model based estimates for levosimendan show that the value with 2 mg is markedly higher compared to the lower doses of levosimendan which strengthens the conclusion that 2 mg dose of levosimendan is not considered safe for long-term treatment in this patient population.

In the levosimendan group, the mean HR was about 72 bpm at baseline and with 0.125 and 0.25 mg doses. Thereafter the mean HR increased to 77, 78 and 80 bpm with 0.5, 1 and 2 mg doses, respectively. In the placebo group, the mean HR varied between 69 and 80 bpm at different time points, without any definite trend. At the dose 0.5 mg and above, the difference between levosimendan and placebo was statistically significant

In the levosimendan group the median NT-pro-BNP values decreased from baseline during all treatment periods by 28-58% except during the end-of-study visit, when the median NT-pro-BNP increased by 1.6% from baseline. There was a statistically significant difference in change from baseline between the treatments with levosimendan doses 0.125, 1 and 2 mg.

Cerebral blood flow and cerebrovascular reactivity to hypercapnia was measured by TCD from the middle cerebral artery. Both the peak systolic flow velocities and the end diastolic flow velocities were significantly higher in subjects in the levosimendan group at rest. The difference in end diastolic flow was higher with the lowest doses of levosimendan and the difference was statistically significant. Similar results were obtained for the mean blood flow velocities at rest, which were significantly higher in subjects treated with levosimendan doses of 0.125 mg and 0.25 mg only. There were also statistically significant differences between the centres in peak systolic flow velocity, end diastolic flow velocity and mean blood flow velocity.

In the 30 second breath holding (BH) tests, the subjects in the levosimendan group showed constantly higher values in peak systolic flow velocities and end diastolic velocities at the end of the 30 second BH period. For peak systolic flow velocity, this difference was statistically significant for 0.125 mg, 0.25 mg, 1 mg and 2 mg. For end diastolic flow velocities, the difference was statistically significant for 0.125 mg, 0.25 mg and 2 mg doses. A constant trend in favour of levosimendan in both velocities was observed with all doses without any distinct dose response. In the mean blood flow velocity after BH there were no statistically significant differences between levosimendan and placebo.

In the statistical analysis of the BHI there was no difference with any dose of levosimendan as compared with placebo.

Safety results: Altogether 74 AEs were reported in 19 subjects during the study. 64 of the AEs were reported in the levosimendan group and 10 AEs in the placebo group. In addition, 19 AEs were reported before start of study treatment and during the placebo run-in period. Most of the AEs were assessed as mild. 5 AEs were assessed as severe (3 in the levosimendan group and 2 in the placebo group) and 8 AEs were assessed as moderate (6 in the levosimendan group and 2 in the placebo group). The investigators assessed 27 AEs as related to levosimendan and 6 AEs as related to placebo.

There were 6 SAEs reported in 3 subjects during the study: 1 subject in levosimendan group had 3 SAEs and 1 subject had 1 SAE, and 1 subject in the placebo group had 2 SAEs.

There were no significant differences between levosimendan and placebo in the percentage of subjects reporting AEs, discontinuing due to AEs or reporting SAEs. There were no clear differences either in the nature of reported AEs between the treatments. Most of the AEs were reported as mild.

Regarding vital signs, higher doses of levosimendan (1 and 2 mg) decreased SBP and/or DBP by approximately 5-7 mmHg compared with baseline with some statistically significant changes compared with placebo as well. 12-lead ECG findings did not differ between the treatments and the abnormalities seen were not considered clinically significant. Levosimendan decreased statistically significantly some conduction parameters (e.g. PR and QT intervals) compared with baseline and/or placebo. This was seen most often with

the 2 mg dose of levosimendan. The shortening of PR and QT intervals was most likely due to the HR increasing effect of levosimendan and was of no clinical relevance. None of the subjects had QTc increase > 60 ms from baseline.

There were no clinically significant changes in the mean laboratory values during the study.

Conclusion: The main objective of the present study was to identify doses of oral levosimendan that would be considered safe for further studies in patients with recent cerebral ischemia. This study showed that levosimendan doses higher than 0.25 mg significantly increased HR, which would probably be unfavourable during chronic treatment. However, from a strictly proarrhythmic point of view, daily doses of up to 1 mg seemed to be safe. On the other hand, already lower doses of levosimendan (0.125 and 0.25 mg) had positive pharmacodynamic effects as indicated by decreased levels of NT-pro-BNP and increased cerebral blood flow. It is concluded that, based on the present results, levosimendan doses of 0.125 and 0.25 mg (and possibly also 0.5 mg dose) could be studied further in the secondary prevention of ischemic stroke.

Date of report: 21 Dec 2009