LeoPARDS

Levosimendan for the Prevention of Acute oRgan Dysfunction in Sepsis

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This protocol describes the LeoPARDS study and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study, but centres entering participants for the first time are advised to contact the trials centre to confirm they have the most recent version.

Problems relating to this trial should be referred, in the first instance, to the study coordination centre.

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.
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GLOSSARY OF ABBREVIATIONS

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse Event</td>
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<tr>
<td>AKI(N)</td>
<td>Acute Kidney Injury (Network)</td>
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<tr>
<td>AR</td>
<td>Adverse Reaction</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Tri-Phosphate</td>
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<tr>
<td>BNP</td>
<td>Brain Natriuretic Peptide</td>
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<td>BP</td>
<td>Blood Pressure</td>
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<td>CCMDS</td>
<td>Critical Care Minimum Data Set</td>
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<tr>
<td>CI</td>
<td>Confidence Intervals</td>
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<tr>
<td>CPAP</td>
<td>Continuous Positive Airway Pressure</td>
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<tr>
<td>CRF</td>
<td>Case Report Form</td>
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<td>CTU</td>
<td>Clinical Trials Unit</td>
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<tr>
<td>DMEC</td>
<td>Data Monitoring and Ethics Committee</td>
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<tr>
<td>DNAR</td>
<td>Do Not Attempt Resuscitation</td>
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<tr>
<td>eCRF</td>
<td>Electronic Case Record Form</td>
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<td>EDC</td>
<td>Electronic Data Capture</td>
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<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
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<td>GMP</td>
<td>Good Manufacturing Practice</td>
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<tr>
<td>HME</td>
<td>Heat and Moisture Exchange</td>
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<tr>
<td>IEC</td>
<td>Independent Ethics Committee</td>
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<tr>
<td>ICU</td>
<td>Intensive Care Unit</td>
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<tr>
<td>I-FABP</td>
<td>Intestinal Fatty Acid Binding Protein</td>
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<tr>
<td>IMP</td>
<td>Investigational Medicinal Product</td>
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<tr>
<td>ISF</td>
<td>Investigator Site File</td>
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<tr>
<td>IV</td>
<td>Intravenous</td>
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<tr>
<td>MAP</td>
<td>Mean Arterial Pressure</td>
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<tr>
<td>MHRA</td>
<td>Medicines and Healthcare products Regulatory Agency</td>
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<tr>
<td>NGAL</td>
<td>Neutrophil Gelatinase-Associated Lipocalin</td>
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<tr>
<td>OR</td>
<td>Odds Ratio</td>
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<tr>
<td>PerLR</td>
<td>Personal Legal Representative</td>
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<tr>
<td>PIS</td>
<td>Patient Information Sheet</td>
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<tr>
<td>PK</td>
<td>Pharmacokinetic</td>
</tr>
<tr>
<td>ProLR</td>
<td>Professional Legal Representative</td>
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<tr>
<td>REC</td>
<td>Research Ethics Committee</td>
</tr>
<tr>
<td>RRT</td>
<td>Renal Replacement Therapy</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
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<tr>
<td>S_{cvo}O_{2}</td>
<td>Central Venous Oxygen Saturation</td>
</tr>
<tr>
<td>SIRS</td>
<td>Systemic Inflammatory Response Syndrome</td>
</tr>
<tr>
<td>SmPC</td>
<td>Summary of Product Characteristics</td>
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<tr>
<td>SOFA</td>
<td>Sequential Organ Failure Assessment</td>
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<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>SUSAR</td>
<td>Suspected Unexpected Serious Adverse Reaction</td>
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<tr>
<td>TSC</td>
<td>Trial Steering Committee</td>
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<tr>
<td>TMF</td>
<td>Trial Master File</td>
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<tr>
<td>TMG</td>
<td>Trial Management Group</td>
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KEYWORDS
Septic shock, levosimendan, acute kidney injury, organ failure
1 STUDY SUMMARY

TITLE Levosimendan for the Prevention of Acute Organ Dysfunction in Sepsis

DESIGN Double-blind randomised placebo-controlled trial

AIMS 1. Does levosimendan reduce the incidence and severity of acute organ dysfunction in adult patients who have septic shock? 2. What is the effect of levosimendan on individual organ function in septic shock? 3. What is the safety profile of levosimendan in this group of patients?

POPULATION Adult patients who have septic shock managed in ICU. 516 patients will be recruited for this trial

ELIGIBILITY Adult patients who have sepsis and cardiovascular failure requiring vasopressors to maintain blood pressure despite adequate fluid resuscitation

TREATMENT Levosimendan 0.05 to 0.2 µg/kg/min vs. placebo within 24 hours of meeting inclusion criteria

DURATION of INTERVENTION 24 hours

PRIMARY OUTCOME Mean SOFA score on ICU after randomisation

SECONDARY OUTCOMES Oxygen Delivery (ScvO2) & Cardiac output
Incidence & duration of renal failure (AKIN criteria)
Serum bilirubin
Time to extubation
28-day, hospital and three & six-month survival
ICU and hospital length of stay, and ICU-free days
Duration of renal replacement therapy
Days free from catecholamine therapy

Secondary mechanistic outcomes:
- Acute kidney injury – serum / urinary biomarkers
- Myocardial dysfunction – serum biomarkers
- Systemic inflammation – multiplex inflammatory biomarker assay
**SCHEMATIC TRIAL PLAN**

**LeoPARDS CONSORT Flow Diagram**

ICU

- Daily screening in 25 participating ICUs
  - N ~14,000

Patients with septic shock assessed for eligibility
- n = 2060

Within 24 h of meeting inclusion criteria

Randomised to LeoPARDS study
- n = 516

- Excluded
  - Failure to fulfill inclusion criteria or presence of exclusion criteria
    - (n = 1030, 50% of shock patients)
  - Consent declined / not obtained
    - (n = 514, 25% of eligible patients)

Placebo
- 24h infusion
  - n = 258

Levosimendan
- 0.05-0.2 μg/kg/min for 24h
  - n = 258

Analysis
- n = 500

**Primary outcome**
- Mean SOFA score in ICU

**Secondary outcomes**
- Organ specific endpoints (cardiovascular, renal, abdominal & respiratory)
  - ICU free days
  - ICU and hospital length of stay
  - Duration of renal replacement therapy
  - Days free from catecholamine therapy
  - Adverse events
  - CCMDS data
  - ICU, hospital, 3 & 6 month mortality
  - Cardiac, renal biomarkers
  - Multiplex cytokine assays

Loss to follow-up for primary outcome measure; withdrawal of consent after recruitment
- Estimated ~3%
2 INTRODUCTION

2.1 BACKGROUND

Severe sepsis is responsible for approximately 30% of all admissions to intensive care in the UK, yet despite improvements in care, the mortality from severe sepsis remains high. According to data from the Intensive Care National Audit and Research Centre, the incidence of severe sepsis has increased by 68% over a nine-year period, such that the total number of severe sepsis cases in the UK is in excess of 45,000 per annum and with a hospital mortality rate of approximately 45% [1]. Mortality rates increase with increasing number of organ failures [2, 3]. In particular, acute renal failure (ARF) in severe sepsis is an independent risk factor for death (odds ratio 2.1) [4].

It was estimated a decade ago that treating critically ill patients who have sepsis costs the NHS more than £700 million [5]. As the population ages and receives more complex medical treatments the incidence of sepsis, associated mortality and morbidity, and costs will continue to rise. Therefore severe sepsis is an extremely important health care problem.

Levosimendan

Levosimendan is a licensed treatment for decompensated heart failure in 48 countries around the world. It acts by sensitising the myocardium to calcium so that a greater ventricular contraction (and thus stroke volume) can be achieved for the same intracellular calcium concentration, thereby reducing the workload of the failing heart [6]. The drug itself has a short plasma half-life of approximately 1 hour, is around 95% bound to plasma proteins and is fully metabolised in the liver and intestine into both active and inactive metabolites. However, the haemodynamic effects are maintained for up to 7 days after a single 24-hour infusion of levosimendan due to the effects of the active metabolite, OR-1896, which has an elimination half-life of approximately 80 hours [7].

Extensive animal and human investigations have concluded that the mechanism of action of levosimendan also includes vasodilatation, mediated by activation of ATP-sensitive sarcolemmal K-channels, and activation of ATP-sensitive mitochondrial K-channels [8]. This in turn, may lead to the maintenance of mitochondrial volume and reduction of calcium overload seen in ischaemia, thereby preserving mitochondrial function [9]. Levosimendan has also been shown to possess anti-inflammatory properties [10, 11]. Post-licensing, levosimendan has been extensively investigated in patients with acute heart failure due to a variety of aetiologies.

As part of the systemic inflammatory response, myocardial dysfunction is seen in over 50% of patients with severe sepsis [12]. The likely mechanism of myocardial dysfunction is a combination of altered calcium trafficking and reduced troponin sensitivity to calcium [13] and its presence contributes to multiple organ failure including acute renal failure. The calcium sensitising and anti-inflammatory actions of levosimendan provide a strong biological rationale for its use in sepsis. In addition, conventional vasoactive support using catecholamines such as noradrenaline and dobutamine may result in sympathetic overstimulation and a range of adverse effects [14]. Evidence of lack of benefit from trials comparing different catecholamine regimens [15], increased mortality in patients exposed to a greater vasopressor load [16] and the observation of higher plasma catecholamine levels in non-survivors compared to survivors of critical illness [17], all provide further evidence of possible harm from conventional catecholamine therapy.

Levosimendan in sepsis – animal studies
The use of levosimendan in severe sepsis has been studied in a range of animal models of sepsis. In an ovine septic shock model, the combination of levosimendan (0.2 µg/kg/min) and vasopressin as opposed to noradrenaline and vasopressin was associated with improved myocardial, pulmonary and renal function [18]. In a mouse model of septic shock, Zager and colleagues observed that levosimendan protected against acute renal failure, likely due to vasodilatation in the kidney as a result of levosimendan inducing K-ATP channel activation [19]. Similarly, in a rat model of sepsis, Fries and colleagues demonstrated an improvement in microvascular perfusion in the buccal mucosa of animals given levosimendan 0.3 µg/kg/min [20]. Dubin and colleagues evaluated higher doses of levosimendan (100µg loading dose followed by an infusion of 1.6 µg/kg/min) in an ovine model, demonstrating that levosimendan prevented the reduction in mesenteric oxygen delivery that was seen in the control group animals [21]. In a porcine septic shock model, high dose levosimendan attenuated the increase in pulmonary vascular resistance and improved both hepato-splanchnic and systemic blood flow compared to control animals [22]. An improved responsiveness to noradrenaline was also seen in this latter study.

In two studies from the same group, both in porcine models of sepsis, levosimendan failed to show improvement in hepato-splanchnic perfusion compared to both placebo and dobutamine as seen in other studies [23, 24]. These results were felt, in part, to be due to the failure to adequately fluid resuscitate the animals and restore mean arterial pressure prior to commencing levosimendan [25].

**Levosimendan in sepsis – human studies**

In man, evidence of potential benefit from levosimendan in severe sepsis comes from a combination of case reports and series [26-29], together with a number of clinical trials [30-32]. In a case series of six patients with refractory septic shock given levosimendan 0.1 to 0.2 µg/kg/min, there was a trend towards improved haemodynamics, associated with a reduction in catecholamine requirements. All but one of these patients survived to hospital discharge despite an Acute Physiology and Chronic Health Evaluation (APACHE II) predicted mortality of 60% [29].

Three of the sepsis trials in man have been led by Dr Morelli, one of the co-investigators for this study. The first trial, published in 2005, compared an infusion of levosimendan 0.2 µg/kg/min to dobutamine 5 µg/kg/min for 24h in 28 patients with septic shock and echocardiographically proven acute left ventricular dysfunction [30]. Statistically significant reductions in both pulmonary artery pressure and pulmonary artery occlusion pressure and an increase in left ventricular stroke work index were seen with levosimendan. In particular, levosimendan increased creatinine clearance by 64% whilst decreasing serum lactate levels as compared to dobutamine [30]. In 35 patients with septic shock and the acute respiratory distress syndrome, levosimendan 0.2 µg/kg/min as compared to placebo increased cardiac index and reduced mean pulmonary artery pressure [31]. More recently, in a study of 40 patients who had septic shock, the effects of levosimendan (0.2 µg/kg/min) on microcirculatory blood flow in the sublingual mucosa were compared to dobutamine (5 µg/kg/min). Blood flow was significantly higher in the levosimendan group (p<0.001) and there was a trend to higher central venous oxygen saturations (ScvO₂) and arterial pH as well as lower noradrenaline requirements in the levosimendan group [32]. These studies were not of sufficient size to detect any differences in any patient focused outcomes.

A number of other small trials have been performed. In 42 patients with septic shock, levosimendan compared to dobutamine reduced the number of patients requiring additional catecholamine support with noradrenaline (p<0.04) [33]. In a similar trial, 42 patients who had severe sepsis and a cardiac index (CI) of less than 2.2 l/min/m² received either levosimendan or dobutamine as additional therapy. CI, ejection fraction and ScvO₂ all increased significantly more in the levosimendan group compared to the dobutamine group [34]. In a recent trial of 30 patients who had septic shock, patients randomised to receive levosimendan (0.1 µg/kg/min), compared to dobutamine (10 µg/kg/min), had a significantly improved splanchnic perfusion as measured by indocyanine green plasma disappearance rate [35].
Further evidence of a beneficial effect of levosimendan on renal function in sepsis comes from a case-control study of 99 patients with septic shock who received levosimendan 0.2 µg/kg/min for 24 hours within 36h of admission to the ICU. When compared to matched controls, a 24% increase in glomerular filtration rate at 96h (p<0.05) was seen in patients who received levosimendan together, with a lower peak serum creatinine concentration (p<0.05) [36]. Similar beneficial effects of levosimendan on renal function have also been demonstrated in patients with acute heart failure. In 88 patients who had acute decompensated heart failure requiring inotropic therapy, levosimendan compared to dobutamine significantly increased calculated glomerular filtration rate (GFR), with an increase of 45% seen at 72h after infusion completion [37].

There are no existing systematic reviews of levosimendan in severe sepsis. However, a recent article reviewing the role of levosimendan in sepsis concluded that “large-scale multicenter clinical trials are now needed to clarify whether levosimendan improves the overall outcome of patients with sepsis and septic shock” [38].

Risks and benefits

The potential benefits have been reviewed above. Levosimendan has been widely used in patients with acute heart failure, has a good safety profile and has no known significant pharmacokinetic drug interactions. According to the levosimendan investigators’ brochure, between September 2000 (when the drug first received a license in Sweden) and November 2010, an estimated 440,000 patients have been treated with levosimendan with a reported serious adverse drug reaction rate of 791/~440,000 (0.2%). The most common events reported were hypotension (0.03%) and serious arrhythmias (0.02%).

Levosimendan has been used in over 200 patients with septic shock in published controlled trials and case series without any reported significant adverse effects. Adequate cardiovascular resuscitation with intravenous fluids and noradrenaline, as well as avoiding an initial bolus dose and high dose infusion (≥0.4 µg/kg/min) help reduce adverse effects when used in sepsis [38].

Levosimendan is currently used in many ICUs within Europe in the treatment of severe sepsis and septic shock, and has recently been recommended as an alternative inotrope in the German Sepsis Society guidelines [39].

As highlighted above, septic shock is associated with a high mortality and many of the drugs required for its treatment, e.g. high dose catecholamine infusions, also have significant risk. Available evidence would suggest that levosimendan has a good safety profile and would not add any additional risk in this population. In fact, levosimendan may reduce the risk associated with standard therapy, if catecholamine use is reduced.

Toxicology

Conventional studies on general toxicity and genotoxicity have revealed no special hazard for humans in short-term use. In animal studies, levosimendan was not teratogenic, but it caused a generalised reduction in the degree of ossification in rat and rabbit foetuses with anomalous development of the supraoccipital bone in the rabbit. Pregnant patients will not be included in this trial.

2.2 RATIONALE FOR CURRENT STUDY

As summarised above, there is a substantial body of research which provides proof of concept that levosimendan improves cardiac output, regional perfusion and other physiological endpoints, including creatinine clearance and glomerular filtration rate, in patients who have septic shock.

We will undertake an exploratory trial designed to identify important clinical outcome benefits and to explore the mechanism of action of levosimendan in septic shock. Given that multiple organ dysfunction is associated with an increased mortality [40], a reduction in the incidence and severity of organ failure would
be associated with meaningful benefits to patients and clinicians alike, along with potential reductions in cost to the NHS.

3 STUDY OBJECTIVES

3.1 Primary objectives

The main objectives of this trial are:

1. To ascertain if levosimendan reduces the incidence and severity of organ dysfunction as compared to placebo in adult patients who have septic shock.
2. To identify the effect of levosimendan on individual organ function in septic shock.
3. To establish the safety profile and pharmacokinetics of levosimendan in this group of patients.

3.2 Secondary objectives

- To identify whether levosimendan reduces the need for and duration of catecholamine support and thus reduces myocardial injury.
- To establish whether levosimendan alters the pro- and anti-inflammatory balance in sepsis.
- To collect long-term (3 and 6 month) survival data to help inform the appropriate long-term outcome measure for a subsequent effectiveness trial, should the efficacy of levosimendan be confirmed in this trial.

4 STUDY DESCRIPTION

4.1 DESIGN

This is a double-blind randomised placebo-controlled parallel group trial. It will be conducted in multiple general adult ICUs within the UK. The study will recruit 516 patients.

4.2 TREATMENT REGIME

Patients will be randomised to receive an intravenous infusion of either levosimendan (0.05 to 0.2 µg/kg/min) or placebo for a duration of 24 hours in addition to standard care.

The study drug infusion will start at 0.1 µg/kg/min and if tolerated will be increased after 2-4 hours to 0.2 µg/kg/min for the remaining 20-22 hours (maximum 24 hour infusion). If there are subsequent adverse effects the rate of infusion will be reduced back to 0.1 µg/kg/min. If there are adverse effects at an infusion rate of 0.1 µg/kg/min (either initially or later) then the rate of infusion will be reduced to 0.05 µg/kg/min or discontinued. Further details are given in section 5.2.4.

5 TRIAL METHODS

5.1 SUBJECT SELECTION

5.1.1 Pre-randomisation evaluations

Patients will only need to be assessed for the inclusion and exclusion criteria detailed below. This will require a history and clinical examination. A full blood count test or an arterial blood gas sample may be needed but would normally be collected as part of routine clinical care.
5.1.2 Inclusion Criteria
The target population is adult patients (≥18 years) who require vasopressor support for the management of sepsis despite fluid resuscitation.

Inclusion criteria will use the internationally established consensus definitions of sepsis. In brief:

- Fulfil 2/4 of the criteria of the systemic inflammatory response syndrome (SIRS) due to known or suspected infection within the previous 24 hours. The SIRS criteria are:
  1. Fever (>38°C) or hypothermia (< 36°C),
  2. Tachycardia (heart rate > 90 beats per minute),
  3. Tachypnoea (respiratory rate > 20 breaths per minute or PaCO₂ < 4.3 kPa) or need for mechanical ventilation,
  4. Abnormal leukocyte count (> 12,000 cells/mm³, < 4000 cells/mm³, or > 10% immature [band] forms).
- Hypotension, despite adequate intravenous fluid resuscitation, requiring treatment with a vasopressor infusion (e.g. noradrenaline / adrenaline / vasopressin analogue) for at least four hours and still having an ongoing vasopressor requirement at the time of randomisation.

5.1.3 Exclusion Criteria

- More than 24 hours since meeting all the inclusion criteria
- End-stage renal failure at presentation (previously dialysis-dependent)
- Severe chronic hepatic impairment (Child-Pugh class C)
- A history of Torsades de Pointes
- Known significant mechanical obstructions affecting ventricular filling or outflow or both.
- Treatment limitation decision in place (e.g. DNAR or not for ventilation/ dialysis)
- Known or estimated weight >135kg
- Known to be pregnant
- Previous treatment with levosimendan within 30 days
- Known hypersensitivity to levosimendan or any of the excipients
- Known to have received another investigational medicinal product within 30 days or currently in another interventional trial that might interact with the study drug. (Potential co-enrollment into other studies will be considered on an individual study basis)

5.2 PROCEDURES AND MEASUREMENTS

5.2.1 Screening
All patients who are clinically judged to have septic shock will be screened against the inclusion and exclusion criteria to be eligible for the study.

5.2.2 Informed Consent
In most cases it will not be possible to obtain prospective consent from the patient at the time of enrolment. This is due to the fact that many patients will have a reduced level of consciousness due to their illness or due to sedative medication used as part of their treatment.

- **Patient Consent**
  If possible, informed consent will be obtained from the patient. The patient will be informed about the trial by the responsible clinician or a member of the research team and given a copy of the Patient Information Sheet (PIS). Informed patients will be given an adequate amount of time to consider their participation in the trial. If the patient decides to participate in the trial they will be asked to sign the Patient Consent Form which will then be countersigned by the responsible clinician / researcher. The patient will retain one copy
of the signed Consent Form. Another copy will be placed in the patient’s medical records whilst the original will be retained in the Trial Site File.

- **Personal Legal Representative Consent**
  If the patient is unable to give consent, informed consent will be sought from the patient’s ‘Personal Legal Representative’ (PerLR) who may be a relative, partner or close friend. The PerLR will be informed about the trial by the responsible clinician or a member of the research team and provided with a copy of the Covering Statement for Personal Legal Representative with an attached PerLR Information Sheet and asked to give an opinion as to whether the patient would object to taking part in such medical research. The PerLR will be given adequate time to consider the patient’s participation in the study. If the PerLR decides that the patient would have no objection to participating in the trial they will be asked to sign the PerLR Consent Form which will then be countersigned by the responsible clinician / researcher. The PerLR will retain a copy of the signed Consent Form. A second copy will be placed in the patients’ medical records whilst the original will be retained in the Trial Site File.

- **Professional Legal Representative Consent**
  If the patient is unable to give informed consent and attempts to meet and discuss with a PerLR have failed then a doctor who is not connected with the conduct of the trial may act as a Professional Legal Representative (ProLR). The doctor will be informed about the trial by a member of the research team and given a copy of the PIS. If the doctor decides that the patient is suitable for entry into the trial they will be asked to sign the ProLR Consent Form. The doctor will retain one copy of the signed Consent Form. A second copy will be placed in the patient’s medical records; the original will be retained in the Trial Site File.

  If a relative, partner or close friend should subsequently visit the patient after enrolment and before the patient has regained capacity they should be informed about the patient’s participation and informed about the retrospective consent process.

- **Retrospective Patient Information / Consent**
  Patients, for whom consent is given by a PerLR or a ProLR, will be informed of their participation in the trial by the responsible clinician or a member of the research team once they regain capacity to understand the details of the trial. The clinician / researcher will discuss the study with the patient and the patient will be given a copy of the PIS to keep. The patient will be asked for consent to continued follow-up in the trial and to sign the retrospective Consent Form. The patient will retain one copy of the signed Consent Form. Another copy will be placed in the patient’s medical records whilst the original will be retained in the Trial Site File. If the patient does not want to continue follow-up in the study no further clinical data beyond that time-point or new samples will be collected.

  The right of the participant to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

**5.2.3 Randomisation**

Randomisation will be by computer generated randomised number and will use the InForm eCRF online system. Randomisation will be stratified by ICU and will occur on a 1:1 basis in permuted blocks.
"VISIT" SCHEDULE
All patients

<table>
<thead>
<tr>
<th>Visit</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8 to 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informed Consent (Patient consent/ PerLR / ProLR / Retrospective Patient Information &amp; consent)</td>
<td>Patient / PerLR / ProLR will be obtained initially. Retrospective patient consent will be obtained when the patient has recovered.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inclusion / Exclusion criteria</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Randomisation</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study drug administration</td>
<td>Study drug infusion for 24 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Follow up

| Blood & Urine sampling             | X     | X     |       | X     | X     |       |       | (see below for PK study) |
| Daily collection of clinical data  | X     | X     | X     | X     | X     | X     | X     | X           |
| Final Visit                        |       |       |       |       |       |       |       | On the day of discharge from the hospital |

Patients in pharmacokinetic study – visits as above plus

<table>
<thead>
<tr>
<th>Visit</th>
<th>Day 8</th>
<th>Day 10</th>
<th>Day 13</th>
<th>Day 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additional blood sampling</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

5.2.4 Treatments

Patients will be randomised to either treatment group or control group.

The study drug should not be started until the treating physician is confident that adequate fluid resuscitation has been achieved and the patient has reached their target mean arterial pressure (suggested target 65-70 mmHg but this may be varied as detailed below). Adequate fluid resuscitation should be achieved using repeated fluid challenges. Examples of appropriate targets include any or all of the following:

- Central venous pressure ≥8mmHg (≥12 mmHg in mechanically ventilated patients)
- Good peripheral perfusion on clinical examination
- Other measures of cardiac output / flow, e.g. Stroke Volume Variability (SVV), Global End-Diastolic Volume Index (GEDVI)

Treatment group.

Patients will receive all normal standard care and in addition they will receive a 24-hour blinded intravenous infusion of levosimendan. The levosimendan infusion will start at 0.1 µg/kg/min and if tolerated will be increased after 2-4 hours to 0.2 µg/kg/min for a further 20-22 hours (total infusion of 24 hours). Levosimendan can cause vasodilatation. Therefore, if there is a mild drop in blood pressure an intravenous fluid bolus should be given (e.g. 250-500mls), fluid status reassessed and treated as necessary. Vasopressor dose should be titrated up if needed once any fluid depletion is corrected. The infusion rate should increase after 2-4 hours once the clinician is satisfied that the drug is well tolerated.

If the dose of 0.2 µg/kg/min is not tolerated (hypotension despite titration of vasopressors, or severe tachycardia) the rate of infusion will be reduced back to 0.1 µg/kg/min. If there is hypotension or tachycardia at an infusion rate of 0.1 µg/kg/min (either initially or later) then the rate of infusion will be reduced to 0.05 µg/kg/min. If the hypotension or tachycardia continues then the infusion should be discontinued (see appendix - flowsheet).
Protocol Number: CRO2047

The summary of product characteristics states that an initial bolus of levosimendan should be given followed by a 24-hour infusion of 0.1 to 0.2 µg/kg/min (reduced if there is hypotension or tachycardia), when treating acute decompensated heart failure. In order to avoid hypotension and to maintain safety in this septic shock population a bolus dose will NOT be given. The infusion will start at the lower dose to ensure that the drug is well tolerated by the patient before being increased to the higher dose. The dose of 0.2 µg/kg/min has led to clinical benefit in the previous septic shock clinical trials and importantly has been shown to be safe using detailed global haemodynamic and microcirculatory monitoring [30-32]. The titration of the dose between 0.05 and 0.2 µg/kg/min will help ensure that patients receive an effective dose while ensuring that any adverse effects are minimised in each individual patient.

Control group.

Patients will receive all normal standard care and in addition they will receive a 24-hour blinded intravenous infusion of matching placebo. The placebo infusion rate will follow the treatment group regimen.

During the study drug administration and especially during the first 6 hours patients must be repeatedly reassessed to ensure adequate fluid resuscitation using any or all of the targets above.

The study drugs will be supplied to the ICU by pharmacy as specific research study drugs and they will be stored in separate research stores (e.g. locked boxes/fridges in ICU). The study drug will be drawn up and administered by the bedside critical care nurse (or critical care research nurses). The study drug will be prescribed on the patient drug chart by the clinical staff as per each ICU’s policy. Pre-printed stickers or preset electronic prescriptions will be provided to ensure standardised prescribing, dilution and administration of the drug.

- Other treatments

Cardiovascular

Fluids

Crystalloid infusions (e.g. 0.9% saline or compound sodium lactate) should be used for intravenous fluid resuscitation. Starch containing colloid solutions (e.g. voluven and volulyte) should NOT be used in view of evidence that they may be associated with adverse outcomes and increased rates of acute kidney injury [41-43]. Gelatin based solutions and human albumin solution may be used as alternative resuscitation fluids.

Fluid resuscitation should be given based on repeated assessment of volume status as detailed above.

Vasoactive drugs - vasopressors

Noradrenaline is the initial vasopressor of choice. After fluid resuscitation it should be titrated to maintain a target mean arterial pressure (MAP) of 65-70mmHg. In individual patients a higher MAP target may be chosen, for instance if the patient is known to be hypertensive, and similarly in a normotensive patient a lower MAP may be chosen. However, it is important to ensure that the lowest dose of vasopressor is used to maintain an acceptable MAP to allow tissue perfusion in that patient.

Vasopressin, or any of its analogues, may be used as an alternative vasopressor or in addition to noradrenaline.

Vasoactive drugs – inotropes
Additional inotropic agents may be used in either treatment group as clinically indicated (i.e. in the presence of low cardiac output after fluid resuscitation). Dobutamine is the inotropic agent of choice as per the surviving sepsis guidelines [44], but other inotropes including adrenaline or milrinone may be used. There is a lack of evidence to recommend a set cardiac output/index target but in general an adequate cardiac output to ensure adequate oxygen delivery should be maintained. A central venous saturation (SvO2) >70% should be targeted in the early stages of septic shock management. Dobutamine (or any other inotropes) should be titrated down and weaned off once an adequate oxygen delivery is achieved.

Corticosteroids

Hydrocortisone should only be used for patients who are poorly responsive to vasopressors, i.e. on high dose vasopressors, as per the surviving sepsis campaign guidelines [44]. Low doses should be used (e.g. hydrocortisone 200mg/day in divided doses or as a continuous infusion) and should be titrated down and weaned off once the shock resolves.

Ventilation

A lung-protective ventilation strategy should be employed; i.e. 6-8mls/kg ideal body weight tidal volume, limiting plateau pressure to ≤30 cmH2O, accepting permissive hypercapnia and ensuring adequate levels of PEEP to prevent extensive lung collapse at the end of expiration.

High frequency ventilation, neuromuscular blockade, inhaled nitric oxide, prone positioning and extracorporeal membrane oxygenation are all permitted as needed to manage severe hypoxaemia.

Renal support

Continuous veno-venous haemo(dia)filtration is the renal replacement therapy (RRT) of choice. It should be initiated to treat the recognised complications of renal failure, i.e. fluid overload, hyperkalaemia, symptomatic uraemia, drug accumulation and severe acid-base disturbance. High volume haemofiltration for the management of sepsis (i.e. RRT not to treat kidney failure) should not be used.

Other management

All other general ICU management should be based on the latest guidance from the surviving sepsis campaign [44] and UK national critical care guidelines (e.g. the ventilator and central line care bundles http://www.patientsafetyfirst.nhs.uk/Content.aspx?path=/interventions/Criticalcare/)

Inevitably there will be minor differences in management between different centres but this will help ensure external generalisability of the results. Stratification of randomisation by treating centre will also ensure balance of small ICU differences between treatment groups.

- Dose modifications for toxicity

The flowsheet (appendix) should be followed to manage the expected pharmacodynamic effects of levosimendan, in particular vasodilatation and tachycardia.

- Premedication

All other drugs should be prescribed as clinically indicated based on the guidance above.

- Interaction with other drugs

In vitro studies have shown that levosimendan, OR-1855 and OR-1896 do not inhibit CYP1A2, CYP2A6, CYP2C19, CYP2D6, CYP2E1 or CYP3A4 at concentrations achieved by the recommended dosing. In addition
levosimendan does not inhibit CYP1A1 and neither OR-1855 nor OR-1896 inhibit CYP2C9. The results of drug interaction studies in humans with warfarin, felodipine and itraconazole confirmed that levosimendan does not inhibit CYP3A or CYP2C9, and metabolism of levosimendan is not affected by CYP3A inhibitors.

The study drug may be administered with the following medications since incompatibilities with levosimendan have not been observed in connected intravenous lines:

- Frusemide 10mg/mL
- Digoxin 0.25mg/mL
- Glyceryl trinitrate 0.1mg/mL

5.2.5 Follow-Up

Participants will be followed up daily whilst on the ICU. Routinely collected clinical data (cardiovascular, respiratory and renal physiological variables as well as haematological, biochemical and microbiological blood test results) will be recorded on a daily basis during this time.

Patients will also be followed up to ascertain survival status at 28 days post recruitment, at hospital discharge, and at 3 and 6 months post-recruitment using The Health and Social Care Information Centre or via their GP.

5.2.6 Lab evaluations

Blood and urine sampling: 25mls of blood and 10mls of urine will be collected on the day of inclusion (day 1, after 24 hours (day 2) and on days 4 and 6 while still in ICU. Samples will be sent to the coordinating centre for storage and analysis. Samples will be kept beyond the end of the trial and stored in accordance with the Human Tissue Act.

5.3 END POINT MANAGEMENT

5.3.1 Primary Outcome

This trial is designed to fully explore the efficacy and mechanism of action of levosimendan, as well as inform a subsequent effectiveness trial, if appropriate. We will therefore examine multiple organ failure, as measured by the SOFA score, as the primary endpoint. We will compare mean SOFA scores between treatment groups. The mean SOFA in ICU has been shown to be closely correlated to mortality and its predictive value was similar regardless of length of stay [3]. This will help solve the “truncated by death” issue, as all patients will contribute scores while alive on the ICU and no imputation of data is needed if a patient dies.

5.3.2 Secondary Outcomes

The SOFA score is a composite of several different organ failures and there may be differential effects of levosimendan in different organ systems. Therefore, in order to gain further insight into the mode of action of levosimendan we will also measure organ specific outcomes.

Cardiovascular

In all patients we will compare oxygen delivery between treatment groups using central venous oxygen saturations (ScvO₂). This should be measured and recorded at baseline, 6 and 12 hours and then 12 hourly in all patients with a jugular or subclavian central line for up to 96 hours and then daily to day 5 if the central line remains in situ.

In pre-specified ICUs we will measure cardiac output using calibrated devices (eg PiCCO, LiDCOplus, oesophageal Doppler or pulmonary artery catheter) in all patients included in the study. Cardiac output data
will be measured and recorded at baseline, 6 and 12 hours and then 12-hourly for up to 96 hours (as long as the device is clinically required).

Renal
In view of the importance of acute kidney injury as an independent determinant of outcome in septic shock and the previous data demonstrating a beneficial effect of levosimendan on kidney function in sepsis we will compare rates of renal failure using the AKIN definitions [45]:

- Increase in serum creatinine to >300% (>3-fold) from baseline
- or serum creatinine ≥354 μmol/L with an acute rise of at least 44 μmol/L
- or initiation of renal replacement therapy (for AKI)
- or a urine output of <0.3 mL/kg/h ≥24 h
- or anuria ≥12 h

Abdominal
As poor mesenteric perfusion and bowel ischaemia are believed to be major contributors to the pathogenesis of multi-organ failure in septic shock we will analyse bilirubin (hepatic SOFA score) over time.

Respiratory
Recent evidence has suggested levosimendan may also enhance calcium sensitivity in diaphragm muscle and thus improve diaphragm contractility [46]. In those patients who require intubation and mechanical ventilation we will assess time to successful liberation from mechanical ventilation. This will be defined as

- Extubated with face mask, nasal cannulae or room air OR
- T-piece / HME filter breathing OR
- Tracheostomy mask breathing OR
- CPAP breathing ≤5 cmH₂O WITHOUT any pressure support or mandatory ventilation

Pharmacokinetic / pharmacodynamic study
The initial 80 patients enrolled in the study will have an additional 3mls of blood collected while on ICU on days 2, 4, 6, 8, 10, 13 and 16, for assays of levosimendan and its active metabolites OR-1896 and OR-1855. Orion Corp will undertake these assays. We will then compare the area under the curve (AUC) and the maximum concentration (Cₘₐₓ) between patients with and without acute renal failure and also with previous PK data from other studies. We will collect the later samples to ensure we can calculate the half-life of the active metabolites (follow-up for at least 4 half lives).

The drug and metabolite levels will be correlated to possible adverse haemodynamic events, namely tachycardia and hypotension. As an additional safety assessment, in subsequently recruited patients blood samples will be collected if a haemodynamic serious adverse event occurs so that any correlation between drug / metabolite levels and adverse events can be explored further. The DMEC will be requested to suggest alternative dosing regimens if drug or metabolite levels are unexpectedly high in certain patient groups or if there is an association between infusion rates / drug levels and adverse events. They will advise if any alternative dosing regimens should alter the sample size for the trial.

Other secondary clinical outcomes
- 28-day, hospital and three & six-month survival
- ICU and hospital length of stay, and ICU-free days
- Duration of renal replacement therapy
- Days free from catecholamine therapy
Secondary Mechanistic outcomes

Serial blood and urine samples will be collected from patients as detailed above. Assays will include markers of acute kidney injury, myocardial dysfunction, inflammation and intestinal perfusion. Possible assays are given below but as biomarker discovery is a very active area of research we will re-evaluate the state-of-the-art in the field at the time that study enrollment is complete and consider the use of additional or complementary biomarkers.

- Acute kidney injury (AKI) biomarkers in urine & plasma. Urinary NGAL is currently the best validated biomarker of AKI [47]. Alternative biomarkers of AKI (e.g. Kidney Injury molecule-1 [48]) may be better characterised and validated by the end of the trial.

- Markers of myocardial dysfunction. Serum BNP has been demonstrated to be a reliable biomarker of myocardial injury, ischaemia and dysfunction in septic patients and also as a prognostic marker for a poor outcome [49, 50]. Although troponin is a well-validated marker of myocardial ischaemia it is also elevated in renal failure. As ARF is so common in septic shock and levosimendan is expected to alter kidney function we feel we cannot use troponin as a useful independent marker of cardiac dysfunction in this study.

- Biomarkers of systemic inflammation. This will be measured using a multiplex inflammatory biomarker assay. One possible assay is the flowCytomix 11plex Th1/Th2 cytokine panel including IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 (p70), TNF-alpha, TNF-beta, and IFN-gamma. This focused array will allow an assessment of the pro / anti-inflammatory balance over time in patients with septic shock and allow more detailed study of the other potential mechanisms of action of levosimendan.

- Intestinal perfusion. Plasma citrulline and/or intestinal-fatty acid binding protein (I-FABP) levels may be measured as markers of intestinal epithelial cell damage.

- In addition samples will be stored for subsequent analysis (e.g. genetics / proteomics / metabonomics) as appropriate.

6 PHARMACOVIGILANCE

6.1 DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical trial subject administered a medicinal 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 (IMP), whether or not considered related to the IMP.

Adverse Reaction (AR): all untoward and unintended responses to an IMP related to any dose administered. All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

 Unexpected Adverse Reaction: an AR, the nature or severity of which is not consistent with the applicable product information (e.g. investigator’s brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product). When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected. Side effects documented in the SmPC which occur in a more severe form than anticipated are also considered to be unexpected.

Serious Adverse Event (SAE) or Serious Adverse Reaction: any untoward medical occurrence or effect that at any dose
• Results in death  
• Is life-threatening – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe  
• Requires hospitalisation, or prolongation of existing inpatients’ hospitalisation  
• Results in persistent or significant disability or incapacity  
• Is a congenital anomaly or birth defect

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR): any suspected adverse reaction related to an IMP that is both unexpected and serious.

### 6.2 CAUSALITY

The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below.

If any doubt about the causality exists the local investigator should inform the study coordination centre who will notify the Chief Investigator. The pharmaceutical companies and/or other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrelated</td>
<td>There is no evidence of any causal relationship</td>
</tr>
<tr>
<td>Unlikely</td>
<td>There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant’s clinical condition, other concomitant treatment).</td>
</tr>
<tr>
<td>Possible</td>
<td>There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant’s clinical condition, other concomitant treatments).</td>
</tr>
<tr>
<td>Probable</td>
<td>There is evidence to suggest a causal relationship and the influence of other factors is unlikely.</td>
</tr>
<tr>
<td>Definitely</td>
<td>There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.</td>
</tr>
<tr>
<td>Not assessable</td>
<td>There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.</td>
</tr>
</tbody>
</table>

### 6.3 REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the study coordination centre in the first instance. A flowchart is given below to aid in the reporting procedures.
Non serious AR/AEs

All such toxicities, whether expected or not, should be recorded in the adverse event section of the relevant case record form within one month of the form being due.

Serious AR/AEs

Fatal or life threatening SAEs should be reported on the day that the local site is aware of the event. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator should assign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

An SAE form should be completed and entered into the eCRF for all SAEs within 24 hours. This will automatically send alert emails to the Chief Investigator, the Project Manager and the sponsor. However, relapse, organ failure and death due to sepsis (see definitions below), and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

Clinical outcomes

Clinical outcomes from sepsis are exempt from adverse event reporting, unless the investigator deems the event to be related to the administration of the study drug. The following events will be considered clinical outcomes:

- Death related to sepsis
- Cardiovascular failure, including the need for vasopressors / inotropes
- Respiratory failure, including mechanical ventilation and acute lung injury
- Hepatic failure
- Renal failure, including the need for renal replacement therapy
- Haematological / Coagulation failure, including thrombocytopenia

Clinical details about these clinical outcomes will be routinely collected in the case record form.

In relation to the study drug in this trial the following specific serious adverse events will be recorded in the eCRF:

- Myocardial infarction / acute coronary syndrome
- Life threatening arrhythmia (e.g. ventricular fibrillation, ventricular tachycardia, or atrial fibrillation that leads to hypotension)

SUSARs

In the case of suspected unexpected serious adverse reactions, the staff at the site should:

Complete the SAE eCRF (within 24 hours). The study coordination centre will be notified by email and may contact the local site for further information and may require anonymised copies of all relevant investigations and documents.

Or

Contact the study coordination centre by phone and complete the SAE form within the following 24 hours as above.

The study coordination centre will notify the MHRA, REC and the Sponsor of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and non-
life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study.

Local investigators should report any SUSARs and / or SAEs as required by the protocol and study specific SOPs.

### Safety Reporting Overview

![Safety Reporting Diagram](image)

**6.4 DATA MONITORING & ETHICAL COMMITTEE**

An independent Data Monitoring and Ethical Committee (DMEC) will be set up to monitor progress, patient safety and any ethical issues involved in this trial. They will review trial progress, recruitment rates, event rates and safety data. A separate charter will be drawn up defining their exact remit and criteria for reporting to the trial steering committee. There will be 6-monthly meetings of the independent DMEC.

**Contact details for reporting SAEs and SUSARs**

Complete an SAE form & submit to the Study Coordination Centre as soon as possible (within 24 hr)

Attention LeoPARDS Trial Manager

Tel: TBC
7 EARLY DISCONTINUATION OF THE STUDY OR INDIVIDUAL SUBJECTS

7.1 EARLY DISCONTINUATION OF THE STUDY

If, in the opinion of the chief investigator, clinical events indicate that it is not justifiable to continue the trial, the Trial Steering Committee may terminate the trial following consultation with the Sponsor.

7.2 EARLY DISCONTINUATION OF THE SUBJECTS

Withdrawal criteria

Patients will be free to withdraw at any stage of the study.

If the patient wishes to withdraw from the study during the treatment period the treating physician will no longer follow the trial protocol and the study drug will be stopped. The patient’s data may or may not be included in the final analysis according to the patient’s wishes.

If the patient wishes to withdraw from the study after the treatment period no further data collection will continue. The patient’s data may or may not be included in the final analysis according to the patient’s wishes.

7.3 LOSS TO FOLLOW-UP

Patients will be followed post-hospital discharge using the The Health and Social Care Information Centre and so loss to follow-up should be low. If patients cannot be traced using this system they will be contacted directly or via their GP.

8 STATISTICS AND DATA ANALYSIS

8.1 OUTCOME MEASURES

The primary outcome will compare mean SOFA score after randomisation between treatment groups. As this trial is designed to fully assess the efficacy and mechanism of action of levosimendan we will also examine, in more detail, its effect in several organ systems as part of the secondary outcome measures. A full statistical analysis plan will be developed with the trial statistician. The primary analyses will be carried out on an intention-to-treat basis.

In the primary analysis, mean SOFA score will be compared between treatment and control groups using regression techniques. The mean SOFA is predictive of mortality regardless of ICU length of stay and therefore no imputation for death is required. All SOFA scores while alive in ICU (excluding neurological component) will be used for all patients to calculate the mean SOFA score for each patient.

In the renal assessment, each patient will be categorised at day 14 into the increasing stages of renal failure according to the international AKIN definitions (0,1,2,3). As death is clearly a worse outcome than renal failure, patients who have died will be categorised into stage 4. These five groups will be analysed as ordinal categorical data, using a constrained non-proportional odds model such that the log-odds ratios are assumed constant across all the cumulative probabilities except the last, death.

For the respiratory end-point, successful liberation from mechanical ventilation requires that the patient is still alive, and death is a competing risk. There are two ways of handling competing risks: modelling the cause-specific hazard or the cumulative incidence function, and their suitability depends on the question of
interest. As an exploratory analysis, we will produce plots showing the probability of survival and successful liberation from mechanical ventilation. For the primary analysis we propose to compare the cause-specific hazards for the two treatments, since we are interested in estimating the difference between groups in coming off the ventilator conditional on survival. However, an alternative analysis will use the cumulative incidence functions to compare whether one treatment group has more successful liberations taking death into account.

The detailed cardiovascular assessment will occur over the first 96 hours after enrollment. Death rates are likely to be low (<10%) in this early period. However, as with all outcomes, we will assess the level, pattern and plausible causes of any missing data, and consider whether the results from the proposed analysis are likely to suffer from any bias. If necessary, sensitivity analyses will be carried out, jointly modeling death and the cardiovascular outcomes under different assumptions as appropriate.

8.2 POWER CALCULATIONS

Our planned sample size is 500 patients. This will provide more than 90% power to detect a 0.5 point difference in mean SOFA score assuming a SD of 1.5 [3]. In this previous validation study a 1 point rise in mean SOFA score was associated with a significant mortality increase (mean SOFA 2.1-3.0=20%, 3.1-4.0=36.1% and 4.1-5.0=73.1% mortality; OR 3.06, 95% CI 2.36-3.97). We will recruit an additional 3% (16 patients) to account for potential loss to follow-up and withdrawal of consent as seen in previous UK ICU trials [51].

Renal

Due to the non-parametric nature of the renal outcome data, calculation of the power for the renal outcome has been undertaken using simulation with multi-state modelling. The event rates and frequencies of moving between the state of no renal failure, developing renal failure, recovery and death have been informed from mortality and organ failure data from the VASST trial [52-54], the CORTICUS trial [55], from local data from the three adult ICUs within Imperial College Healthcare NHS Trust and from Dr Gordon’s recently completed septic shock study (http://www.controlled-trials.com/ISRCTN66727957).

Patients will be classified according to the three stages of renal dysfunction as per AKIN definitions [45] and analysed as ordinal categorical data. Death, which is clearly the worst outcome, is taken into account by classifying it as stage 4. The simulation using multi-state models have suggested that the maximum difference in rates of acute renal failure and death between treatment groups might be seen around day 14. We will therefore compare the ordinal categorical data of death and acute renal failure at day 14. Based on a range of different transition probabilities we would have 65–90% power to detect a 25-35% improvement with levosimendan (previous studies have demonstrated a 24-64% improvement in renal function).

Cardiovascular

Oxygen delivery – 500 patients will provide more than 95% power to detect a 5% difference in ScvO2, assuming a SD of 15% [54].

Cardiac output – Within pre-specified ICUs we will measure cardiac output and this will provide a subgroup of at least 150 patients who will have detailed serial haemodynamic data collected. This will provide more than 85% power to detect a 0.66 l/min/m² difference in cardiac index between treatment groups over the first four days assuming a SD of 1.3 [54].

Respiratory

More than 90% of patients who have septic shock will require intubation and mechanical ventilation. Time to final successful liberation from mechanical ventilation (i.e. without re-ventilation or death) will be compared between the two treatment groups using survival analysis. 450 patients (90% of 500) will provide 80% power to detect a hazard ratio of 1.4, based on the assumption that overall 63% of patients will be successfully liberated from mechanical ventilation by day 28.
The DMEC, including an independent statistician, will be instructed within their charter to check these planning assumptions and undertake an interim sample size review.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

9  TREATMENT

9.1  INVESTIGATIONAL MEDICINAL PRODUCT DETAILS

Orion Corporation will supply the study drugs for this trial.

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Dosage</th>
<th>Description</th>
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<tbody>
<tr>
<td>Levosimendan</td>
<td>0.05 to 0.20 µg/kg/min</td>
<td>Supplied as 8ml vials with nominal filling volume of 5 ml containing levosimendan 12.5mg and inactive ingredients (povidone, anhydrous citric acid and anhydrous ethanol)</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.05 to 0.20 µg/kg/min (equivalent)</td>
<td>Supplied as 8ml vials with nominal filling volume of 5 ml containing riboflavin, sodium phosphate, anhydrous ethanol and water for injections</td>
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</table>

9.2  LABELLING, STORAGE AND DISPENSING

Orion Corporation will be responsible for assuring that the quality of all IMPs are adequate for the duration of the trial and in compliance with the Good Manufacturing Practice (GMP) standards.

Levosimendan and the matched placebo will be imported from Finland. All study drugs will be packaged, labelled to meet the MHRA requirements and distributed to sites by Victoria Pharmaceuticals, Belfast. The Trial Coordination Centre will keep accurate records of supply to trial centres and destruction of unused IMP at the end of the trial.

It is each trial centre’s responsibility to ensure that accurate records of IMPs dispensed and returned are maintained and reported to the Study Coordination Centre. It is the Trial Investigator’s responsibility to ensure that accurate records of IMPs prescriptions are maintained. The Study Coordination Centre will track supplies of IMPs via information from Victoria Pharmaceuticals and site IMP tracking documents. At the completion of the trial, the Trial Coordination Centre, via the monitor, will ensure the destruction of all returned dispensed IMPs (after close out and before archiving).

9.3  ACCOUNTABILITY

Hospital pharmacies will be responsible for recording study drugs dispensed to the ICU. Preparation of all drug infusions will be recorded on the Nursing Staff Drug Accountability Form and drug administration on the patient’s prescription chart. The study drug stores will include a sheet on which the fate of all ampoules will be recorded (infused, opened but not infused, unused). At the end of the study any remaining unused drug will be returned to the hospital pharmacy for recording.

9.4  UNBLINDING
Emergency envelopes will be supplied to each hospital pharmacy to allow emergency unblinding if needed. The local investigators should discuss the need for unblinding with the trial coordinator or Chief Investigator beforehand. Local SOPs describing the emergency unblinding procedure will be in place.

10 REGULATORY, ETHICAL AND LEGAL ISSUES

10.1 DECLARATION OF HELSINKI

This trial will be conducted in full conformity with the Declaration of Helsinki according to its 1996 version.

10.2 GOOD CLINICAL PRACTICE

The study will be conducted in accordance with the protocol, Good Clinical Practice (ICH GCP E6 guidelines), Imperial Clinical Trials Unit Standard Operating Procedures (ICTU SOPs) and national regulatory requirements and the provisions of relevant ethics committees.

10.3 INDEPENDENT ETHICS COMMITTEE APPROVAL

10.3.1 Initial Approval

Prior to the shipment of IMPs and the enrolment of subjects, the IEC must provide written approval of the conduct of the study at named sites, the protocol and any amendments, the Patient and PerLR Information Sheet and Consent Form, any other written information that will be provided to the subjects, any advertisements that will be used and details of any subject compensation.

10.3.2 Approval of Amendments

Proposed amendments to the protocol and aforementioned documents must be submitted to the IEC for approval as instructed by the Sponsor. Amendments requiring IEC approval may be implemented only after a copy of the IEC’s approval letter has been obtained. Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving Sponsor or IEC approval. However, in this case, approval must be obtained as soon as possible after implementation.

10.3.3 Annual Progress Reports and End of Trial Notification

The regulatory authorities and IEC will be offered annual progress reports and informed about the end of trial, within the required timelines.

10.4 REGULATORY AUTHORITY APPROVAL

The study will be performed in compliance with UK clinical trial regulations. Clinical Trial Authorisation from the Medicines and Healthcare products Regulatory Authority (MHRA) will be obtained prior to the start of the study. In addition, the Regulatory Authority must approve amendments (as instructed by the Sponsor), receive SUSAR reports and annual safety updates, and be notified of the end of the trial.

10.5 TRIAL REGISTRATION

The LeoPARDS trial will be registered with the European Clinical Trial Database and “International Standard Randomised Controlled Trial Number Register” databases.

10.6 INSURANCE & INDEMNITY
Imperial College London, the Sponsor of the trial has civil liability insurance, which covers this study in all participating centres. Imperial College London also holds negligent harm and non-negligent harm insurance policies which apply to this study.

10.7 SUBJECT CONFIDENTIALITY

Participants’ identification data (initials and date of birth) will be required for the registration process. The Study Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

The investigator must ensure that the subject’s privacy is maintained. On the eCRF or other documents submitted to the Sponsors, subjects will be identified by a subject ID number only. Documents that are not submitted to the Sponsor (e.g. signed informed consent form) should be kept in a strictly confidential file by the investigator.

The investigator shall permit direct access to subjects’ records and source document for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, Regulatory Authorities and IECs.

10.8 DATA PROTECTION

All personnel involved in the study will observe or work within the confines of the local data protection guidelines.

10.9 END OF TRIAL

This study will end when the specified number of patients have been recruited, all patients have completed 6 month follow-up and the database is locked.

10.10 STUDY DOCUMENTATION AND DATA STORAGE

The investigator will retain essential documents until notified by the Sponsor, and at least for ten years after study completion, as per Sponsor’s SOPs. Subject files and other source data (including copies of protocols, CRFs, original reports of test results, correspondence, records of informed consent, and other documents pertaining to the conduct of the study) must be kept for the maximum period of time permitted by the institution. Documents will be stored in such a way that they can be accessed/data retrieved at a later date. Consideration should be given to security and environmental risks.

No study document will be destroyed without prior written agreement between the Sponsor and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

11 ADMINISTRATIVE MATTERS

11.1 SOURCE DATA

Source documents include original documents related to the trial, to medical treatment and to the history of the participant, and adequate source documentation must be maintained to allow reliable verification and validation of the trial data. What constitutes the source data for this trial will be outlined in the Source Data agreement.
11.2 ELECTRONIC DATA CAPTURE

Electronic CRF: the principal means of data collection from participant visits will be Electronic Data Capture (EDC) via the internet. Data is entered into the EDC system via site personnel. All source data recorded in the CRF will be signed by the Investigator or his/her appropriate designee. All changes made following the electronic signing will have an electronic audit trail with a signature and date. Specific instructions and further details will be outlined in the CRF manual.

11.3 TRIAL MANAGEMENT

11.3.1 Trial Steering Committee

A Trial Steering committee (TSC) with an independent Chair will be appointed and will be responsible for overseeing the progress of the trial. A TSC Charter will be devised to list the roles and responsibilities of the TSC members. TSC will be convened biannually either in person or by teleconference.

11.3.2 Trial Management Group

The Trial Management Group will be set up by the Chief Investigator. TMG will convene on a monthly basis and will discuss on the recruitment, and other practical aspects of the trial.

The day-to-day management of the trial will be co-ordinated through the Imperial Clinical Trials Unit and the Chief Investigator.

11.3.3 Monitoring

A monitoring plan will be devised based on risk analysis and described in detail in the monitoring manual by the project manager. Trial Monitors will visit all sites and facilities where the trial will take place to ensure compliance with the protocol, GCP and local regulatory compliance. Monitoring visits will be performed by trained monitors before, during and after the trial as required by the protocol and trial procedures according to the monitoring manual to ensure patient safety, accurate data collection and reporting. Monitoring visits will also be dependent on rates of and numbers of participant recruitment per site. Communication with sites by telephone, mail and email will also be made as necessary. Training sessions will be organised for the investigators and all site staff at the beginning of the trial and then as appropriate. Initiation visits will be completed at all trial centres prior to the recruitment of participants, and will consist of review of protocol and trial documents, training with respect to trial procedures (informed consent, SAE reporting, inclusion and exclusion criteria), review of recruitment strategy, review of site facilities and equipment, essential document receipt, collection and filing, and archiving and inspection. Copies of the trial specific procedure manuals and related documents will be given to the investigators. The approved version of the protocol should be followed at all times, and any significant protocol deviations will be documented in a Protocol Violation Form and submitted to the study coordination centre as soon as possible. The investigators will allow the monitors to:

- inspect the site, the facilities, IMP management and materials used for the trial
- meet all members of the team involved in the trial, and ensure all staff working on the trial are experienced and appropriately trained, and have access to review all of the documents relevant to the trial
- have access to the electronic case record forms and source data
- discuss with the investigator and site staff trial progress and any issues on a regular basis

The monitor will ensure that:

- all participant records will be inspected for confirmation of existence, eligibility and informed consent
- there is adherence to the protocol, including consistency with inclusion/exclusion criteria
there is GCP and regulatory compliance

- Trial Documentation is complete and up to date (e.g. correct versions of documents being used, source data captured) and relevant documents are collected for the Trial Master File (TMF)
- The eCRFs have been completed correctly and accurately, and all entries correspond to data captured in source documents
- The IMP accountability records are in order (receipt, dispensing and destruction), storage is under appropriate conditions and secure, expiry dates are being checked and adhered to, and dispensing is according to the protocol and trial procedures.

All information dealt with during such visits will be treated as strictly confidential. At the end of the trial, close out visits will be performed by the monitor after the final participant visit has been completed and prior to database lock. During this visit the monitor will verify that all trial close out activities are completed – all queries resolved, missing data completed, monitoring completed, archiving arrangements in place, IMP accountability complete and all used and unused IMP destroyed, ISF completed and TMF documents collected, and end of trial notification. Each investigator will also be notified that an audit or inspection may be carried out - by the sponsor, sponsor's representatives or the host institution, or regulatory authorities - at any time, before, during or after the end of the trial. The investigator must allow the representatives of the audit or inspection team:

- to inspect the site, facilities and material used for the trial,
- to meet all members of his/her team involved in the trial,
- to have direct access to trial data and source documents, to consult all of the documents relevant to the trial. If an Investigator is informed of an impending audit or inspection, the trial coordination centre should be notified immediately.

11.3.4 Patient and Public Involvement (PPI)

Two PPI representatives will sit on the Trial Steering Committee and will provide input from a patient perspective at trial meetings. Both representatives have reviewed and provided feedback on all of the project documents prior to the ethics and regulatory submissions and their comments have been incorporated.

11.4 QUALITY CONTROL AND QUALITY ASSURANCE

Quality Control will be performed according to Imperial Clinical Trials Unit internal procedures. The study may be audited by a Quality Assurance representative of the Sponsor. All necessary data and documents will be made available for inspection.

11.5 AUDITS AND INSPECTIONS

The study may be subject to inspection and audit by Imperial College London under their remit as Sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

11.6 PUBLICATION POLICY

All publications and presentations relating to the study will be authorised by the Trial Management Group. Authorship will be determined according to the internationally agreed criteria for authorship (www.icmje.org). Authorship of parallel studies initiated outside of the Trial Management Group will be according to the individuals involved in the project but must acknowledge the contribution of the Trial Management Group and the Study Coordination Centre.
12 REFERENCES


13 APPENDIX – INFUSION GUIDELINE

LeopARDS study drug infusion protocol

Levosimendan can cause vasodilation and increased heart rate.
If blood pressure drops a fluid bolus should be given, volume status reassessed and treated, and vasopressors titrated.

- Seek & correct causes of hypotension
  - Ensure adequate volume replacement
  - Start study drug infusion at rate A
  - Note time, HR, MAP & vasopressor infusion rate

- Assess physiological effects over 2-4 hours

- Hypotension and tachycardia definitions
  - Unable to maintain target MAP despite fluids & vasopressors
  - HR >130 or
  - ΔHR >20% (if already >110)

- If infusion stopped but patient is now more stable infusion can be restarted

- Decrease infusion to rate ¾ A

- Increase infusion to rate 2A

- If no hypotension, tachycardia

- Continue to assess physiological effects and continue infusion at rate 2A

- Hypotension or tachycardia

- Infuse study drug at rate A

- If no hypotension, tachycardia

- Assess physiological effects over 4-8 hours

- Stop study drug infusion

- Decrease infusion to rate ¾ A

- If no hypotension, tachycardia

- Assess physiological effects over 4-8 hours

- Hypotension or tachycardia

All study drug infusions should stop at 24 hours
### 13.1 LeoPARDS study drug rates

5mls of study drug (2.5mg/ml of levosimendan or placebo) should be diluted in 500mls 5% Dextrose

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<tr>
<th>Patient weight (kg)</th>
<th>Infusion rate A (mls/hr) 0.1 µg/kg/min</th>
<th>Infusion rate 2A (mls/hr) 0.2 µg/kg/min</th>
<th>Infusion rate ½ A (mls/hr) 0.05 µg/kg/min</th>
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14 APPENDIX 2 – ECHOCARDIOGRAPHIC SUB-STUDY

Title
A prospective observational study: An echocardiographic evaluation of the effects of levosimendan on left and right ventricular function in severe sepsis.
A sub-study to the LeoPARDS trial: Levosimendan for the Prevention of Acute Organ Dysfunction in Sepsis.

Rationale
Severe sepsis is the leading cause for admission to an intensive care unit in the United Kingdom and despite improvements in care the mortality from this condition remains high. In around 50% of patients a reversible impairment of cardiac function can be demonstrated using echocardiography [12]. The likely mechanisms for this is a combination of altered calcium trafficking and reduced troponin sensitivity to calcium [13]. Amongst its effects, levosimendan improves myocardial contractility by increasing the sensitivity of troponin to intracellular calcium, making levosimendan an attractive option for the patient with sepsis-induced myocardial depression.

Transthoracic echocardiography is increasingly used in intensive care to assess cardiac function and guide therapy. It is a totally non-invasive technique and allows formal quantitative measurements of both left and right ventricular function to be made. To date, no studies have been performed that assess the effects of levosimendan on detailed cardiac function using echocardiography in patients with septic shock in a large randomised controlled trial.

Objectives
1. To obtain quantitative measurements of left and right ventricular function using transthoracic echocardiography in patients with septic shock enrolled in the LeoPARDS trial. These measurements will be performed at 3 time points – (1) just prior to initiation of study drug, (2) between 24 and 48h following initiation of study drug and (3) between 5 and 7 days following initiation of study drug.
2. To compare echocardiographic variables between the two treatment groups.

Patient population
Patients with severe sepsis with an ongoing requirement for vasopressor support despite adequate fluid resuscitation will be screened against the existing inclusion and exclusion criteria for the LeoPARDS trial. Having obtained informed consent for enrolment in LeoPARDS patients will undergo a transthoracic echocardiogram prior to the commencement of the study drug to assess the likelihood of achieving the measurements required – this is dependent on the adequacy of the views of the heart obtained. In critically ill patients, particularly those requiring ventilator support, it may not be possible to obtain the particular echocardiographic windows needed. Only those patients with adequate echocardiographic windows will be included in the sub-study. In addition, any patients identified as having severe valvular heart disease on echocardiography will be excluded from this sub-study.

Patient consent
The process of consent for this sub-study will be included in that of the main LeoPARDS trial. In the centres conducting the sub-study echocardiography is routinely practiced without the need for formal informed consent. Specific consent for inclusion will not be sought at the time of enrolment into the LeoPARDS trial, however, the patient information sheet (PIS) will be amended to include a description of the sub-study.

Centres
The sub-study will be conducted in selected sites as part of the LeoPARDS trial. These centres have significant experience in echocardiography and have personnel with the relevant skills to perform advanced
echocardiography (equivalent to the level of British Society of Echocardiography accreditation in adult echocardiography).

**Echocardiographic variables**

The following quantitative measurements will be obtained during the echocardiogram:

**Left ventricle**
- End-diastolic & end-systolic diameters
- Ejection fraction (Simpson’s biplane technique)
- Lateral and septal annulus E’ and Sm using tissue Doppler
- Mitral inflow E & A peak velocities, E deceleration time
- Left ventricular outflow tract (LVOT) diameter & velocity time integral (VTI) for calculation of LV cardiac output

**Right ventricle**
- RV end-diastolic diameter
- TAPSE (Tricuspid annular plane systolic excursion) at the lateral annulus
- Tricuspid annular Sm using tissue Doppler
- Tricuspid regurgitation peak gradient; record CVP simultaneously to calculate RV systolic pressure
- Pulmonary acceleration time (PAT)

Studies will be performed using the system available to each site. Each study will be archived locally to permit peer review if indicated.

**Time points**

Echocardiograms will be performed at three time points. This will allow the investigators to compare both between groups and over time the changes in cardiac function. The choice of a 3rd time point will allow us to identify any longer lasting effects that might be related to the active metabolite of levosimendan, OR-1896 and also whether there is any difference in the recovery from sepsis-induced myocardial dysfunction between the groups. The time points will be:

1. Post randomisation and prior to the commencement of study drug
2. At 24 to 48 hours following commencement of study drug
3. At 5 to 7 days following commencement of study drug.

**Data collection**

Data will be recorded using a separate data collection sheet. No personal identifiable data will be collected other than the patient’s initials and their study number. In addition to the echocardiographic variables the following clinical parameters will be recorded at the time of each echocardiogram:

- Blood pressure
- Heart rate
- Central venous pressure
- Details of vasoactive drugs being administered
- Use of mechanical ventilation

These data will then subsequently be uploaded to the main electronic case report form.

**Results**

The data will be analysed at the end of the LeoPARDS trial.
SIGNATURE PAGE - CHIEF INVESTIGATOR

The signature below constitutes approval of this protocol by the signatory and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol including all statements regarding confidentiality.

Signed:

Dr Anthony Gordon
Department of Surgery & Cancer
Imperial College London

Date: ___________________
SIGNATURE PAGE - SPONSOR

The signature of the below constitutes agreement of this protocol by the signatory.

Signed: ____________________________________________

Tharani Thurairajah
Clinical Trial Manager, Joint Research Compliance Office
Imperial College London

Date: ________________________________
SIGNATURE PAGE - STATISTICIAN

The signature of the below constitutes agreement of this protocol by the signatory.

Signed:  

___________________________________________

Dr Alexina Mason  
Statistician, School of Public Health  
Imperial College London

Date:  

____________________
SIGNATURE PAGE - INVESTIGATOR

The signature of the below constitutes agreement of this protocol by the signatory and provides the necessary assurance that this study will be conducted at his/her investigational site according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: LeoPARDS: Levosimendan for the Prevention of Acute Organ Dysfunction in Sepsis

Protocol Number: CRO2047

Address of Institution: ______________________________________________

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____________________________________________

Signed: ______________________________________________

Print Name and Title: ______________________________________________

Date: ______________________