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Title:	Effects of oral levosimendan (ODM-109) on respiratory function in patients with ALS
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CLINICAL STUDY PROTOCOL – AMENDMENT 3

[redacted]

EFFECTS OF ORAL LEVOSIMENDAN (ODM-109) ON RESPIRATORY FUNCTION IN PATIENTS WITH ALS

Study code:	3119002
Study design:	Randomised, double-blind, placebo-controlled, parallel-group, multicentre study
Short study title:	REFALS
Phase:	III
Standard:	GCP
EudraCT number:	2017-002754-36
IND number:	134169
Study Coordinating Investigator:	Merit Cudkowicz, MD
Sponsor:	Orion Corporation Orion Pharma Orionintie 1, FI-02200 Espoo, Finland
Date:	26 March 2020

[redacted]

Sponsor approvals:

[redacted]

[redacted]

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SYNOPSIS

Sponsor: Orion Corporation Orion Pharma
Finished product: Not applicable
Active pharmaceutical ingredient: Oral levosimendan (ODM-109)
Study title: Effects of oral levosimendan (ODM-109) on respiratory function in patients with ALS
Study code: 3119002
Investigator: The study coordinating investigator is Merit Cudkowicz.
Study centres: This will be a multinational study in approximately 100 centres.
Development phase: III
Objectives: The primary objective of this study is to confirm that levosimendan can significantly improve respiratory function measured by supine slow vital capacity (SVC) in amyotrophic lateral sclerosis (ALS) patients. The secondary objective is to confirm that levosimendan improves the functionality of subjects, measured by Revised ALS Functional Rating Scale (ALSFRS-R), Clinical Global Impression (CGI), Borg Category Ratio 10 (CR10) scale on dyspnoea, Epworth Sleepiness scale (ESS) and Pittsburgh Sleep Quality Index (PSQI). The latter two are sleep scales assessing daytime somnolence and sleep quality, respectively. In addition, the long-term tolerability and safety of levosimendan in ALS patients will be evaluated, assessing up to 48 weeks of exposure. Other objectives: For the purposes of potential later pharmacoeconomic analysis, the use of specific health and home care resources and assistive devices will be quantified. This will include both in and outpatient care, as well as formal and informal home care. Exploratory objectives: The plasma concentrations of levosimendan and the metabolites OR-1855 and OR-1896 will be determined. In addition, a population pharmacokinetic/pharmacodynamic (PK/PD) model between OR-1855 and OR-1896 exposure and efficacy related endpoints and heart rate (HR) will be explored. Plasma concentrations of riluzole will also be determined. The effects of levosimendan, OR-1855 and OR-1896 on plasma trough concentrations of riluzole will be evaluated. The acetylation status will be determined for all subjects to assess whether it affects the PD responses of levosimendan in patients with ALS.
Methodology: This is a randomised, double-blind, placebo-controlled, parallel-group, multinational, multicentre study. The subjects will be allocated to 2 parallel groups receiving either levosimendan 1-2 mg daily or placebo in 2:1 ratio. There will be a screening visit, a baseline visit followed by visits at 2, 4, 8, 12, 24, 36 and 48 weeks, and telephone contacts during weeks 18, 30 and 42. An end-of-study visit will take place 14-25 days after the last study treatment administration for each subject. The total study duration for each subject will be about 51-52 weeks including the end-of-study visit.
Number of subjects: The planned number of subjects is approximately 450.
Diagnosis and main criteria for inclusion: Male or female subjects with diagnosis of ALS, disease duration from symptom onset of 12-48 months, written or verbal informed consent (IC) obtained from the subject. Age at least 18 years. Able to swallow study treatment capsules, and in the opinion of the investigator, is expected to continue to do so during the study. Sitting SVC between 60-90% of the predicted value for age, height and sex at screening visit. Able to perform supine SVC at screening and baseline visits. Subjects with or without riluzole and/or edaravone. If using riluzole (any daily dose up to 100 mg), the dose must have been stable for at least 4 weeks before the screening visit and should not be changed during the study. If using edaravone, the treatment should have been started at least 4 weeks before the screening visit (at least one 28-day treatment cycle as indicated) and should not be changed during the study. If not on riluzole and/or edaravone, the respective treatments should not be started during the study.
Investigational medicinal product, dose and mode of administration: Levosimendan 1 mg capsule
Reference product, dose and mode of administration: Placebo Levosimendan capsule
Frequency/duration of treatment: The daily doses of oral levosimendan will be 1-2 mg depending on the tolerability (mainly judged by HR). The subjects will start with a 1 mg dose (in the morning) for 2 weeks. Administration of placebo (1 or 2 capsules per day) will be based on the same criteria as for levosimendan.
Bioanalytical methods: The concentrations of levosimendan, OR-1855, OR-1896 and riluzole in plasma will be determined by validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods.

Variables and methods of assessments:

Efficacy:

- The primary efficacy variable will be the change from baseline at 12 weeks in SVC measured in supine position, SVC (supine). All SVC measurements prior to 12 weeks will be included in the statistical model. SVC is the maximum volume of air that can be exhaled slowly after slow maximum inhalation.
- ALSFRS-R will be assessed. The variables derived will be the scores of the 12 separate items, the total scores of each subdomain and the total score of ALSFRS-R.
- The 'Time to respiratory event' composite endpoint will be used to validate the changes seen in supine SVC. This variable consists of the following events:
  - At least 1 point decrease in ALSFRS-R respiratory function score 10, 11 or 12
  - Meeting 'protocolised' criteria for NIV: supine SVC ≤ 50% predicted
  - Starting NIV (actual start or attempt to start NIV)
  - Invasive mechanical ventilation by intubation or tracheostomy or death

  Time to respiratory event will be reached whenever any of the 4 criteria listed above has been first met.
- CGI is used to rate the severity of subjects' clinical condition. Clinical condition is assessed by the subjects themselves with a visual analogue scale (VAS).
- Perception on the intensity of dyspnoea will be assessed by Borg CR10 scale in supine and sitting position.
- Daytime somnolence will be assessed by ESS.
- Sleep quality and disturbances will be assessed by PSQI.
- Health care and home care resource use will be assessed for possible later use in pharmacoeconomic analysis.
- Other assessments: Subject's status for NIV support, invasive mechanical ventilation by intubation or tracheostomy and survival will be recorded at all visits.

Pharmacokinetics (PK): Blood samples for the determination of levosimendan, its metabolites OR-1855 and OR-1896 and riluzole concentrations in plasma will be collected.

Biomarkers: Blood samples will be collected for exploratory biomarker analyses which may give supportive data related to the disease state of the subjects.

Pharmacogenomics (PG): All subjects will provide a blood sample for determination of subjects' acetylation status (NAT polymorphisms). The DNA of subjects who have given a separate PG IC will be stored to allow possible exploratory PG research to assess whether genetic polymorphisms relate to the absorption, distribution, metabolism, excretion, pharmacodynamics or safety of levosimendan.

Safety: Safety will be assessed by adverse events (AEs), vital signs, 12-lead electrocardiograms (ECGs), physical examination, weight, laboratory tests and by assessment of suicidality.

Statistical methods: The primary efficacy variable SVC (supine) will be compared between the treatment arms using the mixed model for repeated measures (MMRM) with between subject effects for treatment, centre and randomisation strata, time as within subject effect, and baseline value (visit 1) as a covariate. Primary efficacy will be evaluated at 12 weeks using contrasts to obtain estimates from the primary model including all SVC measurements prior to 12 weeks. Any missing data will be imputed using multiple imputation method. Effect of missing data imputation will be evaluated analysing observed data only and estimating the slope of decline using the random slope model. Several sensitivity subgroup analyses will be performed and defined in the statistical analysis plan (SAP). Additional evaluations will be performed including all data through 48 weeks, using MMRM.

Secondary efficacy endpoints will be tested in following hierarchy to preserve the overall alpha level:

1. Combined assessment of ALSFRS-R function and survival through 48 weeks
2. Time to respiratory event through 48 weeks
3. CGI through 48 weeks
4. Supine Borg CR 10 scale at 12 weeks
5. Slope of decline in respiratory function of ALSFRS-R through 48 weeks

The efficacy variables time to respiratory event, time to NIV or death, time to SVC (supine) decline of 20%, time to SVC (supine) ≤ 50% of predicted, time to ALSFRS-R total score decline of 20% and time to decline in ALSFRS-R respiratory domain will be evaluated using Cox's proportional regression model. The model will include effect for treatment and will be adjusted for randomisation strata. Premature discontinuations will be censored from the analyses. Censoring rules will be defined in the SAP.

The efficacy variables ALSFRS-R total score and its subdomain scores will be performed through 48 weeks. Primary analysis will combine change from baseline to 48 weeks and survival through 48 weeks in joint rank analysis using Wilcoxon-Mann-Whitney test. Slope of decline will be estimated using the random slope model. Additional analyses will be conducted using the MMRM and estimates for individual time points will be done using contrasts. Imputation of missing data using the multiple imputation method will be considered.

CGI, Borg CR10 (sitting, supine and orthostatic changes), ESS and PSQI will be evaluated using the MMRM and estimates for individual time points will be done using contrasts. In addition categorical analyses for ESS and PSQI will be performed using repeated measures logistic regression using the generalised estimating equation (GEE) approach.

All efficacy data will be listed and summarised over the time and by treatment groups using appropriate descriptive statistics.

Standard safety evaluations will be performed for AEs, vital signs, 12-lead ECG, safety laboratory variables and suicidality.

ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
ALP	Alkaline phosphatase
ALS	Amyotrophic lateral sclerosis
ALSFRS-R	Revised ALS Functional Rating Scale
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AV	Atrioventricular
b.i.d.	Twice a day
BMI	Body mass index
BP	Blood pressure
bpm	Beats per minute
CA	Competent authority
CAFS	Combined assessment of function and survival
CHF	Chronic heart failure
CGI(-C)	Clinical Global Impression (of Change)
C_{max}	Maximum concentration
CMV	Cytomegalovirus
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CR10	Category Ratio 10
CRO	Contract research organisation
CRF	Case report form
DSMB	Data and safety monitoring board
EBV	Epstein-Barr virus
EC	Ethics committee
ECG	Electrocardiogram

eCRF	Electronic case report form
EDC	Electronic data capture
EMG	Electromyogram
ESS	Epworth Sleepiness Scale
FAS	Full analysis set
FDA	U.S. Food and Drug Administration
GCP	Good clinical practice
GGT	Gamma-glutamyl transferase
GMP	Good manufacturing practice
HADS	Hospital Anxiety and Depression Scale
HAV	Hepatitis-A virus
HBc	Hepatitis B virus core antigen
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HCVAb	Hepatitis C virus antibody
HEV	Hepatitis-E virus
HF	Heart failure
HIV	Human immunodeficiency virus
HR	Heart rate
IC	Informed consent
ICD	Implantable cardioverter defibrillator
INR	International normalised ratio
IRB	Institutional review board
ITT	Intent-to-treat
i.v.	Intravenous
IWRS	Interactive web response system
LC-MS/MS	Liquid-chromatography-tandem mass spectrometry
MedDRA	Medical Dictionary for Regulatory Activities

MG	Myasthenia gravis
MHRA	The Medicines and Healthcare Products Regulatory Agency
MMRM	Mixed model for repeated measures
NIV	Non-invasive mask ventilation
PD	Pharmacodynamic(s)
PG	Pharmacogenomic(s)
PK	Pharmacokinetic(s)
PP	Per-protocol
QTcF	QTc Fridericia
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SNP	Sniff nasal pressure
SVC	Slow vital capacity
$t_{1/2}$	Terminal elimination half-life
TdP	Torsades de Pointes
TIA	Transient ischemic attack
TSH	Thyroid-stimulating hormone
VAS	Visual analogue scale
ULN	Upper limit of normal

Note on usage of terms:

Orion Corporation Orion Pharma is hereafter in this document called “Orion” or sponsor.

The term ‘investigator’ in the text of the protocol refers to the principal investigator or co-investigator.

1. INTRODUCTION

1.1 Background

Amyotrophic lateral sclerosis (ALS) is a rare, rapidly progressive neurological disease characterised by degeneration of upper and lower motor neurons with subsequent muscle atrophy and weakness and loss of respiratory function. The latter is due to the weakness and loss of diaphragm muscle strength. The median survival time from symptom onset is approximately 3 years (Turner MR et al., 2013). There is an unmet need for new treatments in ALS. There are 3 drugs currently indicated for ALS. The first was riluzole, which has only modest effects (approx. 3 months) on the survival of ALS patients (Bensimon G et al., 1994) and no symptomatic effects. Riluzole is globally available. In addition, two new drugs have recently been approved for ALS in the US. Edaravone has modest effects on the Revised ALS Functional Rating Scale (ALSFRS-R), but does not specifically address muscle weakness and respiratory insufficiency in ALS. The other one is dextromethorphan/quinidine indicated for the symptomatic treatment of pseudobulbar affect. In later stage patients with significantly compromised respiratory function, it is a common practice to initiate non-invasive mask ventilation (NIV) to improve patients' quality of life and survival (Andersen PM et al., 2012, NICE guideline [NG42], 2016).

Almost all of the new molecules under development are disease modifying in nature (Zinman L et al., 2011). Exceptions are fast skeletal muscle troponin activators, tirasemtiv and reldesemtiv (CK-2127107), which increase the efficiency of muscle contraction. Despite promising effects on respiratory function (slow vital capacity [SVC]) in a 3-month phase II study in patients with ALS (Shefner JM et al., 2016) tirasemtiv did not show benefits over placebo in any of the primary or secondary endpoints in a recently completed phase III study (Press release, 2017). A phase II study investigating the effects of reldesemtiv on measures of respiratory function and skeletal muscle function in patients with ALS is currently ongoing.

Levosimendan is an inodilator originally developed for the treatment of heart failure (HF). The clinical intravenous (i.v.) programme included about 3400 patients, of whom almost 2000 patients received levosimendan. Currently i.v. formulation of levosimendan (Simdax®) is registered in 60 countries. In these patients, levosimendan significantly increases cardiac output thus providing inotropic support to overcome the acute worsening of HF. The main mechanism of action of levosimendan is a selective binding to troponin C (Haikala H et al., 1995) and subsequent sensitisation of cardiac and fast and slow skeletal muscles to calcium. In addition, levosimendan opens ATP-sensitive potassium channels on the sarcolemma of smooth muscle cells and in the mitochondria of cardiomyocytes and has vasodilatory effects via potassium channel opening and antiaggregatory effects on thrombocytes. Levosimendan does not increase consumption of ATP or oxygen (Deschodt-Arsac V et al., 2010) and does not have any direct central nervous system (CNS) effects in laboratory animals with plasma concentrations up to > 10-100 times higher than those seen with daily oral levosimendan doses of 1-2 mg in man.

Levosimendan is metabolised by intestinal bacteria to OR-1855, which is further acetylated to OR-1896. About 5% of the levosimendan dose is converted to OR-1896. Both metabolites are active, but OR-1855 to a much lesser extent.

The acetylation status of a subject affects the formation of the active metabolites so that rapid acetylators produce more OR-1896 than slow acetylators. However, the haemodynamic effects

in rapid and slow acetylators have been similar in clinical studies. The effect of levosimendan is thus the sum of the effect of the parent drug and its metabolites; the main effect thought to be derived mainly from the long-acting metabolite OR-1896.

After oral administration in healthy subjects, the maximum concentration (C_{max}) of levosimendan (mean approx. 45 ng/ml) in plasma was seen at 1 hour after doses of 1 mg once a day or 1 mg twice a day (b.i.d.). However, the active metabolite (OR-1896) concentrations increase slowly and steady-state is achieved after 12-14 days with constant daily dosing. The mean C_{max} of OR-1896 after 14 days of dosing with daily levosimendan doses of 1 and 2 mg were 1.6 and 2.8 ng/ml, respectively. The elimination half-life ($t_{1/2}$) of levosimendan is about 1 hour and that of the active metabolites 60 hours in healthy subjects.

Before the first phase IIa study in ALS (LEVALS, 3119001), oral levosimendan has been studied in healthy subjects, in patients with HF and in a small number of patients with a recent history of ischemic stroke/transient ischemic attack (TIA).

In the 2 earlier dose-ranging studies in 90 patients with HF, 5 doses of oral levosimendan from 0.25 to 4 mg or placebo were administered 4 times daily for 2-4 weeks. Daily doses of 4-16 mg had beneficial haemodynamic effects, but daily doses of 8-16 mg were associated with increased (> 10 beats per minute [bpm]) heart rate (HR). Despite high plasma levels of the active metabolite OR-1896 (1.3 to 28.5 ng/ml in the different dose groups), no signs of acute tolerability problems were seen.

The largest study with oral levosimendan, the PERSIST study (Nieminen MS et al., 2008), was a placebo-controlled study in 307 patients with severe chronic heart failure (CHF). The studied levosimendan doses were 1 mg once a day or 1 mg b.i.d. and the duration of exposure was at least 180 days. The results were inconclusive; quality of life (QoL) improved and the number of worsening HF events decreased, but mortality was numerically higher in levosimendan treated patients. The low overall mortality and the fact that the highest number of deaths was seen in the levosimendan 1 mg dose group with the most severe HF at baseline suggests that chance may have played an important role in distribution of deaths. HR increased by approximately 8 bpm compared to placebo in both levosimendan groups. In this study, HR increase and palpitations were the only adverse events (AEs) reported statistically more frequently with levosimendan compared to placebo. The numbers of atrial fibrillations or ventricular arrhythmias reported were similar between patients receiving levosimendan and placebo.

In a small study (5 and 16 patients randomised to placebo and levosimendan, respectively) conducted in TIA/stroke patients with increasing doses of oral levosimendan (from 0.125 mg up to 2 mg daily), all doses increased cerebral blood flow velocity (Kivikko M et al., 2015). Systolic and/or diastolic blood pressure (BP) decreased by approx. 5-7 mmHg with daily levosimendan doses of 1-2 mg. In turn, HR increased by approx. 6-8 bpm with daily doses of 0.5-2 mg. Only the 2 mg daily dose increased the number of ventricular extrasystoles per hour above a predefined limit (set to reflect the possible increased risk of proarrhythmic effects in cardiovascular patients) compared to baseline. There were no significant differences in arrhythmias or adverse events (AEs) reported between placebo and levosimendan.

Levosimendan has been shown to improve the force generation of diaphragm muscle fibres obtained from a rat model of HF (van Hees HW et al., 2011). The same has been reported with diaphragm muscle fibres obtained from lung cancer patients during surgery (van Hees HW et al., 2009). Both of these studies reported that the beneficial force generating effects of

levosimendan were seen both in slow and fast muscle fibres. In addition, the same group demonstrated that levosimendan (i.v.) improved the neuromechanical efficiency and contractility of human diaphragm function in healthy subjects (Doorduin J et al., 2012). In an antibody-induced myasthenia gravis (MG) rat model, levosimendan (0.25 mg/kg orally) and its long-acting metabolite OR-1896 (0.025 mg/kg) given as a single dose significantly improved motor performance in the accelerating RotaRod test as well as exercise capacity in the Treadmill test. These beneficial effects are thought to be due to improved skeletal muscle strength and endurance (Orion Report).

LEVALS (3119001) was a randomised, double-blind, placebo-controlled, crossover, 3-period, multicentre phase IIa study in 66 ALS patients with a 6-month open-label part. Randomised treatments for the crossover part were placebo and levosimendan with 1 mg and 2 mg daily doses. Each treatment period during the double-blind, placebo-controlled part lasted for 14 (± 2) days separated by a 19-23 days wash-out period.

In the primary efficacy variable, SVC% (sitting) there was a clear period effect in the data. Therefore, period-wise baselines were justified and used in efficacy analyses (post-hoc). The estimated mean changes from baseline were -0.67, -0.98 and -0.01% points for placebo, levosimendan 1 mg daily (p = 0.97 vs. placebo) and levosimendan 2 mg daily (p = 0.85 vs. placebo), respectively.

Secondary and other efficacy variables were analysed using period-wise baselines (post-hoc analysis). In the secondary efficacy variable SVC% (supine), the estimated mean differences from baseline were -3.62, +0.77 and +2.38 % points for placebo, levosimendan 1 mg daily (p = 0.018 vs. placebo) and levosimendan 2 mg daily (p = 0.001 vs. placebo), respectively. In other words, both levosimendan doses (in a dose-dependent manner) improved SVC% (supine) values during the 2-week treatment period, whereas SVC% (supine) decreased during placebo. The treatment effects between placebo and levosimendan 1 mg and 2 mg were 4.39% and 6.00%, respectively, favouring levosimendan. Both levosimendan doses were numerically better compared to placebo in the ALSFRS-R total score. The estimated mean differences from baseline were -0.82, -0.46 and -0.37 for placebo, levosimendan 1 mg daily (p = 0.49 vs. placebo) and levosimendan 2 mg daily (p = 0.34 vs. placebo), respectively. For the ALSFRS-R respiratory domain the estimated mean differences from baseline were -0.22, +0.04 and +0.05 for placebo, levosimendan 1 mg daily (p = 0.13 vs. placebo) and levosimendan 2 mg daily (p = 0.12 vs. placebo), respectively. These small numerical trends seen in the ALSFRS-R total and respiratory domain scores provide support for supine SVC results.

There were no statistically significant differences seen between placebo and levosimendan 1 mg and 2 mg daily treatments in sniff nasal pressure (SNP), visual analogue scale (VAS) of fatigue, overnight oxygen saturation (SpO_2) measured by a pulse oximeter, maximal hand grip strength and submaximal hand grip endurance or Clinical Global Impression of Change (CGI-C) assessed by the investigator and the subject.

Tolerability and safety were good in general in the LEVALS study and similar to previous studies with oral levosimendan. As expected, AEs of headache and increased HR were more common during levosimendan (in a dose-dependent manner) and the latter led to study treatment discontinuations based on a predefined criterion (increase in HR > 15 bpm compared with screening). The numbers of severe AEs or serious adverse events (SAEs) were similar between levosimendan and placebo. There were no differences either in supraventricular or ventricular tachyarrhythmias between the treatments.

In conclusion, there is both experimental and clinical evidence that levosimendan improves diaphragmatic function. In addition, levosimendan improved motor function and endurance in a rat model of MG functionally mimicking impaired motoneuron function in ALS. The phase IIa placebo-controlled results from the LEVALS study also indicated that there was a dose-related treatment effect favouring levosimendan in SVC% supine after 2 weeks treatment.

Collectively these results indicate that levosimendan is able to improve the muscle strength endurance of diaphragm and therefore respiratory function in patients with ALS.

1.2 Rationale of the study

1.2.1 Rationale of the study design

The aim of this pivotal phase III study is to confirm the positive findings of the supine SVC% results seen in the LEVALS phase IIa study and to demonstrate benefit on supporting endpoints such as ALSFRS-R and the composite of 'time to' respiratory events.

The target population is subjects with diagnosis of laboratory supported probable, probable or definite ALS according to the El Escorial revised criteria (Brooks BR et al., 2000). The main inclusion criterion will be SVC% (sitting) between 60-90% of predicted. It is believed that enrolling patients who already have some respiratory compromise will increase the ability to identify positive effects of levosimendan. This might be the case particularly on respiratory symptoms, which are not present early in the disease. The SVC% (supine) results in the LEVALS study support using this SVC% range also in this phase III study.

SVC% assessed in the supine position will be the primary efficacy variable. In the LEVALS study, both levosimendan doses were superior to placebo in supine SVC%. Assessing SVC in the supine position is also clinically relevant, because the first clinical signs of respiratory insufficiency often appear during sleep or when lying down. There is also evidence that vital capacity measured in the supine position correlates better with diaphragmatic weakness than that measured in upright position (Lechtzin N et al., 2002) and is a better predictor of survival compared with upright vital capacity (Schmidt EP et al., 2006, Baumann F et al., 2010).

There will be 2 futility/interim analyses, first when about 50% and second when 100% of subjects have been treated for 12 weeks. Based on these analyses, the study will be stopped in case the futility/interim analysis indicates that statistically significant differences between the treatment arms are unlikely at the planned study end.

The double-blind treatment duration will be 48 weeks in order to collect controlled long-term safety and efficacy data. The primary efficacy variable (SVC% supine) will be analysed at 12 weeks. Supporting secondary variables such as 'time to' respiratory endpoints and ALSFRS-R require longer treatment duration to demonstrate benefit and will be examined at 48 weeks.

1.2.2 Rationale for selected doses

The LEVALS phase IIa study included treatment periods with 1 mg once a day and 1 mg b.i.d. of levosimendan. These two daily doses of levosimendan showed significant benefits over placebo in supine SVC in a dose-dependent manner. Based on the LEVALS results and studies in cardiac and other patient populations, higher doses are associated with increased HR. Therefore Orion concludes that no further dose finding is needed. The target maintenance dose

of levosimendan in the phase III study will be 2 mg per day administered as 1 mg b.i.d. Using flexible dosing, the levosimendan arm will start with a 1 mg daily dose for 2 weeks. Previous studies have shown that levosimendan may be associated with headache in a dose-dependent manner, lasting for about 2-3 days. Starting with 1 mg per day will therefore decrease the occurrence of headache. HR has also been shown to increase dose-dependently with levosimendan. If tolerability allows (e.g. based on HR) at 2 weeks, the dose will be increased to 1 mg b.i.d. If required due to any tolerability issues (e.g. based on HR) during 1 mg b.i.d. dosing, the dose can be decreased to 1 mg per day. During b.i.d. dosing the 2nd daily dose will normally be taken at 12 hours, but to best meet the clinical needs of the subject, the investigator may instead specify taking the 2nd daily dose at 6 hours after the morning dose or when the subject goes to bed.

1.3 Benefit-risk assessment

1.3.1 Potential benefits associated with levosimendan

SVC assessed in the supine position in the LEVALS study indicated that levosimendan can significantly improve the skeletal muscle function of the diaphragm, thereby supporting respiratory function in ALS patients. This in turn is expected to improve patients' well-being and functional ability.

1.3.2 Potential risks associated with levosimendan

Although the extent of safety data with oral levosimendan is still limited, available data suggest that levosimendan or its active metabolites are not likely to cause clinically significant direct CNS effects. Myocardial and arterial changes such as hypertrophy, myocarditis and vasculitis have been observed in toxicity studies in animal species, dog being the most sensitive species with first signs of toxicity appearing at 2-5 fold clinical exposure. The clinical relevance of these findings is not known. Based on the safety data originating from the completed clinical trials with oral levosimendan (administered 1-2 mg daily for longer than 180 days), safety signals of increased heart rate and palpitations have been detected and are considered as potential risks for oral levosimendan use. No other safety concerns related to cardiac function have been identified from these data. Arrhythmias are known AEs for i.v. levosimendan in patients with severe CHF. However, there is no such evidence for oral levosimendan in healthy subjects. In addition, the LEVALS phase IIa study conducted in ALS patients did not find any differences in supraventricular or ventricular tachyarrhythmias between placebo and levosimendan. There were no differences either in the numbers of severe AEs or SAEs between the treatments. However, special attention should be paid to symptoms potentially connected with cardiac arrhythmias, such as palpitations, dizziness and loss of consciousness. Headache (due to vasodilatation), increased HR and palpitations have been the most commonly reported adverse drug reactions in healthy subjects and/or ALS patients treated with levosimendan. In laboratory parameters, slight decreases in red cell parameters and serum potassium have been observed in some studies.

1.3.3 Potential risks associated with the study assessments

Blood samples will be collected for safety laboratory, pharmacokinetic (PK) and pharmacogenomic (PG) analyses. The risks of blood sampling include fainting and pain,

bruising, swelling or rarely infection of the injection site. Electrocardiogram (ECG) pads can cause skin irritation and the removal of the pads can be painful. Repeated respiratory assessments may be burdensome for patients with ALS.

2. STUDY OBJECTIVES

2.1 Primary objective

The primary objective of this study is to confirm that levosimendan can significantly improve respiratory function measured by supine SVC in ALS patients.

2.2 Secondary objective

The secondary objective is to confirm that levosimendan improves the functionality of subjects measured by ALSFRS-R, CGI, Borg Category Ratio 10 (CR10) scale on the intensity of dyspnoea, Epworth Sleepiness scale (ESS) and Pittsburgh Sleep Quality Index (PSQI). The latter two are sleep scales assessing daytime somnolence and sleep quality, respectively. In addition, the long-term tolerability and safety of levosimendan in ALS patients will be evaluated, assessing up to 48 weeks of exposure.

2.3 Other objectives

For the purposes of potential later pharmacoeconomic analysis, the use of specific health care resources will be quantified on weekly basis, including:

- The number of days spent in hospital care.
- The number of additional visits to a hospital emergency unit.
- The average number of formal home care hours per day on the given week (paid/public services obtained) and the number of times such care was received during a usual night.
- The average number of informal home care hours per day on the given week (unpaid; by family, other relative, friend or volunteer) and the number of times such care was received during a usual night.
- The number of days spent in institutional care other than hospital (e.g. respite/hospice/nursing home/rehabilitative care).
- Receipt of new, non-medicinal assistive devices and other aids.

2.4 Exploratory objectives

The plasma concentrations of levosimendan and the metabolites OR-1855 and OR-1896 will be determined. In addition, a population pharmacokinetic/pharmacodynamic (PK/PD) model between OR-1855 and OR-1896 exposure and efficacy related endpoints and HR will be explored. Plasma concentrations of riluzole will also be determined. The effects of levosimendan, OR-1855 and OR-1896 on plasma trough concentrations of riluzole will be evaluated.

The acetylation status will be determined for all subjects to assess whether it affects the PD responses of levosimendan in patients with ALS.

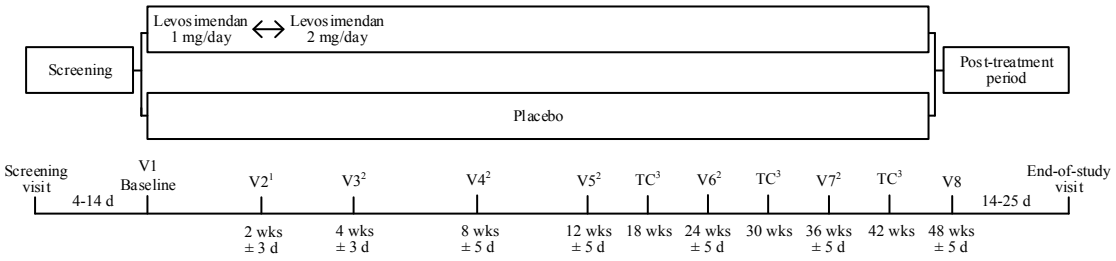
3. OVERALL STUDY DESIGN AND PLAN

This is a randomised, double-blind, placebo-controlled, parallel-group multinational, multicentre phase III study. The subjects will be allocated to 2 parallel groups receiving either levosimendan 1-2 mg daily or placebo in 2:1 ratio. The study will be conducted globally. The estimated number of sites will be approximately 100.

The total duration for double-blind comparison will be 48 weeks. A futility/interim analysis will be conducted when approximately 50% and all of the subjects have been treated for 12 weeks. An independent data and safety monitoring board (DSMB) will be established for this and for safety monitoring purposes.

There will be a screening visit, a baseline visit (V1) followed by visits at 2 weeks (V2), 4 weeks (V3), 8 weeks (V4), 12 weeks (V5), 24 weeks (V6), 36 weeks (V7) and 48 weeks (V8), and telephone contacts during weeks 18, 30 and 42. An end-of-study visit will take place 14-25 days after the last study treatment administration for each subject. Additional visits may take place after visit 3 and after later visits (see section 5.3.2). The total study duration for each subject will be about 51-52 weeks including the end-of-study visit. The study design is presented in Figure 1.

Figure 1. Study design



[1] Dose will be increased if the 1 mg/day dosing was well tolerated

[2] Dose can be decreased back to 1 mg/day if required for any reason In case the dose was decreased due to HR increase > 30 bpm, additional visit will take place after approx 2 weeks

[3] Any time during the week

4. SELECTION OF STUDY POPULATION

4.1 Number of subjects

The planned number of subjects is approximately 450.

4.2 Inclusion criteria

The subjects must meet all of the following criteria to be included into the study:

1. Written or verbal informed consent (IC) for participation in the study will be obtained from the subject. In case that the study subject him/herself cannot sign the IC due to severe muscle weakness, a witness may sign the consent form to indicate that the subject has given verbal consent.
2. Age at least 18 years.
3. Male or female subjects with diagnosis of laboratory supported probable, probable or definite ALS according to El Escorial revised criteria (Brooks BR et al., 2000). Full electromyogram (EMG) report available consistent with ALS (but not necessarily fulfilling the electrodiagnostic criteria for ALS) from an experienced neurophysiologist.
4. Able to swallow study treatment capsules, and in the opinion of the investigator, is expected to continue to do so during the study.
5. Sitting SVC between 60-90% of the predicted value for age, height and sex at screening visit.
6. Disease duration from symptom onset (defined by first muscle weakness or dysarthria) 12-48 months at the time of visit 1 (baseline).
7. Able to perform supine SVC in an adequate and reliable way at screening and baseline visits as judged by the investigator.
8. Subjects with or without riluzole and/or edaravone. If using riluzole (any daily dose up to 100 mg), the dose must have been stable for at least 4 weeks before the screening visit and should not be changed during the study. If using edaravone, the treatment should have been started at least 4 weeks before the screening visit (at least one 28-day treatment cycle as indicated) and should not be changed during the study. If not on riluzole and/or edaravone, the respective treatments should not be started during the study.

4.3 Exclusion criteria

Subjects will not be included into this study if they meet any of the following criteria:

1. Subject in whom other causes of neuromuscular weakness have not been excluded.
2. Subject with a diagnosis of another neurodegenerative disease (e.g. Parkinson's or Alzheimer's disease).
3. Assisted ventilation of any type within 3 months before the screening visit or at screening.
4. Any use of a diaphragm pacing system (DPS) within 3 months before the screening visit.
5. Any form of stem cell or gene therapy for the treatment of ALS.
6. Known hypersensitivity to levosimendan.
7. Administration of levosimendan within 3 months before the screening visit or previous participation in the present phase III study or earlier study with oral levosimendan in ALS patients (LEVALS).
8. Any use of tirasemtiv or reldesemtiv within 1 month before the screening visit.

9. Participation in a clinical trial with any experimental treatment within 30 days or within 5 half-lives of that treatment (whichever is longer) before the screening visit.
10. Any botulinum toxin use within 3 months before the screening visit.
11. Recorded diagnosis or evidence of major psychiatric diagnosis, significant cognitive impairment or clinically evident dementia that may interfere with the patient's ability to comply with study procedures.
12. Pulmonary illness (e.g. asthma or COPD) requiring regular treatment.
13. Haemodynamically significant uncorrected valve disease or hypertrophic cardiomyopathy or restrictive cardiomyopathy.
14. Any cardiovascular event (e.g. myocardial infarction, HF, arrhythmia or stroke) requiring hospitalisation within 3 months before the screening visit.
15. History of Torsades de Pointes (TdP) or diagnosed long QT-syndrome.
16. History of life-threatening ventricular arrhythmia, unless treated with reliable measures to prevent recurrence (e.g. with placement of implantable cardioverter defibrillator [ICD] or catheter ablation).
17. History of second or third degree atrioventricular (AV) block or sinus node disease at screening, if not treated with pacemaker.
18. HR repeatedly > 100 bpm in the 12-lead ECG after a 5-minute rest at screening. If the HR is > 100 bpm in the first recording, then the second recording must be done after another 5 min rest to confirm HR > 100 bpm.
19. Systolic blood pressure (SBP) < 90 mmHg at screening.
20. Potassium < 3.7 mmol/l or > 5.5 mmol/l at screening.
21. Severe renal impairment (creatinine clearance < 30 ml/min at screening), creatinine > 170 µmol/l at screening or on dialysis.
22. Blood haemoglobin < 10 g/dl at screening or blood donation or loss of significant amount of blood within 60 days before the screening visit.
23. Clinically significant hepatic impairment at the discretion of the investigator.
24. Body mass index (BMI) $\leq 18.5 kg/m^2$ (BMI = weight/height^2).
25. Women who are lactating or of reproductive age without a negative pregnancy test and without a commitment to using a highly effective method of contraception (e.g. oral hormonal contraceptives associated with inhibition of ovulation, intrauterine devices and long acting progestin agents), if sexually active during the study, and for 1 month after the last dose of the study treatment. Women who are postmenopausal (1 year since last menstrual cycle), surgically sterilised or who have undergone a hysterectomy are considered not to be reproductive and can be included.
26. Patient judged to be actively suicidal by the investigator during 3 months before the screening visit.

27. Patients with known history of human immunodeficiency virus (HIV) infection.

28. Any other clinically significant cardiovascular, gastrointestinal, hepatic, renal, neurological or psychiatric disorder or any other major concurrent illness that in the opinion of the investigator could interfere with the interpretation of the study results or constitute a health risk for the subject if he/she took part in the study.

4.4 Information collected on screening failures

For subjects screened but not included in the study, the following case report forms (CRFs) will be completed: date of the screening visit, IC, demography (birth year, age, sex and race), criteria causing the exclusion and decision of entry. In addition, all information (including concomitant treatments and medical history) about AEs related to study assessments and SAEs must be collected. If according to the source data there are no AEs, the AE CRF can be inactivated.

4.5 Removal of subjects from treatment or assessment

Study subjects are free to discontinue the study at any time without providing a reason. However, the investigator should try to identify the reason and document it in the CRF.

A subject must discontinue the study treatment for the following reasons:

- HR increase of > 30 bpm from baseline (visit 1) at the dose level of 1 mg once daily in 3 separate ECG recordings, taken within 5-10 minutes from each other before the morning dose of the study drug (see also section 6.5.4).
- Life-threatening supraventricular or ventricular arrhythmia.
- The investigator judges that it is in the best interest of the subject to stop the study treatment due to any other abnormality compromising patient safety. However, the investigator should consult the medical monitor whenever possible in these instances.
- Pregnancy.
- Invasive mechanical ventilation by intubation or tracheostomy.

The subjects who have discontinued the study medication, but continue in the study, are encouraged to follow the study visit and phone call schedule according to the study protocol for recording ALSFRS-R, ventilatory support and survival status. End-of-study visit assessments for these subjects will be performed 14-25 d after the last study treatment administration.

A subject must discontinue the study if the investigator or the sponsor considers the discontinuation to be medically necessary or in the best interest of the subject.

Irrespective of the reason for discontinuation, the subject should be invited to end-of-study assessments as soon as possible. As long as the subject consents, all relevant assessments, at least those of safety, should be performed, preferably according to the schedule for the end-of-study assessments.

The study monitor should be notified about premature discontinuations by email/phone/fax within 24 hours in the event of discontinuation due to an SAE (see section 6.5.1.3) or within 7 days in the event of discontinuation due to another reason.

Subjects who prematurely discontinue will not be replaced. Discontinued subjects are not allowed to re-enter the study.

5. STUDY TREATMENTS

Manufacturing, packaging, and labelling of the study treatments will comply with good manufacturing practice (GMP) regulations (Annex 13 of EU guide to GMP). The capsules will be stored below 30°C and the container kept tightly closed.

5.1 Investigational product

Investigational product will be Levosimendan 1 mg capsules for oral administration.

5.2 Reference product

Placebo Levosimendan capsule for oral administration containing only excipients.

5.3 Dosing

5.3.1 Selection and timing of doses

The subjects will be randomised centrally to receive either levosimendan or placebo. The daily dose of oral levosimendan will be 1-2 mg (see section 5.3.2). The subjects will take the study treatment in the morning and when the dose is increased to 1 mg b.i.d., the second dose will normally be taken 12 hours later. Alternatively the investigator may specify to take the 2nd daily dose 6 hours after the morning dose or when going to bed, according to the clinical needs of the subject. The prescribed time of the second dose will be recorded in the CRF and should be the same each day unless changed by the investigator. It is recommended to take the study treatment about 1 hour before food intake or on an empty stomach. The morning dose of study treatment will be administered at the study centre during the visit days.

The total duration of study treatments will be 48 weeks.

5.3.2 Dose adjustment

The daily doses of oral levosimendan will be 1-2 mg depending on the tolerability (mainly judged by HR). The subjects will start with a 1 mg dose (in the morning) for 2 weeks.

HR will be assessed in the morning before study drug intake:

- At visit 2 (2 weeks):
  - If the HR in the 12-lead ECG has increased ≤ 15 bpm from baseline, the subject will increase the dose to 1 mg b.i.d.
  - If the HR in the 12-lead ECG has increased > 15 bpm from mean baseline value, the subject will continue with the 1 mg once daily. If the increase in HR is > 30 bpm in 3 separate recordings, the subject must discontinue the study treatment (see section 4.5).

- At visit 3 (4 weeks) or later visits:
  - If the HR in the 12-lead ECG has increased ≤ 20 bpm from baseline, no changes to the dosing regimen are made.
  - If the HR in the 12-lead ECG has increased > 20 bpm from baseline in 3 separate recordings taken within 5-10 minutes from each other, the subject will decrease the dose back to 1 mg once daily if treated with 1 mg b.i.d. In case the increase in HR is > 30 bpm in each of the 3 separate ECG recordings, the HR must be checked again at an additional visit approximately 2 weeks after the dose decrease. If the increase > 30 bpm is recorded at the dose level of 1 mg once daily in 3 separate recordings, the subject must discontinue the study treatment (see section 4.5).

Administration of placebo (1 or 2 capsules per day) will be based on the same criteria as described for levosimendan above.

5.4 Method of assigning study subjects to treatment groups

The study subjects will be randomly allocated to treatment groups by interactive web response system (IWRS) using a complete randomisation scheme to eliminate the possible bias due to predictability of treatment assignment (Hewitt CE et al., 2006). The subjects will be allocated at random to the levosimendan and placebo groups at a ratio of 2:1 using permuted blocks.

The subjects will be stratified via IWRS for bulbar and spinal onset patients, use of edaravone and in regard to geographical region for Northern America vs. regions outside Northern America, so that in these groups the treatment allocations will be balanced. Stratification will occur on a study level rather than on a centre level. Enrolment by stratum is not constrained.

To ensure random allocation of the study treatments, all subjects to be randomly allocated will receive the treatment that corresponds to the next consecutive subject number.

5.5 Blinding

Placebo levosimendan capsules are identical in appearance to the active treatment. Packaging and labelling will ensure the blinding.

None of the persons directly involved in the conduct of the study will have access to the treatment code. The DSMB will have access to the treatment code. In addition, the bioanalytical laboratories will have access to the treatment code. The treatment code will be opened after the study database has been locked.

5.6 Emergency procedures

In case of an emergency requiring immediate knowledge of the study treatment, the treatment code may be broken by the investigator through the IWRS. As a back-up to the IWRS, there will be a 24x7x365 Customer Help Desk available to support the investigators in unblinding. Whenever possible, the investigator should discuss the case with the medical monitor prior to unblinding. If not possible, the medical monitor should be notified immediately after breaking the treatment code.

The sponsor's personnel may unblind the treatment assignment for an individual subject's SAE in order to fulfil expedited regulatory reporting requirements.

5.6.1 Treatment of emergencies

Emergencies will be treated according to the decision of the physician in charge or the investigator, when available.

5.7 Prior and concomitant treatments

All concomitant treatments during the study, including the post-treatment period, must be recorded. No other investigational medicinal product is allowed to be used concomitantly with the study treatment. The study subject must not have been administered another investigational medicinal product within 30 days before the screening visit. The use of tirasemtiv or reldesemtiv within 1 month or botulinum toxin within 3 months before the screening visit is prohibited.

5.7.1 Prohibited treatments during the study

The use of tirasemtiv or reldesemtiv is prohibited during the study due to their similar mechanism of action with levosimendan.

The subjects should avoid starting supplements (such as creatine, N-acetyl-cysteine, coenzyme Q10) as well as off-label uses of drugs for ALS (such as memantine and tamoxifen) during the study.

Use of botulinum toxin is not allowed during the study.

If the permissibility of a specific drug/treatment is questionable, the medical monitor must be contacted.

5.7.2 Permitted treatments during the study

Commonly used supplements such as vitamin C or multivitamin tablets are permitted during the study.

If using riluzole, the dose must have been stable for at least 4 weeks before the screening visit and should not be changed during the study. If using edaravone, the treatment should have been started at least 4 weeks before the screening visit (at least one 28-day treatment cycle as indicated) and should not be changed during the study. If not on riluzole and/or edaravone, the respective treatment(s) should not be started during the study. If riluzole and/or edaravone needs to be discontinued due to an AE, the medical monitor must be contacted.

If necessarily required, benzodiazepines can be started during the study to treat e.g. panic attacks or anxiety.

Any other treatments, with the exceptions noted in section 5.7.1, which are considered necessary for the subject's welfare, and which will not interfere with the study treatment, may be given at the discretion of the investigator.

If the permissibility of a specific drug/treatment is questionable, the medical monitor must be contacted.

5.8 Treatment compliance and exposure

Treatment compliance will be assessed by study treatment accountability. The morning dose of study treatment will be administered at the study centre during the visit days. Treatment deviations must be recorded. Subjects should be asked about the reason for non-compliance.

Drug accountability records will be kept. The investigator must maintain accurate records demonstrating the date and amount of study treatments received, to whom and by whom dispensed (drug dispensing list) and accounts of study treatments accidentally or deliberately destroyed.

At the end of the study, any remaining study treatments will be collected and returned to the sponsor. Any discrepancies between the returned and expected returned study treatments should be explained.

5.9 Availability of investigational medicinal product after termination of study

There is an option to continue levosimendan treatment once the study subject has completed 48 weeks of treatment and the end-of-study visit of the current study, either in the form of an open-label extension study or compassionate use program.

6. STUDY PROCEDURES AND ASSESSMENTS

6.1 Study procedures

Table 1 lists all study procedures and indicates with an ‘x’ during which visit a particular procedure is performed.

Table 1. Schedule of study events

Protocol activities	Screening period	Baseline	Treatment period										Post-treatment period
	Scr. visit 4-14 d before V1	V1 Baseline D1	V2 Week 2 D15 ± 3 d	V3 Week 4 D29 ± 3 d	V4 Week 8 D57 ± 5 d	V5 Week 12 D85 ± 5 d	TC Week 18	V6 Week 24 D169 ± 5 d	TC Week 30	V7 Week 36 D253 ± 5 d	TC Week 42	V8 Week 48 D337 ± 5 d	End-of-study visit 14-25 d after V8[1]
IC	x												
Demography	x												
Physical examination	x												x
Weight	x	x		x	x	x		x		x		x	x
Height and BMI	x												
HR and BP	x	x	x	x	x	x		x		x		x	x
12-lead ECG	x	x[2]	x	x	x	x		x		x		x	x
Laboratory safety assessments													
Haematology	x	x	x	x	x	x		x		x		x	x
Chemistry	x	x[3]	x[3]	x[3]	x[3]	x[3]		x[3]		x[3]		x[3]	x[3]
Urinalysis	x	x		x		x		x		x		x	x
Serology	x												
Pregnancy test for females of childbearing potential	x	x		x	x	x		x		x		x	x
Testing ability to swallow capsules	x												
SVC sitting	x												
SVC supine	x	x	x	x	x	x		x		x		x	x
Eligibility criteria and decision of entry	x												
Study treatment		x											
ALSFRS-R		x		x	x	x	x	x	x	x	x	x	
CGI		x		x		x		x		x		x	

Protocol activities	Screening period	Baseline	Treatment period										Post-treatment period
	Scr. visit 4-14 d before V1	V1 Baseline D1	V2 Week 2 D15 ± 3 d	V3 Week 4 D29 ± 3 d	V4 Week 8 D57 ± 5 d	V5 Week 12 D85 ± 5 d	TC Week 18	V6 Week 24 D169 ± 5 d	TC Week 30	V7 Week 36 D253 ± 5 d	TC Week 42	V8 Week 48 D337 ± 5 d	End-of-study visit 14-25 d after V8[1]
Borg CR10 scale		x	x	x	x	x		x		x		x	x
Epworth Sleepiness Scale (ESS)		x		x		x		x		x		x	
Pittsburgh Sleep Quality Index (PSQI)		x		x		x		x		x		x	
Use of health care and home care resources and non-medical assistive devices		x[4]	x	x	x	x		x		x		x	
Blood sampling for levosimendan, OR-1855 and OR-1896 PK			x	x	x	x		x		x		x	
Blood sampling for riluzole PK		x	x	x								x	
Blood sampling for exploratory biomarkers		x		x	x	x		x		x		x	
Assessment of suicidality	x	x	x	x	x	x		x		x		x	x
Blood sampling for PG		x[5]											
Status for NIV support, invasive mechanical ventilation by intubation or tracheostomy and survival	x												
AEs and SAEs	x												
Medical history and current medical conditions	x												
Concomitant treatments	x												

[1] Also for subjects who discontinue treatment but continue in the study, end-of-study visit assessments will be performed 14-25 d after the last study treatment administration

[2] 3 baseline recordings pre-dose

[3] Except thyroid-stimulating hormone (TSH)

[4] Data requested for the past 7 days prior to baseline visit

[5] One sample during the study preferably at visit 1 (baseline)

6.1.1 Procedures during the screening period

A screening visit will take place 4-14 days before the first study treatment administration. A prospective subject will receive both written and verbal information about the study, and will have an opportunity to ask questions and sufficient time to decide whether or not to participate in the study. A signed and dated written IC will be obtained (a witness may sign the consent form to indicate that the subject has given verbal consent).

Re-screening (one occasion, unless otherwise authorised by the Medical Monitor) is allowed for example in case the patient has been taking prohibited treatment within 3 months before the screening visit or the sitting SVC is not within the inclusion criterion limits (60-90%).

The following procedures will be performed at the screening visit to ensure fulfilling the inclusion/exclusion criteria:

- Demographic data (birth year, age, sex, race, geographic region) and weight and height will be recorded. BMI will be derived from weight and height automatically in electronic case report form (eCRF).
- ALS history (date of diagnosis, ALS family history, site of onset [spinal/bulbar] and start date of ALS symptoms) will be recorded.
- HR and BP will be recorded in supine position.
- 12-lead ECG will be recorded in supine position.
- Physical examination will be performed.
- A blood sample for haematology, clinical chemistry and serology will be collected and a urine sample will be collected for urinalysis. A pregnancy test will be performed for females of childbearing potential.
- Ability to swallow capsules will be tested with a placebo capsule.
- SVC will be measured in the sitting and supine positions. Sitting SVC% will be assessed first and used for inclusion criterion. Thereafter, SVC supine will be assessed only to ensure successful assessment.
- Status for NIV support, invasive mechanical ventilation by intubation or tracheostomy and survival will be recorded.
- AEs will be inquired.
- Medical history, current medical conditions and concomitant treatments will be recorded.
- Suicidality will be assessed.

6.1.2 Procedures during the treatment period

Procedures during visit 1 (baseline) are presented in Table 2 and procedures during visits 2-8 in Table 3. The morning dose of study treatment will be administered at the study centre during the visit days.

Table 2. Procedures at visit 1 (baseline)

Time	Pre-dose	0 h	45-120 min
Weight	x		
HR and BP	x		x
12-lead ECG	x[1]		x
Laboratory safety assessments			
Haematology	x		
Chemistry	x[2]		
Urinalysis	x		
Pregnancy test for females of childbearing potential	x		
SVC supine	x		
Study treatment		x	
ALSFRS-R	x		
CGI	x		
Borg CR10 scale	x		
ESS	x		
PSQI	x		
Use of health care and home care resources and non-medical assistive devices	x[3]		
Blood sampling for riluzole PK	x		
Blood sampling for exploratory biomarkers	x		
Blood sampling for PG	x[4]		
Assessment of suicidality	x		
Status for NIV support, invasive mechanical ventilation by intubation or tracheostomy and survival	x		
AEs and SAEs	x		
Medical history and current medical conditions	x		
Concomitant treatments	x		

[1] 3 baseline recordings
[2] Except TSH
[3] Data requested for the past 7 days prior to baseline visit
[4] One sample during the study preferably at visit 1

Table 3. Procedures at visits 2-8

Time	Pre-dose	0 h	30-90 min	120-240 min
Weight	x[1]			
HR and BP	x		x	
12-lead ECG	x		x	
Laboratory safety assessments				
Haematology	x			
Chemistry	x[2]			
Urinalysis	x[3]			
Pregnancy test for females of childbearing potential	x[4]			
SVC supine	x			
Study treatment		x		
ALSFRS-R	x[1]			
CGI	x[3]			
Borg CR10 scale	x			
ESS	x[3]			
PSQI	x[3]			
Use of health care and home care resources use and non-medical assistive devices	x			
Blood sampling for levosimendan, OR-1855 and OR-1896 PK			x	x[5]
Blood sampling for riluzole concentration	x[5]			
Blood sampling for exploratory biomarkers	x[1]			
Assessment of suicidality	x			
Status for NIV support, invasive mechanical ventilation by intubation or tracheostomy and survival	x			
AEs and SAEs	x			
Medical history and current medical conditions	x			
Concomitant treatments	x			

[1] Not at visit 2
[2] Except TSH
[3] Not at visits 2 and 4
[4] Not at visit 2
[5] Not at visits 4, 5, 6 and 7

6.1.3 Telephone contacts

The study centre personnel will phone the subjects during weeks 18, 30 and 42. During the phone call ALSFRS-R will be assessed and AEs inquired.

6.1.4 Additional visits

Additional visits will be performed in case dose decreases were triggered by predefined HR increase criteria (see section 5.3.2). Additional visits may take place approximately 2 weeks after the dose decrease. Procedures during additional visits are presented in Table 4.

Table 4. Procedures during additional visits

Time	Pre-dose	0
HR and BP	x	
12-lead ECG	x	
Study treatment		x
AEs and SAEs	x	

6.1.5 Procedures during the post-treatment period

An end-of-study visit will take place 14-25 days after the last study treatment administration for each subject. The following procedures will be performed:

- HR and BP will be recorded.
- 12-lead ECG will be recorded.
- SVC supine will be assessed.
- The Borg CR10 scale to assess the intensity of dyspnoea will be completed at rest in sitting and supine positions (immediately before SVC measurement).
- Physical examination will be performed.
- Weight will be recorded.
- A blood sample for haematology and clinical chemistry and a urine sample for urinalysis will be collected. A pregnancy test will be performed for females of childbearing potential.
- AEs, current medical conditions and concomitant treatments will be recorded.
- Status for NIV support, invasive mechanical ventilation by intubation or tracheostomy and survival will be recorded
- Suicidality will be assessed.

6.1.6 Follow-up period for subjects who have discontinued the study medication

The subjects who have discontinued the study medication, but continue in the study, are encouraged to follow the study visit and phone call schedule according to the study protocol for recording ALSFRS-R, ventilatory support and survival status.

6.2 Efficacy assessments

6.2.1 Slow Vital Capacity

The primary efficacy variable will be the change from baseline at 12 weeks in SVC measured in the supine position, SVC (supine). All SVC measurements prior to 12 weeks will be included in the statistical model. SVC is the maximum volume of air that can be exhaled slowly after slow maximum inhalation. The best read from 3 attempts will be chosen. The volume is measured in litres and the SVC variable will be % of predicted (normal) value for age, height and sex. In

addition, time to decline in supine from baseline SVC by ≥ 20% and time to supine SVC ≤ 50% of predicted will be assessed.

SVC (supine) will be assessed once at each visit during the study including the baseline (visit 1), i.e. in the morning before the study treatment administration.

SVC will be assessed in the sitting and supine position at screening visit. Sitting SVC will be one of the inclusion criteria. Supine SVC is assessed at screening and baseline to ensure that the subject is able to perform it successfully when entering the study.

6.2.2 Revised ALS Functional Rating Scale

ALSFRS-R (Cedarbaum JM et al., 1999) will be assessed. This scale includes 12 items: speech, salivation, swallowing, handwriting, cutting food and handling utensils, dressing and hygiene, turning in bed and adjusting sheets, walking, climbing stairs, dyspnea, orthopnea, and respiratory insufficiency. Each item will be scored from 0 to 4. The variables derived will be the scores of the 12 separate items, the total scores of each subdomain (bulbar, fine motor, gross motor and respiratory) and the total score of ALSFRS-R.

In addition the following variables will be assessed:

- Combined assessment of function and survival (CAFS)
- Change from baseline in ALSFRS-R total score
- Time to decline from baseline in ALSFRS-R total score by ≥ 20%
- Time to decline from baseline in the respiratory domain of ALSFRS-R (items 10, 11, and 12).

6.2.3 Time to respiratory event

The ‘Time to respiratory event’ composite endpoint will be used to validate the changes seen in supine SVC. This variable consists of the following events:

- At least 1 point decrease in ALSFRS-R respiratory function score 10, 11 or 12
- Meeting ‘protocolised’ criteria for NIV: supine SVC ≤ 50% predicted
- Starting NIV (actual start or attempt to start NIV)
- Invasive mechanical ventilation by intubation or tracheostomy or death.

Time to respiratory event will be reached whenever any of the 4 criteria listed above has been first met.

6.2.4 Clinical Global Impression

CGI is used to rate the severity of subjects’ clinical condition. Clinical condition is assessed by the subjects themselves with a VAS. Score of 0 indicates (in the 100 millimetre scale) that the subject is completely well without any disability and score of 100 indicates the worst possible severity of the condition. This rating is based on symptoms and function during the past 1 week and the score should reflect the average clinical condition during the week.

6.2.5 Borg Category Ratio 10 Scale

Borg Category Ratio 10 (CR10) scale will be used in the assessment of dyspnoea (Borg GA, 1982). The subjects will be asked to point out a score quantifying their perception on the intensity of dyspnoea on the CR10 scale at rest in sitting and supine positions (immediately before SVC measurements). Orthostatic changes between sitting and supine positions will be calculated.

6.2.6 Epworth Sleepiness Scale

Daytime somnolence will be assessed by the ESS (Johns MW, 1991). The ESS is a self-administered questionnaire with 8 questions. Subjects are asked to rate, on a 4-point scale (0-3), their usual chances of dozing off or falling asleep while engaged in eight different activities. Score 0 indicates 'no chance of dozing' and 3 indicates 'high chance of dozing'. Most people engage in those activities at least occasionally, although not necessarily every day. If one has not engaged recently in a certain activity, the subject will estimate how sleepy he/she would have been during that activity. The ESS score (the sum of 8 item scores, 0-3) can range from 0 to 24. The higher the ESS score, the more pronounced the subject's average daytime sleepiness. The sum score 10 or more suggests medical attention may be required.

6.2.7 Pittsburgh Sleep Quality Index

PSQI is a self-rated questionnaire which assesses sleep quality and disturbances over a preceding 1 month period (Buysse DJ et al., 1989). There are a total of 19 self-rated questions/items which are included in the scoring. These 19 items are combined to form seven component scores (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, daytime dysfunction) each of which has a range of 0-3 points. In all components, a score of 0 indicates no difficulty, while a score of 3 indicates severe difficulty. The seven component scores are then added to yield one global score, with a range of 0-21 points, 0 indicating no difficulty and 21 indicating severe difficulties in all items. The derived variable will be the PSQI global score. Global score of >5 indicates poor sleep quality.

6.2.8 Health care and home care resource use

For possible later use in pharmacoeconomic analysis, the subjects will be given a home diary for recording their use of health and home care resources. The diary will be filled in on a weekly basis. Any hospital inpatient days, other institutional care, any additional hospital emergency unit visits, days in other institutional care, and any outpatient visits to physicians or other relevant service providers will be recorded. In addition, all non-medical assistive devices which the subjects have in use at baseline, are recorded and thereafter all new devices that they start using due to ALS will be recorded in the diary each week. Regarding home care, the diary will contain information of the number of formal (paid/public services) and informal (unpaid e.g. by spouse, other family members, friends or other volunteers) home care hours received. Additionally, information will be collected about the number of times such home care has been received during the night time. Details of the home diary will be described in a separate document.

6.2.9 Other assessments

Subject's status for NIV support, invasive mechanical ventilation by intubation or tracheostomy and survival will be recorded at all visits.

6.3 Pharmacokinetic assessments

6.3.1 Blood sampling

Blood samples for the determination of levosimendan and its metabolites OR-1855 and OR-1896 will be collected. Blood samples will be drawn for the first time at visit 2 (2 weeks) and thereafter during all consecutive visits (except at end-of-study visit and possible additional visits). The samples will be collected in the morning after study drug intake (Table 5).

Table 5. Blood sampling for the determination of levosimendan and its metabolites concentrations

Blood sampling	Visit						
	V2 Week 2	V3 Week 4	V4 Week 8	V5 Week 12	V6 Week 24	V7 Week 36	V8 Week 48
First sample after dosing (min)	30-90	30-90	30-90	30-90	30-90	30-90	30-90
Second sample after dosing (min)	120-240	120-240	-	-	-	-	120-240

Blood samples will be collected also to measure trough concentrations of riluzole before study treatment dosing at baseline and at visits 2, 3 and 8.

Instructions for the collection, handling, storage and transportation of samples will be provided before the start of the study.

6.3.2 Determination of levosimendan, OR-1855 and OR-1896 concentrations in plasma

The concentrations of levosimendan, OR-1855 and OR-1896 in plasma will be determined with a validated liquid-chromatography-tandem mass spectrometry (LC-MS/MS) method.

Bioanalytical details and criteria for acceptance of the results will be described in the bioanalytical plan and reported in the bioanalytical report.

6.3.3 Determination of riluzole concentrations in plasma

The concentrations of riluzole in plasma will be determined with a validated LC-MS/MS method.

Bioanalytical details and criteria for acceptance of the results will be described in the bioanalytical plan and reported in the bioanalytical report.

6.3.4 Calculation of pharmacokinetic variables

Due to the sparse blood sampling, no PK variables will be calculated. However, plasma concentrations of levosimendan, OR-1855 and OR-1896 after the morning dose of the study

treatment at visit 2 (2 weeks) and thereafter during all consecutive visits (excluding the end-of-study visit) and plasma concentrations of riluzole before study treatment dosing at baseline (visit 1) and at visit 2, visit 3 and visit 8 will be collected.

6.4 Biomarker, pharmacogenomic and other specific biological variables

6.4.1 Biomarkers

Spare plasma taken originally for the analysis of levosimendan and its metabolites may be used for exploratory research purposes. The plasma samples will be stored in the sponsor's sample repository to allow possible exploratory biomarker analyses related to the disease state of the subjects and the pharmacodynamics of levosimendan.

A 5 ml blood samples will be collected before dosing at visits 1, 3, 4, 5, 6, 7 and 8. The samples may be used for exploratory biomarker analyses that may give supportive data related to the disease state of the subjects.

Instructions for the collection, handling, storage and transportation of samples will be provided before the start of the study. The plasma samples will be stored in the sponsor's sample repository until analysis.

6.4.2 Pharmacogenomics

A 5 ml blood sample for DNA extraction will be taken once during the study preferably at visit 1 (baseline).

Uses of the extracted DNA sample are:

1. Determination of subject's acetylation status (NAT polymorphisms). This will be determined from all subjects participating in the study and the results will be source data. The samples will be destroyed 6 months after the sponsor has received analysis results.
2. PG assessment, but only if the subject has signed the PG IC.

The PG assessment is done as exploratory research. The objective of the PG research is to assess whether genetic polymorphisms relate to the absorption, distribution, metabolism, excretion, pharmacodynamics or safety of levosimendan. The extracted DNA will be stored in the sponsor's DNA repository for future use.

Instructions for the collection, handling, storage and transportation of samples will be provided before the start of the study.

6.5 Safety assessments

6.5.1 Adverse events

6.5.1.1 Definitions

An AE is any untoward medical occurrence in a study subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not related to the investigational medicinal product. The definition also covers medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

Thus, an AE may be an appearance or worsening of any undesirable sign or symptom, any worsening of the current medical conditions or onset of a new disease, compared with the previous observations or a clinically significant adverse change in a laboratory variable or other diagnostic finding (e.g. ECG).

In this study, day-to-day fluctuation in ALS signs/symptoms (e.g. dysphagia, muscular weakness) not indicating clinically significant progression of the disease should not be reported as an AE.

An SAE is any untoward medical occurrences that at any dose

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation (see section 6.5.1.3 for exclusions)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect, or
- is an important medical event jeopardizing the patient or requiring intervention to prevent a serious outcome (examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; development of drug dependency or drug abuse; overdose or interaction).

Other significant AEs are AEs (other than those meeting the definition of serious) that are of clinical importance and lead to:

- a diagnostic or therapeutic intervention
- discontinuation of the investigational medicinal product
- reduction of its dose
- significant additional concomitant treatment, or

- marked haematological and other laboratory abnormalities

6.5.1.2 Assessment of adverse events

All AEs must be elicited, documented and reported by the investigator to the sponsor from the time that a study subject signs the IC form until the end-of-study visit (14-25 days after the last study treatment administration).

SAEs and other significant AEs should be followed up until resolved or until the event is considered a chronic or stable outcome, or both.

An AE may be notified to the investigator by the study subject (or his/her caregiver) or observed by the investigator clinically, or be an adverse change in laboratory assessment results. The investigator will evaluate the subject's AEs at each visit by asking a standard question such as "Since you were last asked, have you felt unwell or different from the usual in any way?"

The investigator will assess and record the causality and severity of the AEs. Causality should be assessed in relation to the investigational medicinal product (see criteria for causality and severity below).

Causality criteria:

Related: The temporal relationship of the AE/SAE onset to the administration of the investigational medicinal product makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the AE/SAE.

Not related: The temporal relationship of the AE/SAE onset to the administration of the investigational medicinal product makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the AE/SAE.

Severity criteria:

Mild: Discomfort noticed, but it does not affect normal activity.

Moderate: Discomfort sufficient to reduce or affect normal daily activity.

Severe: Incapacitating with inability to work or perform normal daily activity.

From the time that a study subject candidate signs the IC form, newly appearing diagnosed diseases will be recorded on the AE CRF.

Investigators must report all AEs to the sponsor on a specific AE CRF irrespective of their assessment of the causal relationship of the investigational medicinal product to the event.

6.5.1.3 Reporting of serious adverse events by the investigator

The investigator must report all SAEs within 24 hours of becoming aware of an SAE. SAEs must be reported within 24 hours regardless of the time that may have elapsed since the time the event occurred and regardless of the causal relationship of investigational medicinal product to the event.

In this study, the following events will not be reported as SAEs:

- hospitalisation and elective surgery for treatment of pre-existing condition that has not exacerbated during the study
- hospitalisation for procedures and/or evaluations in connection with ALS such as evaluation for the need or initiation of ventilatory support (e.g. NIV) or gastrostomy.

All SAEs should be reported electronically by the investigator or other relevant study centre personnel and submitted by the investigator. Optionally, if the investigator is not able to submit the SAE electronically, a paper version of the SAE form can be completed and sent by e-mail or fax. The SAE reporting contact information can be found on the SAE form and will be filed in the investigator's study file.

If the initial report is reported by phone or e-mail to the study monitor or other contract research organisation (CRO) personnel and the study centre personnel are unable to fill in the SAE electronically within 24 hours, a paper SAE form will be initiated by the person receiving the report. The investigator must report the SAE electronically as soon as possible.

The minimum criteria for SAE reporting are: the event or outcome meets the SAE definition, the event happens to an identifiable study subject, and the event is reported by an identifiable and qualified reporter (usually an investigator or other qualified study centre personnel)

A follow-up report to an SAE should be prepared if any relevant change in the condition of the study subject occurs after the initial report. The follow-up report should be documented as an update to the initial report.

SAEs that occur after the end-of-study visit (14-25 days after the last study treatment administration), should be reported if the investigator feels that there is a reasonable possibility for the event to have been caused by the study subject's participation in the study.

6.5.1.4 Reporting of serious adverse events to competent authorities and ethics committees

The sponsor is responsible for expediting all suspected unexpected serious adverse reactions (SUSARs) as well as other safety issues requiring expedited reporting to the relevant authorities within applicable timelines. These tasks can also be carried out by a delegate of the responsible sponsor.

Notification of the ethics committees (ECs) and institutional review boards (IRBs) about all relevant events (SUSARs, other relevant safety information) will be performed by the sponsor or delegate of the responsible sponsor and/or by the investigator according to applicable regulations.

The Sponsor will inform all investigational sites about suspected unexpected serious adverse reactions (SUSARs) as well as other safety issues according to all applicable regulations.

The expectedness evaluation is required for regulatory reporting and it is performed by the sponsor. The expectedness in this study is evaluated against the Reference safety information section in the current ODM-109: oral levosimendan capsule for treatment of ALS investigator's brochure.

6.5.2 Special situations

Special situations with study treatment are defined as:

- medication error
- overdose
- abuse
- misuse
- interaction

These special situations with study treatment are reported on the Special situations with study treatment CRF even if there is no accompanying AE. All clinical manifestations in relation to these special situations will be reported as AEs or SAEs at the same time using the corresponding section of the CRF.

6.5.3 Pregnancy during the study

Whenever it becomes known that a study subject or a partner of a study subject was pregnant during the exposure to study treatments, the outcome of the pregnancy, delivery, postpartum recovery and the clinical condition of the offspring during the neonatal period should be reported, subject to the study subject's or the partner's consent. A pregnancy follow-up form will be provided to the investigator for completion after the sponsor has received the initial report.

Any case of pregnancy during a clinical study should be reported by the investigator in the same way as an SAE.

6.5.4 Clinical safety assessments

Vital signs (HR, systolic and diastolic BP) and standard 12-lead ECG will be assessed at screening, at each visit during the treatment period before and 30-90 minutes after the morning dose of the study drug, and at the end-of-study visit. At baseline visit, vital signs and ECG will be assessed before and 45-120 minutes after the morning dose of the study drug.

At the screening visit, only supine HR and BP will be measured. At baseline visit, each visit during the treatment period and end-of-study visit, supine HR and BP will be assessed after a 5-minute rest. Subjects will then be asked to stand up and after 3 minutes, standing HR and BP will be measured (orthostatic test). In case the subject is not able to stand, only supine HR and BP will be measured.

A standard 12-lead ECG (central reading) will be recorded with subject lying supine and after a 5-minute rest. 3 baseline recordings of ECG in 1-3 minutes intervals will be done before dosing at visit 1 (baseline). The HR, RR, PR and QT intervals and QRS duration will be analysed in the central ECG laboratory. QTc will be calculated using both Bazett's and Fridericia's correction. Each ECG will be assessed as normal/abnormal by the central ECG laboratory. Clinical significance of the abnormalities will be assessed by the investigator.

Additional 1-2 assessments of 12-lead ECG should be performed within 5-10 minutes from each other (all before the morning dose), if the HR in the first recording was increased from baseline (visit 1) as follows:

- > 20 bpm if the subject is receiving the study treatment 1 mg b.i.d., to assess the need for dose reduction (see section 5.3.2)
- > 30 bpm if the subject is receiving the study treatment 1 mg once daily, to assess the need for discontinuation (see section 4.5)

If the HR increase is not repeated in the second ECG, no further ECG recordings are needed.

Physical examination will be performed at screening and the end-of-study visit. Weight will be measured at screening, at visits 1, 3, 4, 5, 6, 7, 8 and at the end-of-study visit. Height will be recorded at screening only.

In case there are abnormal findings, control assessments can be performed according to the judgement of the investigator.

6.5.5 Assessment of suicidality

Suicidality will be assessed by asking the following standard questions at screening, at each visit during the treatment period before dosing, and at the end-of-study visit:

Screening:

1. During the past 3 months, have you wished you were not alive anymore or had any thoughts about ending your life?
2. During the past 3 months, have you done anything or prepared to do anything to end your life?

Treatment period and end-of-study:

1. Since the last visit, have you wished you were not alive anymore or had any thoughts about ending your life?
2. Since the last visit, have you done anything or prepared to do anything to end your life?

The second question (2) will be asked only in cases where the answer to the first question (1) has been ‘yes’.

6.5.6 Laboratory safety assessments

The following laboratory tests will be taken at the screening visit after at least 10 hours of fasting:

Haematology:

- Haemoglobin
- Haematocrit
- Erythrocyte count
- Leukocyte count
- Platelet count
- Differential count (lymphocytes, monocytes, eosinophils, neutrophils, basophiles)
- Mean corpuscular volume

- Mean corpuscular haemoglobin

Chemistry:

- Albumin
- Alkaline phosphatase (ALP)
- Bilirubin total
- Bilirubin conjugated
- Calcium
- Creatine kinase
- Creatinine
- Creatinine clearance
- C-reactive protein
- Gamma-glutamyl transferase (GGT)
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Glucose
- Lactate dehydrogenase
- Potassium
- Sodium
- Troponin T
- TSH

Urinalysis:

- Glucose
- Protein
- Erythrocytes
- Leucocytes

Serology:

- Hepatitis B surface antigen (HBsAg)
- Hepatitis C virus antibody (HCVAb)

Haematology, chemistry (except TSH) and urinalysis will be repeated at each visit during the treatment period and at the end-of-study visit.

A pregnancy test for female subjects of childbearing potential will be performed at screening and baseline and at each visit during the treatment period (except visit 2, i.e. 2 weeks after baseline) and at the end-of-study visit.

The investigator will review all laboratory results and assess them for clinical significance. In case there are abnormal findings, control assessments can be performed according to the judgement of the investigator.

Instructions for the collection, handling, storage and transportation of samples will be provided before the start of the study.

6.6 Changes implemented due to COVID-19 pandemic

In case visits 7 (Week 36), 8 (Week 48) and/or end-of-study visit (14-25 days after visit 8) described in Table 1 and in sections 6.1.2 and 6.1.5 cannot be conducted at the study centre

within the visit window due to restrictions related to COVID-19, a telephone visit (could also be a home visit or video call) should be arranged.

The telephone visit should include all protocol assessments that can be performed remotely (see table below) and should take place within the original visit window. The decision to replace the study centre visit with a telephone contact will be made by the investigator case by case, and the sponsor should be informed of each case as early as possible.

In addition to the telephone visit, the investigator should make reasonable efforts to obtain 12-lead ECG, laboratory safety assessments and vital signs for each visit. These measurements may include local practitioners and resources. In case these assessments, at least vital signs, cannot be obtained within a reasonable time after the visit 7 telephone contact, the benefit-risk ratio for the subject to continue in the study should be reassessed.

Visit 8 telephone contact should take place at the time of stopping study medication. All safety data which are possible to obtain locally should be collected prior (on treatment) or at the remote visit: the study treatment should be stopped at the time of the remote visit.

The following assessments will not be performed at telephone visits: SVC supine, weight, sitting or orthostatic changes in vital signs, blood sampling for levosimendan, OR-1855 and OR-1896 PK, for riluzole concentration and for exploratory biomarkers, and physical examination.

All arrangements described in this section apply only to the extent that protocol requirements cannot be met because of COVID-19 restrictions. Study centre visits should take place to the extent possible and usual protocol requirements adopted for all subjects as soon as COVID-19 limitations permit.

Exceptional measures taken in response to COVID-19 and their impact on study results, such as tests done in a local laboratory, will be explained, assessed and reported in the clinical study report following ICH E3.

Procedures at remote visits

Time	Visit 7 Week 36	Visit 8 Week 48	End-of-study
HR and BP	x[1]	x[1]	x[1]
12-lead ECG	x[2]	x[2]	x[2]
Laboratory safety assessments[3]			
Haematology	x	x	x
Chemistry	x	x	x
Urinalysis	x	x	x
Pregnancy test for females of childbearing potential	x[4]	x[4]	x[4]
Study treatment	x	x	
ALSFRS-R	x	x	
CGI	x	x	
Borg CR10 scale	x	x	x
ESS	x	x	
PSQI	x	x	
Use of health care and home care resources use and non-medical assistive devices	x	x	
Assessment of suicidality	x	x	x
Status for NIV support, invasive mechanical ventilation by intubation or tracheostomy and survival	x	x	x
AEs and SAEs	x	x	x
Medical history and current medical conditions	x	x	x
Concomitant treatments	x	x	x

[1] Only in supine position after at least 5 min rest before the morning dose of the study treatment. The assessment should preferably be done by a health care professional. The use of alternative methods, including home devices are subject to sponsor approval.

[2] The measurement should be taken using the local ECG device, preferably before the morning dose of the study treatment, after at least 5 min rest. A copy of the trace (paper or electronic) should be provided.

[3] Standard biochemical, electrolyte and haematological test panels of the local laboratory will be accepted. A copy of the laboratory report with local reference range should be provided.

[4] Test kits for urine pregnancy test should be provided to the subject for testing at home (unless taken in a local laboratory).

7. DATA COLLECTION AND MANAGEMENT

The investigators and study centre personnel will prepare and maintain accurate source data for each study subject about clinical findings specified in the protocol. Source data include patient records, laboratory results and questionnaires if not collected electronically. The data from source documents will be recorded into an electronic data capture (EDC) system, Medidata Rave (Medidata Inc), using eCRFs at the study centre. Safety laboratory, spirometry and ECG will be analysed centrally and results will be uploaded to the Rave system and electronically signed by the investigator. All data on the eCRFs must be verifiable in the source data or patient records unless eCRF data are declared as source data.

Investigators and other relevant study centre personnel will be trained to use the eCRFs. After completion of training, they are provided with user names and authorised access to enter and correct data on the eCRFs.

Electronic queries about missing, misleading, incomplete or illogical data will appear in the EDC system. An audit trail within the system will track all changes/corrections made. The investigator has to confirm the content of the eCRF with an electronic signature.

Individual data fields in the EDC system may be locked on an ongoing basis during the study. The fields may be unlocked if further updates are needed. When all data have been entered and all queries resolved, the whole database will be locked. Only authorised and well-documented updates to the study data are possible after the database lock.

Further details regarding data collection and management are presented in the data management plan.

8. STATISTICAL METHODS

8.1 Statistical hypotheses

The main statistical hypothesis of the study is to show the superiority of levosimendan against matching placebo in SVC (supine) at 12 weeks. The statistical hypothesis will be two-sided comparing change from baseline to 12 week pre-dose sample using the mixed model for repeated measures (MMRM) at a nominal 5% significance level.

$$H0: \Delta SVC\%_{\text{levosimendan}} = \Delta SVC\%_{\text{placebo}}$$

$$H1: \Delta SVC\%_{\text{levosimendan}} \neq \Delta SVC\%_{\text{placebo}}$$

8.2 Estimation of sample size

Sample size estimation is primarily based on the study 3119001 LEVALS SVC (supine) results (Table 6). The LEVALS study had placebo controlled data only up to 2 weeks and therefore extrapolation of the results is needed. The placebo effect from phase II tirasemtiv trial BENEFIT-ALS (Shefner JM et al., 2016) is also evaluated.

Assuming at least 3.6% difference between treatment arms and common standard deviation of 10%, 164 evaluable subjects have 90% power to detect a statistically significant difference at two-sided 5% level using 1:1 allocation ratio and two group T-test. Randomisation of 450 subjects to study using a 2:1 allocation ratio (approximately 300 levosimendan and 150 placebo treated subjects) allows approximately 20% drop out rate.

Based on dexpramipexole phase III study EMPOWER (Cudkowicz ME et al., 2013) 12 point reduction in total ALSFRS-R score and a 15% mortality rate at 12 months can be assumed in placebo treated patients. Assuming 10 point decline, with 8 point standard deviation, in ALSFRS-R and 20% relative improvement in survival 450 patients provide approximately 80% simulated power to detect statistically significant difference using joint rank test. With 450 subjects and assuming 48 week levosimendan event rate of 73% and hazard ratio of 0.70, time to respiratory event has approximately 70% power to detect statistically significant difference using log-rank test.

Table 6. Mean (SD) change from baseline SVC (supine)

Treatment	2 weeks	12 weeks
Levosimendan 1 mg x 2	+2.4 (8.3)	-4.9
Levosimendan 1 mg x 1	+0.8 (8.6)	
Placebo	-3.6 (9.4)	-17.7[1]
Placebo (BENEFIT-ALS)	-1.4	-8.5

[1] Extrapolated from the data
SD = standard deviation

8.3 Analysis populations

Intent-to-treat (ITT) dataset, including all randomised subjects, will be used for the primary evaluation. Sensitivity analyses using full analysis set (FAS) and per-protocol (PP) dataset will be performed and described in the SAP. FAS includes all treated subjects with at least one post baseline SVC measurement. PP dataset includes all randomised subjects excluding subjects with relevant major protocol deviations. All subjects who received any study treatment will be included in the evaluation of safety. Subject classification will be completed during the blind review before the database lock and opening the treatment code.

8.4 Statistical analyses

Statistical analyses will be described in more detail in the SAP.

8.4.1 Demographic and other baseline characteristics

All relevant demographic and baseline characteristics will be summarised using descriptive statistics. The number and reasons for discontinuations will be listed and tabulated by treatment groups.

An authorised person will code medical history and concomitant diseases using standard coding dictionaries.

8.4.2 Treatment compliance and extent of exposure

The number of exposed subjects, the number of dispensed and returned study treatments and the duration of study treatment exposure will be tabulated with descriptive statistics.

8.4.3 Analysis of efficacy

The primary efficacy variable SVC (supine) will be compared between the treatment arms using the MMRM with between subject effects for treatment, centre and randomisation strata, time as within subject effect, and baseline value (visit 1) as a covariate. Primary efficacy will be evaluated at 12 weeks using contrasts to obtain estimates from the primary model including all SVC measurements prior to 12 weeks. Any missing data will be imputed using multiple imputation method. Effect of missing data imputation will be evaluated analysing observed data only and estimating the slope of decline using the random slope model. Several sensitivity subgroup analyses will be performed and defined in the SAP. Additional evaluations will be performed including all data through 48 weeks, using the MMRM.

Secondary efficacy endpoints will be tested in following hierarchy to preserve the overall alpha level:

1. Combined assessment of ALSFRS-R function and survival through 48 weeks
2. Time to respiratory event through 48 weeks
3. CGI through 48 weeks
4. Supine Borg CR 10 scale at 12 weeks
5. Slope of decline in respiratory function of ALSFRS-R through 48 weeks

The efficacy variables time to respiratory event, time to NIV or death, time to SVC (supine) decline of 20%, time to SVC (supine) ≤ 50% of predicted, time to ALSFRS-R total score decline of 20% and time to decline in ALSFRS-R respiratory domain will be evaluated using the Cox's proportional regression model. The model will include effect for treatment and will be adjusted for randomisation strata. Premature discontinuations will be censored from the analyses. Censoring rules will be defined in the SAP.

The efficacy variables ALSFRS-R total score and its subdomain scores will be performed through 48 weeks. Primary analysis will combine change from baseline to 48 weeks and survival through 48 weeks in joint rank analysis (Berry JD et al., 2013) using Wilcoxon-Mann-Whitney test. Slope of decline will be estimated using the random slope model. Additional analyses will be conducted using the MMRM and estimates for individual time points will be done using contrasts. Imputation of missing data using the multiple imputation method will be considered.

CGI, Borg CR10 (sitting, supine and orthostatic changes), ESS and PSQI will be evaluated using the MMRM and estimates for individual time points will be done using contrasts. In addition categorical analyses for ESS and PSQI will be performed using repeated measures logistic regression using the generalised estimating equation (GEE) approach.

All efficacy data will be listed and summarised over the time and by treatment groups using appropriate descriptive statistics. Health and home care resource use data will be summarised in the study report, but any further analysis for potential pharmacoeconomic purposes will be reported separately.

8.4.4 Pharmacokinetic analysis

Levosimendan, OR-1855, OR-1896 and riluzole concentrations will be summarised using descriptive statistics. Metabolites OR-1855 and OR-1896 will be further summarised by subject acetylation status.

8.4.5 Population PK/PD modelling

A population pharmacokinetic/pharmacodynamic (PK/PD) model between OR-1896 and OR-1855 exposure and efficacy related endpoints will be explored. This will include building population PK model, and characterising the concentration effect relationship with PD endpoints.

8.4.6 Evaluation of pharmacogenomics

Acetylation status will be determined from all subjects participating in the study and the results will be source data. Acetylation status will be summarised by treatments groups. The effects of

acetylation status on OR-1896 and OR-1855 pharmacokinetics and on SVC and HR will be evaluated using analysis of variance (ANOVA).

In the context of this study, genetic polymorphisms will be analysed in relation to significant variation or specific scientific questions in PK, PD or safety variables of levosimendan. These pharmacogenomic analyses apply only to those subjects who have signed a separate IC for PG. If such analysis is performed, the results will be reported in a separate report.

8.4.7 Evaluation of exploratory biomarkers

In the context of exploratory biomarker analyses, microRNA, proteins or biochemical metabolites may give supportive data related to the disease state of the patients and pharmacodynamics of levosimendan. If such analysis will be performed, the results will be reported in a separate report.

8.4.8 Safety analysis

An authorised person will code AEs, prior and concomitant medications using standard coding dictionaries.

8.4.8.1 Analysis of adverse events

AEs reported during the study will be classified by system organ classes and preferred terms using the Medical Dictionary for Regulatory Activities (MedDRA) coding system. The AEs will be displayed in a frequency table. The number and proportion (%) of subjects having each AE will be given, the severity of AEs (mild, moderate, severe) and causality to the study treatment will be evaluated. SAEs and other significant AEs will be evaluated case by case. AEs occurring before and after the start of study treatment will be reported separately.

8.4.8.2 Clinical safety analysis

Vital signs (supine and orthostatic HR, systolic and diastolic BP) as well as standard 12-lead ECG variables will be tabulated by treatment. Potential treatment effects will be analysed using the MMRM. The number of subjects with QTc interval prolongation will be classified and summarised. The details of the MMRM model and categorical analyses will be defined in the SAP. Abnormal ECG findings, special situations and physical examination findings will be summarised by treatment group using descriptive statistics.

8.4.8.3 Laboratory safety analysis

Safety laboratory results will be primarily evaluated using descriptive statistics and line plots only. Potential treatment effects may be analysed using the MMRM, if deemed necessary. The frequencies of normal and abnormal safety laboratory findings will be summarised based on the provided reference ranges of the respective laboratory, and the clinical significance of the findings will be evaluated.

8.4.8.4 Analysis of prior and concomitant treatments

Prior and concomitant treatments will be coded using the anatomical therapeutic chemical (ATC) classification system. The number and percentage of subjects using concomitant treatments will be summarised by pharmacological subgroup and chemical substance.

8.4.8.5 Analysis of suicidality

Suicidality will be summarised by treatment group using descriptive statistics.

8.5 Interim analyses

Interim/futility analyses will be done when approximately 50% and 100% of the subjects have been treated for 12 weeks. Futility will be evaluated using SVC% response and time to respiratory event endpoint. Based on the estimation, approximately 35% and 75% of the respiratory events have cumulated at the time of planned analyses. Stopping criteria will be based on O'Brien-Fleming type of boundaries (O'Brien PC et al., 1979, Lan GKK et al., 1983). Exact alpha spending for futility analyses will be based on the actual amount of information used and are detailed in a DSMB charter. An independent DSMB will perform and evaluate the interim/futility analyses and make recommendations for the study conduct.

9. DATA QUALITY ASSURANCE

9.1 Training

An investigators' meeting will be arranged for the investigators and other relevant study centre personnel. This meeting will include a review of the protocol, CRF completion and study procedures as well as training on efficacy assessments like supine SVC and ALSFRS-R.

The investigators will ensure that appropriate training relevant to the study is given to the medical, nursing and other personnel involved in the study. The investigators will also ensure that any information relevant to the conduct of the study is forwarded to other relevant study centre personnel.

9.2 Case report forms

Electronic queries about missing, misleading, incomplete or illogical data will appear in the EDC system. An audit trail within the system will track all changes/corrections made. The investigator has to confirm the content of the eCRF with an electronic signature.

9.3 Monitoring, audits and inspections

The study monitor will visit the study centre regularly as agreed by the investigator and the sponsor. The study monitor will ensure that the study complies with good clinical practice (GCP) and applicable regulatory requirements and that the protocol is followed in all aspects, including the randomisation procedure, accurate recording of results, reporting of AEs, drug accountability and record keeping. Furthermore, it will be verified that the clinical facilities remain appropriate,

and that the CRFs correspond with source data. Further details regarding monitoring are presented in the monitoring manual.

The study may be audited by independent representative(s) of the sponsor or inspected by the competent authorities (CAs). For these purposes, the study monitor, auditors and inspectors will be allowed direct access to hospital or patient records/source data of the study subjects, original laboratory data etc., as far as they are related to the study.

It is essential that the investigator and other relevant members of the study centre team are available during the monitoring visits, audits and inspections, and that they devote sufficient time to these processes.

9.4 Laboratories and other vendors

A central laboratory will be used for laboratory safety measurements, ECG analyses and spirometry.

Details regarding laboratory safety measurements, ECG analyses and spirometry are presented in the separate instructions. Quality certificates are required from all safety laboratories.

Bioanalytics will be performed using validated methods.

10. FURTHER REQUIREMENTS AND GENERAL INFORMATION

10.1 Investigators and study administrative structure

10.1.1 Investigators

Should the investigator transfer one of his/her responsibilities to other members of the study centre team, he/she must have this approved by the representative of the sponsor and documented.

In the event of changes in key study centre team members, the responsible investigator must ensure that the successor is fully informed and capable of following the procedures.

A curriculum vitae in English must be obtained from all investigators who sign the protocol, and from other relevant persons.

10.1.2 Data and safety monitoring board

An independent DSMB will be established for the study. The duty of the DSMB is to protect the ethical and safety interests of the study subjects and all others who may possibly be exposed to study treatments. The DSMB safety meetings will occur by approximately bimonthly frequency. The DSMB will also perform and evaluate formal futility/interim analyses and make recommendations for the study conduct.

Further details regarding the composition and responsibility of the DSMB are presented in a DSMB charter.

10.2 Amendments to the study protocol

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by the sponsor, and when required by the EC/IRB or CA. Only in the event of a need to eliminate an immediate hazard(s) to study subjects, the investigator may implement a deviation from the protocol without prior approval.

Any change or addition to the approved study protocol may lead to suspension of the study or its results.

Any changes to the study protocol are subject to prior discussion with, and approval by, the sponsor and the study coordinating investigator. As a general rule, protocol amendments should be approved according to the same procedures as the study protocol.

Amendments are regarded as substantial, where they are likely to have a significant impact on the safety, physical or mental integrity of the study subjects, or the scientific value of the study. An approval of the responsible EC/IRB or CA shall be obtained before substantial amendments may be implemented, unless local regulations are different.

If an amendment contains only minor changes (typically administrative) not affecting the safety, physical or mental integrity of the study subjects, or the scientific value of the study, the EC/IRB or CA need not to be notified and in-house approval (including approval by the study coordinating investigator) is adequate.

10.3 Insurance

The sponsor will provide clinical trial liability insurance for study subjects in all participating countries according to local regulations.

10.4 Financial disclosure

The investigator will be required to disclose any financial arrangement whereby the value of the compensation for conducting the study could be influenced by the outcome of the study; any significant payments of other sorts from the sponsor, such as a grant to fund on-going research, compensation in the form of equipment, retainer for on-going consultation, or honoraria; any proprietary interest in oral levosimendan; any significant equity interest in the sponsor as defined in the US Code of Federal Regulations [21 CFR 54 2(b)].

In consideration of participation in the study, the sponsor will pay the investigator, or nominated payee the sums set out in the payment schedule attached to the Investigator Agreement.

10.5 Completion of the study

The end of the study is defined as the date of the last subject's last visit or last contact with the study site.

Study centres will be closed upon study completion. A study centre is considered closed when all required documents and study supplies have been collected and a study completion/termination visit has been performed.

The sponsor reserves the right to prematurely terminate the study, or recruitment to the study, for valid scientific or administrative reasons. After decision to prematurely terminate the study, the investigator must contact all participating study subjects within 7 days, and invite them for an end-of-study visit.

10.6 Reports, publications and communication of results

Orion wishes to collaborate with the investigators to publish the results as timely as possible, without compromising accuracy or industrial property rights. The preparation, submission and authorship for publications containing the study results shall be in accordance with a process determined by mutual written agreement among the sponsor and participating investigators/institutions, and in accordance with international criteria for authorship; see International Committee of Medical Journal Editors recommendations, available at http://www.icmje.org.

Orion remains the exclusive owner of the study data defined by the protocol.

This study will be registered in one of the acceptable registries before the enrolment of the first subject.

10.7 Retention of records

The investigator agrees to keep the following documentation in the investigator's study file: study subject records, including a subject screening log, a subject identification list, all original signed IC forms, a copy of CRFs and records of drug dispensing.

The study files at the study centres will be stored in the respective archives for 15 years, after which the sponsor will be contacted and the possibility of future archiving will be mutually agreed upon.

11. ETHICS

11.1 Ethics committee

The study protocol, subject information sheet, IC form, and all other necessary documents will be submitted to an independent EC/IRB for review according to local regulations.

The investigator is responsible for obtaining a favourable opinion from the EC/IRB for the study, submitting any amendment(s), and communicating study-related safety issues as requested by the EC/IRB. The investigator should file all correspondence with the EC/IRB in the investigator's study file. Relevant copies of this correspondence should be forwarded to the sponsor.

11.2 Ethical conduct of the study

The study will be conducted in accordance with the Declaration of Helsinki guiding physicians in biomedical research involving human subjects.

The study shall not be initiated before favourable opinion from the EC/IRB and approval from the CA has been obtained for the protocol, including its appendices.

The study will be conducted in compliance with the protocol, GCP (ICH/135/95) and applicable regulatory requirements. A substantial amendment shall not be implemented until the protocol amendment has received favourable opinion from the EC and approval from the CA. Only in case of the need to eliminate an immediate hazard(s) to study subjects, the investigator may implement deviation from the protocol without prior favourable opinion from the EC and approval from the CA for the protocol amendment.

In case of serious breaches, the Medicines and Healthcare Products Regulatory Agency (MHRA) GCP Inspectorate must be notified according to MHRA Guidance for the notification of serious breaches of GCP or the study protocol (see http://www.mhra.gov.uk).

11.3 Subject information and informed consent

The investigator will ensure that each subject candidate is fully informed about the objectives and procedures of the study. The investigator will also explain any possible risks with participating in the study and answer all questions regarding the study. In the case that the subject is unable to sign the consent form due to muscular weakness, a witness may sign the form to indicate that the subject has given oral consent to participate in the study. In such cases, the investigator must also explain the study to the witness at the same time as they explain the study to the participant. After this, the subject will be given sufficient time to make a decision regarding participation in the study.

Subjects will be informed of their right to discontinue the study at any time without their medical care or legal rights being affected. Subjects will also be informed that representatives of the sponsor or CA may inspect relevant parts of their medical records and study data.

The investigator will obtain a signed and dated consent from each subject before any study related procedures are performed. A copy of each signed and dated IC will be given to the subject. The investigator should confirm the receipt of every IC by entering the date of the consent both on the subject's CRF and also on the subject screening log and identification list.

11.4 Subject data protection

Information collected during the course of the study will be stored in a database and used in the further development of oral levosimendan and thereafter for as long as the information is relevant to patient care. The use includes the transfer of data to CAs in the European Union, the USA or other countries for the purpose of obtaining and maintaining marketing authorisations. All information is handled confidentially and according to local laws and regulations.

The study subjects can be identified in the CRFs only by study subject number, birth year and sex.

The confidentiality of PG data will be protected according to local laws and regulations.

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13. SUMMARY OF CHANGES TO AMENDMENT 2

13.1 Revision history

Protocol and amended protocols	Date	Applies to
Clinical study protocol	20 October 2017	Global
Amendment 1 - Amended clinical study protocol	05 January 2018	Global
Amendment 2 - Amended clinical study protocol	17 April 2018	Global
Amendment 3 - Amended clinical study protocol	26 March 2020	Global

Country specific amendments	Date	Applies to
Amendment 1	27 Mar 2018	Sweden
Amendment 1	06 Apr 2018	The United Kingdom
Amendment 1	16 Apr 2018	Belgium

13.2 Summary of changes

The changes incorporated in this amended protocol from the Amendment 2 – Amended clinical study protocol dated 17 April 2018 are described in the following. The modifications of the text are indicated by ~~strikethrough~~ (for deletions) and underline (for additions).

The following changes were made due to COVID-19 pandemic:

Section	Previous text	New text	Rationale for the change
6.6 Changes implemented due to COVID-19 pandemic	-	Whole text is new, see the section 6.6.	New section describing changes due to COVID-19 pandemic added

In addition,

- the table of contents was updated to reflect the headings and pagination of the current document.
- the title page and header and footer sections were updated to reflect the version details of the current document.
- the approver information was updated.

14. APPENDICES

Appendix 1. Investigator signature

3119002 clinical study protocol amendment 3

Written by: [redacted]

Date dd.mm.yyyy (UTC)	Justification	Electronically signed by
26.03.2020 12:49:48	Approved	[redacted]
26.03.2020 13:07:03	Approved	[redacted]
26.03.2020 13:26:45	Approved	[redacted]