A Double-blind, Randomised, Placebo-controlled, Phase 2b/3 Adaptive Clinical Trial Investigating the Efficacy and Safety of Selepressin as Treatment for Patients with Vasopressor-dependent Septic Shock

SEPSIS-ACT

Selepressin **E**valuation **P**rogramme for **S**epsis-**I**nduced **S**hock - **A**daptive **C**linical **T**rial

This supplement contains:

Original Protocol	27 May 2015; Ver. 3.0
Clinical Trial Protocol Amendment (Summary of Changes)	8 Jul 2016; Ver. 0.1
Final Protocol	8 Jul 2016; Ver.4.0
Original Statistical Analysis Plan (SAP)	17 Dec 2014; Ver. 1.0
Final Statistical Analysis Plan (SAP)	20 Nov 2017; Ver. 9.0
Statistical Analysis Plan (SAP) Addendum	20 Nov 2017; Ver. 1.0

CLINICAL TRIAL PROTOCOL

A Double-blind, Randomised, Placebo-controlled, Phase 2b/3 Adaptive Clinical Trial Investigating the Efficacy and Safety of Selepressin as Treatment for Patients with Vasopressor-dependent Septic Shock

SEPSIS-ACT

Selepressin Evaluation Programme for Sepsis-Induced Shock - Adaptive Clinical Trial

Trial Code: 000133

EudraCT Number:	2014-003973-41
IND Number:	77246
Investigational Medicinal Products:	Selepressin; concentrate for solution for infusion Placebo; sterile 0.9% sodium chloride solution
Indication:	Vasopressor-dependent septic shock
Phase:	2b/3
Name and Address of Sponsor:	Ferring Pharmaceuticals A/S Clinical Research and Development Kay Fiskers Plads 11 2300 Copenhagen S, Denmark Telephone number: +45 88 33 88 34
GCP Statement:	This trial will be performed in compliance with GCP

SYNOPSIS

TITLE OF TRIAL

A Double-blind, Randomised, Placebo-controlled, Phase 2b/3 Adaptive Clinical Trial Investigating the Efficacy and Safety of Selepressin as Treatment for Patients with Vasopressor-dependent Septic Shock

SEPSIS-ACT / Selepressin Evaluation Programme for Sepsis-Induced Shock - Adaptive Clinical Trial

SIGNATORY INVESTIGATORS

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[redacted] Medical-surgical Intensive Care Unit, Saint Luc University Hospital at the Université Catholique de Louvain, Brussels, Belgium.

TRIAL SITES

The trial will be conducted predominately across Europe and North America at approximately 60-100 trial sites in total.

PLANNED TRIAL PERIOD	CLINICAL PHASE
First visit for the first patient is planned for Q3 2015. Last visit for the last patient is expected in Q4 2018.	2b/3

OBJECTIVES

Primary objective

- To demonstrate superiority of selepressin plus standard care versus placebo plus standard care in the number of vasopressor- and mechanical ventilator-free days (with penalty for mortality) in patients with vasopressor-dependent septic shock

Secondary objectives

- To determine the efficacy of selepressin on:
    - Organ dysfunction
    - Morbidity and mortality
    - Fluid balance
    - Health-related quality of life
- To determine the safety profile of selepressin
- To determine the pharmacokinetics of selepressin
- To determine the health economics of selepressin
- To further evaluate a range of biomarkers in relation to the mode of action of selepressin

ENDPOINTS

Primary endpoint

- Vasopressor- and mechanical ventilator-free days (P&VFDs) up to Day 30

  This composite endpoint is defined as the number of days (reported to one decimal place [0.0 to 30.0 days]) from start of treatment with the investigational medicinal product (IMP) [selepressin or placebo] to 30.0 days thereafter during which the patient is: 1) alive; 2) free of treatment with intravenous vasopressors; and 3) free of any invasive mechanical ventilation (see definition below).

  Any patient that dies within this 30-day period is assigned zero P&VFDs, even if there is a period during which the patient is free of both vasopressor treatment and mechanical ventilation. If vasopressors need to be restarted or mechanical ventilation needs to be initiated or restarted, and the use of either is greater than 60 minutes within a 24-hour period, then the clock is reset and the patient is not considered free of vasopressors and/or mechanical ventilation until after those therapies are again finally discontinued. Vasopressor use or mechanical ventilation during - and up to three hours after - surgery / procedure (including bedside) is exempt from this rule (i.e. does not reset the calculation of P&VFDs). The intent is for the endpoint to reflect the speed of recovery from septic shock and respiratory failure, with appropriate penalties for recurrent shock, new or recurrent respiratory failure, and death.

  Vasopressor use is defined as any intravenous dose of norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, and IMP (i.e. selepressin and placebo).

  Mechanical ventilation is defined as use of endotracheal or tracheostomy tube assisted ventilation (>5 cm H_2O continuous positive airway pressure and >5 cm H_2O of pressure support from the ventilator in tracheostomy patients). End of mechanical ventilation is defined as: 1) extubation of intubated patients or 2) ≤5 cm H_2O continuous positive airway pressure and ≤5 cm H_2O of pressure support from the ventilator in tracheostomy patients. If non-invasive ventilation by mask or bag (>5 cm H_2O of pressure support) is deployed to avoid (re)intubation, it also counts as mechanical ventilation. However, all other uses of non-invasive ventilation such as chronic night-time use of positive airway pressure for chronic obstructive pulmonary disease (COPD) or sleep apnea does not count as mechanical ventilation (regardless of pressure).

Key secondary endpoints

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Day 90
- Renal replacement therapy (RRT)-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)
- Intensive care unit (ICU)-free days up to Day 30

Secondary efficacy endpoints

Organ dysfunction

- Vasopressor-free days up to Day 30
- Mechanical ventilator-free days up to Day 30
- Duration of septic shock (i.e. vasopressor use) up to Day 30
- Duration of mechanical ventilation up to Day 30
- Incidence of RRT up to Day 30 (counting patients who die as on RRT and excluding patients on RRT for chronic renal failure at time of randomisation)
- Duration of RRT up to Day 90 (excluding patients on RRT for chronic renal failure at time of randomisation)
- Daily overall and individual organ (cardiovascular, respiratory, renal, hepatic, coagulation) scores using a modified version of the Sequential Organ Failure Assessment (SOFA) until ICU discharge
- Incidence of new organ dysfunction and new organ failure (based on the SOFA score) up to Days 7 and 30

Morbidity and mortality

- ICU length of stay up to Day 30
- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Days 30 and 180

Fluid balance

- Daily and cumulative fluid balance until ICU discharge (for a maximum of 7 days)
- Daily and cumulative urine output until ICU discharge (for a maximum of 7 days)

Health-related quality of life

- Change in utility, based on the EuroQol group's 5-dimension 5-level (EQ-5D-5L) questionnaire, up to Day 180

Safety endpoints

- Incidence of adverse events (type, frequency, and intensity) with specific emphasis on:
  - Ischaemic events (e.g. myocardial, skin, cerebral, mesenteric, and limb ischaemia)
  - Changes in vital signs assessed as unanticipated in the setting of septic shock
  - Changes in safety laboratory variables assessed as unanticipated in the setting of septic shock
- Changes in vital signs and safety laboratory variables
- Episodes of hypotension (mean arterial pressure <60 mmHg for longer than one hour)

Additional endpoints

- Hospital-free days up to Day 90
- Hospital length of stay up to Day 90
- Patient residence at Day 30, Day 60, Day 90, and Day 180
- Health economic evaluation - to be reported separately according to a pre-specified health economic analytical plan
- Mean arterial pressure (MAP), until ICU discharge (for a maximum of 7 days)
- Norepinephrine/noradrenaline and other vasopressor doses
- Pharmacokinetic response (in a subset of approximately 200 patients) - to be reported separately according to a pre-specified pharmacokinetic analysis plan (including plasma level of selepressin at the first attempt to wean the IMP infusion)
- Creatinine clearance
- Ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2 ratio)
- Extravascular lung water and pulmonary permeability index (in a subset of 100-350 patients)
- Cardiac output (in a subset of 100-350 patients)
- Cytokines (in a subset of 100-350 patients)
- Angiopoietin-1 and -2 (in a subset of 100-350 patients)

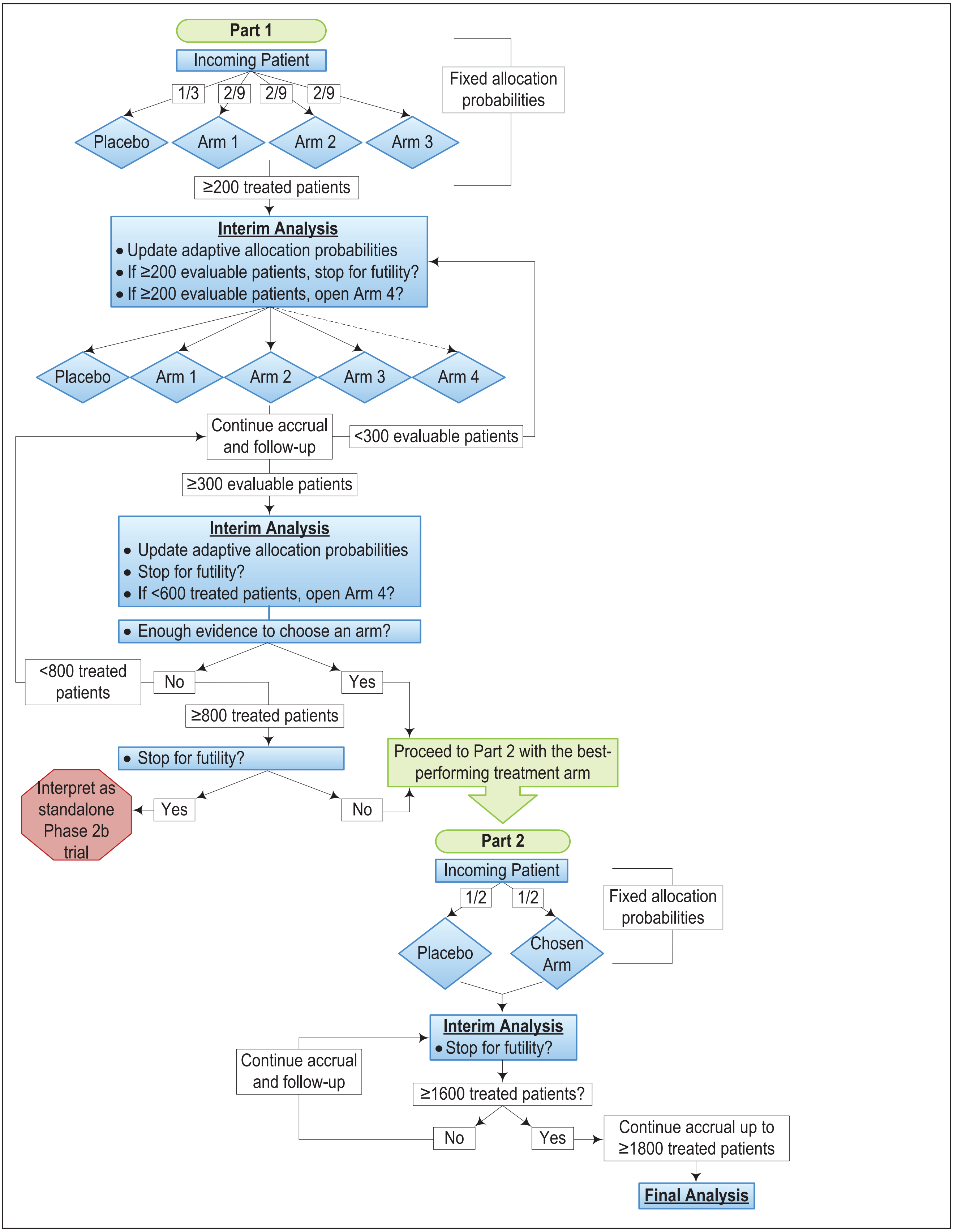
METHODOLOGY

This is a double-blind, randomised, placebo-controlled, two-part adaptive clinical trial. The trial is designed to investigate the efficacy and safety of multiple dosing regimens of selepressin and to confirm the efficacy and safety of one dosing regimen in treatment of adult patients with septic shock requiring vasopressor.

Up to four dosing regimens of selepressin will be investigated:

Treatment Arm	Starting Dose (ng/kg/min)	Maximum Dose (ng/kg/min)	Range (ng/kg/min)
Arm 1	1.7	2.5	0-2.5
Arm 2	2.5	3.75	0-3.75
Arm 3	3.5	5.25	0-5.25
Arm 4	5.0	7.5	0-7.5

The overall trial design includes two parts (Part 1 – Phase 2b and Part 2 – Phase 3) as illustrated on the next page.


Part 1
Incoming Patient
1/3
2/9
2/9
2/9
Fixed allocation probabilities
Placebo
Arm 1
Arm 2
Arm 3
≥200 treated patients
Interim Analysis
• Update adaptive allocation probabilities
• If ≥200 evaluable patients, stop for futility?
• If ≥200 evaluable patients, open Arm 4?
Placebo
Arm 1
Arm 2
Arm 3
Arm 4
Continue accrual and follow-up
<300 evaluable patients
≥300 evaluable patients
Interim Analysis
• Update adaptive allocation probabilities
• Stop for futility?
• If <600 treated patients, open Arm 4?
• Enough evidence to choose an arm?
<800 treated patients
No
Yes
≥800 treated patients
• Stop for futility?
Proceed to Part 2 with the best-performing treatment arm
Interpret as standalone Phase 2b trial
Yes
No
Part 2
Incoming Patient
1/2
1/2
Fixed allocation probabilities
Placebo
Chosen Arm
Interim Analysis
• Stop for futility?
Continue accrual and follow-up
≥1600 treated patients?
No
Yes
Continue accrual up to ≥1800 treated patients
Final Analysis

Part 1 is a dose-finding stage with response-adaptive randomisation to preferentially allocate patients to the dosing regimens that appear to have the maximum benefit with respect to the primary endpoint. Interim analyses will be conducted regularly in Part 1 to optimise the efficiency of selecting the optimal dosing regimen and to allow early termination of the trial for futility. If Part 1 results in a decision to run the second part of the trial, Part 2 will be a 1:1 randomised comparison of the best-performing dosing regimen of selepressin selected in Part 1 versus placebo on top of standard care. All patients will be on norepinephrine/noradrenaline treatment as part of standard of care at the time when the IMP (selepressin or placebo) infusion is initiated.

Before administration, selepressin will be diluted to one of four specific concentrations to allow for blinded administration using similar infusion rates. The infusion of IMP (selepressin or placebo) will start as early as possible and no later than 12 hours after initiation of continuous infusion of vasopressor treatment for septic shock. To ensure start of IMP treatment without delay, informed consent will be obtained, in compliance with local regulations, as early as possible.

During the course of treatment, the IMP infusion rate will be adjusted within pre-defined infusion rates to keep the MAP at the target of 65 mmHg. A different MAP target is acceptable, if it is pre-specified and if it is appropriate, as judged by the investigator, for the clinical management e.g. previous history of hypotension or hypertension (if deemed necessary to maintain adequate organ perfusion). A detailed IMP and vasopressor administration guide will be provided to trial sites. If the administration of IMP in addition to norepinephrine/noradrenaline increases MAP to above the target, norepinephrine/noradrenaline will be weaned first while aiming to keep MAP at the target. Norepinephrine/noradrenaline must be completely weaned prior to weaning of IMP. If norepinephrine/noradrenaline cannot be completely weaned, the infusion rate of IMP is to be increased up to the maximum allowed infusion rate in an attempt to use IMP as the sole vasopressor. If infusion of IMP alone increases the MAP to above the target, the IMP will be weaned step-wise while aiming to keep MAP at the target. If the maximum allowed infusion rate of the IMP is not sufficient to maintain MAP at the target, norepinephrine/noradrenaline will be added to achieve the targeted MAP. If target MAP cannot be maintained despite maximum allowed infusion rate of IMP and ≥1 μg/kg/min norepinephrine/noradrenaline base (or a total catecholamine equivalent of 1 μg/kg/min norepinephrine/noradrenaline base), vasopressin may be added.

The IMP infusion will continue as long as blood pressure support is deemed necessary with a maximum period of 30 days. After complete weaning, IMP infusion may be re-started during this 30 day-period if necessary. Patients who still need vasopressor treatment after 30 days will be switched to other vasopressors at the discretion of the investigator.

NUMBER OF PATIENTS

Enough patients will be enrolled in the trial to obtain 1800 evaluable patients in the entire programme (including both parts of the trial).

CRITERIA FOR INCLUSION / EXCLUSION

The intention is to enrol a typical sample of patients presenting with septic shock and commence treatment with the IMP during the initial hours of resuscitation, within 12 hours from onset of vasopressor treatment, targeting those who do not respond rapidly to fluids and whose vasopressor-need persists for at least one hour.

Inclusion criteria

1. 18 years of age or older.
2. Proven or suspected infection.
3. Septic shock defined as hypotension (systolic blood pressure less than 90 mmHg OR MAP less than 65 mmHg) requiring vasopressor treatment (i.e. any dose of norepinephrine / noradrenaline base greater than 5 µg/min) despite adequate fluid resuscitation (at least one litre for hypotension).
4. Informed consent obtained in accordance with local regulations.

Exclusion criteria

1. Not possible to initiate IMP treatment within 12 hours from onset of vasopressor treatment for septic shock.
2. Primary cause of hypotension not due to sepsis (e.g. major trauma including traumatic brain injury, haemorrhage, burns, or congestive heart failure/cardiogenic shock).
3. Previous severe sepsis with ICU admission within this hospital stay.
4. Known/suspected acute mesenteric ischaemia.
5. Suspicion of concomitant acute coronary syndrome based on clinical symptoms and/or ECG during this episode of septic shock.
6. Chronic mechanical ventilation for any reason OR severe COPD requiring either continuous daily oxygen use during the preceding 30 days or mechanical ventilation (for acute exacerbation of COPD) during the preceding 30 days.
7. Received bone marrow transplant during the preceding 6 months or chemotherapy during the preceding 30 days for lymphoma or leukemia.
8. Known to be pregnant.
9. Decision to limit full care taken before obtaining informed consent.
10. Use of vasopressin in the past 12 hours prior to start of the IMP infusion or use of terlipressin within 7 days prior to start of the IMP infusion.
11. Prior enrolment in the trial.
12. Prior use of an investigational medicinal product within the last month OR planned or concurrent participation in a clinical trial for any investigational drug or investigational device.

Eligibility criteria – post-randomisation / before start of IMP infusion

In addition, the following criteria must be met at start of IMP infusion:

1. Received a minimum of 30 mL/kg fluid in total from the onset of hypotension (or less if evidence of fluid replete/overload).
2. Received a continuous infusion of norepinephrine/noradrenaline base greater than 5 µg/min for at least one hour and is still receiving at least 5 µg/min norepinephrine/noradrenaline base.
3. Less than 12 hours since onset of vasopressor treatment for septic shock.

INVESTIGATIONAL MEDICINAL PRODUCTS

- Selepressin 0.3 mg/mL (10 mM acetate buffer, pH 4). Selepressin is provided as a stock solution which will be diluted with sterile 0.9% sodium chloride solution prior to infusion according to a specific dilution protocol.
- Placebo: sterile 0.9% sodium chloride solution.

DURATION OF TREATMENT

All patients will receive IMP (selepressin or placebo) as an intravenous infusion until recovery from the need of vasopressor treatment or for 30 days, whichever comes first. If the need of vasopressor treatment subsides and recurs within the 30-day treatment period, IMP should be used when possible.

STATISTICAL METHODS

The trial is powered to demonstrate superiority of selepressin plus standard care versus placebo plus standard care in the number of P&VFDs in patients with vasopressor-dependent septic shock.

Primary analysis

If Part 2 of the trial is run, the primary endpoint, P&VFDs, will be analysed using a van Elteren test. The primary analysis will compare all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) to all patients on the placebo arm from both parts of the trial.

The primary analysis will be a test of superiority using a two-sided 5% significance level test. This test, within the trial, controls the type 1 error at a two-sided 5% level.

The analysis will be based on both the full analysis set (FAS) and the per protocol (PP) analysis set, with the FAS being considered the primary analysis to judge statistical significance and the PP analysis considered as supportive. The FAS comprises all randomised (as planned) patients who have received IMP treatment.

Power

The overall power for obtaining statistical significance based on combined evidence from Part 1 and Part 2 is 91% in situations where all 4 arms have a true underlying 1.5% lower mortality rate and a 1.5-day higher expected number of P&VFDs for survivors (corresponding to an overall treatment effect of 1.5 P&VFDs) as compared to placebo. If the effect sizes are 2% on mortality and 2 days for P&VFDs in survivors for all 4 arms (corresponding to an overall treatment effect of 2 P&VFDs) then the overall power is 98%. In this latter case the probability of engaging into Part 2 is ~99%.

Secondary analyses
The secondary endpoints are aimed at supporting primary efficacy by further demonstrating treatment effect accompanied by an acceptable safety profile.

Endpoints defined as '-free days' will be defined and analysed in a similar manner as the primary endpoint. Endpoints addressing SOFA score, fluid balance, and health-related quality of life as well as all endpoints measuring duration and split in survivors/non-survivors will be analysed by analysis of covariance (ANCOVA) methods and presented graphically. Mortality, incidence of new organ dysfunction, and new organ failure will be analysed by logistic regression. Mortality will be presented graphically by a Kaplan-Meier plot. All secondary endpoints will be analysed using both the FAS and the PP analysis set.

For the purpose of a possible label inclusion, the Hochberg procedure for adjustment on multiplicity will be implemented to selected secondary endpoints. Only if the primary efficacy analysis leads to a statistically significant result at the (one-sided) 2.5% level, then the Hochberg procedure, which is described below, will be applied to selected secondary analyses. If the primary efficacy analysis does not result in statistical significance at the (one-sided) 2.5% level, then statistical significance (for the purpose of a possible label inclusion only) will not be declared for any of the secondary analyses, regardless of their p-values.

The selected key secondary endpoints aimed at further demonstrating treatment effect are:

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Day 90
- RRT-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)
- ICU-free days up to Day 30

In this application of the Hochberg procedure there are three hypothesis tests of superiority for each of the selected secondary endpoints. The target alpha level is (one-sided) 2.5%. The Hochberg procedure is as follows:

- Order the p-values from the smallest to the largest value, $p(1) < p(2) < p(3)$, with corresponding null hypothesis $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$.
- Start with the highest p-value. If $p(3) < 2.5\%$ (one-sided), then stop and declare all three comparisons significant at the 2.5% (one-sided) level (i.e. reject $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$). Otherwise, accept $H_{(3)}$ for the endpoint related to p(3), and go to p(2) the second highest p-value.
- If $p(2) < 2.5/2 = 1.25\%$ (one-sided), then stop and declare significance for $H_{(1)}$ and $H_{(2)}$. Otherwise, accept $H_{(2)}$, for the endpoint related to p(2), and go to p(1) the lowest p-value.

- If p(1) < 2.5/3 = 0.833% (one-sided), then stop and declare significance for $H_{(1)}$. Otherwise, accept $H_{(1)}$, for the endpoint related to p(1).

Regardless of the statistical significance declared according to the Hochberg procedure, all analysis will be included and presented in the statistical report.

The safety profile, including adverse events, vital signs, and safety laboratory variables, will be summarised descriptively. The safety analyses will be performed using the safety analysis set. The safety analysis set comprises all IMP-treated patients and are analysed according to the actual treatment received.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

List of Abbreviations

ANCOVA	Analysis of Covariance
ATC	Anatomical Therapeutic Chemical (Classification System)
CCC	Clinical Coordinating Centre
CCL	CC Chemokine Ligand
COPD	Chronic Obstructive Pulmonary Disease
CSF	Colony-stimulating Factor
CVP	Central Venous Pressure
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ED	Emergency Department
EQ-5D-5L	EuroQoL group's 5-dimension 5-level Questionnaire
EudraCT	European Union Clinical Trial Database
EVLW	Extravascular Lung Water
FAS	Full Analysis Set
FiO_2	Fraction of Inspired Oxygen
FPFV	First Patient First Visit
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HCO_3	Bicarbonate
ICMJE	the International Committee of Medicinal Journal Editors
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IL	Interleukin
IMP	Investigational Medicinal Product
IND	Investigational New Drug

IRB	Institutional Review Board
ITT	Intention-to-Treat
KM	Kaplan Meier
LOCF	Last Observation Carried Forward
LPLV	Last Patient Last Visit
LTA	Lymphotoxin Alpha
MAP	Mean Arterial Pressure
MedDRA	Medical Dictionary for Regulatory Activities
P&VFD	Vasopressor- and Mechanical Ventilator-free Day
$PaCO_2$	Arterial Carbon Dioxide Partial Pressure
PaO_2	Arterial Oxygen Partial Pressure
PK	Pharmacokinetic
PP	Per Protocol
PPI	Pulmonary Permeability Index
PT	Preferred Term
QALY	Quality-adjusted Life Years
RRT	Renal Replacement Therapy
SaO_2	Arterial Oxygen Saturation
$ScvO_2$	Central Venous Oxygen Saturation
SOC	System Organ Class
SOFA	Sequential Organ Failure Assessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
TSC	Trial Steering Committee
VEGFA	Vascular Endothelial Growth Factor

Definition of Terms

Audit	A systematic and independent examination of trial-related activities and documents to determine whether the evaluated trial-related activities were conducted, and the data were recorded, analysed, and accurately reported according to the protocol, sponsor's standard operating procedures, good clinical practice, and the applicable regulatory requirement(s).
Clinical Coordinating Center	A third party contracted by Ferring to provide support to the trial sites for certain aspects of conduct of the trial, such as assessment of eligibility and medical support.
Compliance	Adherence to all the trial-related requirements, good clinical practice requirements, and the applicable regulatory requirements.
Evaluable Patient	A patient who has been treated with the investigational medicinal product (IMP) and 30 days have passed since initiation of IMP infusion.
Good Clinical Practice	A standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of trial participants are protected.
Investigator	The person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator.
Legal Representative	An individual or juridical or other body authorised under applicable law to consent, on behalf of a prospective patient, to the patient's participation in the clinical trial.
Treated Patient	A patient who has been treated with the IMP.
Trial Entry Terms	*Screening* The act of determining if an individual meets requirements for participation in the clinical trial. *Enter (=Consent)* The act of obtaining informed consent for participation in the clinical trial from patients deemed eligible or potentially eligible to participate in the clinical trial. Patients who have entered into a trial are those who sign the informed consent document directly or through their legally acceptable representatives. *Enrolment (=Randomisation)* The act of assigning a patient to a treatment. Patients who are enrolled in the trial are those who have been assigned to a treatment.

1 INTRODUCTION

1.1 Background

Ferring Pharmaceuticals A/S (hereinafter called Ferring) is currently developing selepressin, a novel vasoconstrictor agent for treatment of patients with vasopressor-dependent septic shock.

Patients suffering from septic shock, defined by the need for vasopressor treatment despite adequate fluid resuscitation, represent an extremely ill patient population with a common need for prolonged intensive care, frequent multisystem organ failure, and a high mortality. Septic shock is one of the most common causes of death in intensive care units (ICUs) and its incidence is rising. The growing incidence is most likely due to increased use of invasive devices and immunosuppressive therapies, higher numbers of immunocompromised patients, more antibiotic resistance, and an aging population.

Selepressin is a cyclic nonapeptide vasopressin analogue with high affinity and selectivity for the human vasopressin V_{1a}-receptor versus the V_{1b}-, V_2-, and oxytocin receptors and no known affinity to other receptors, ion-channels, and transporters (Laporte R et al, 2011). The lack of V_{1b} and V_2 activity is the main differentiator of selepressin from existing vasopressin analogues such as arginine vasopressin.

In addition to its vasopressor activity, one of the key characteristic of selepressin is its apparent anti-leakage properties (Maybauer et al., 2014; Rehberg et al., 2012(a); Rehberg et al., 2011; Su et al., 2012). The ability to limit vascular leakage is a clinically relevant and very important feature of selepressin. While fluid therapy is fundamental to the acute resuscitation of critically ill patients, in those with sepsis accompanied by increased capillary permeability from microvascular injury, it can contribute to tissue oedema and eventually organ dysfunction.

Thus, selepressin may serve a dual role of providing haemodynamic benefit while reducing the leakage of intravascular fluid into the extracellular space. It is believed that these unique characteristics could help address the unmet need in the treatment of vasopressor-dependent septic shock and provide significant benefit for the patients.

Moreover, due to the lack of V_2 activity, selepressin does not cause V_2-mediated antidiuresis and release of coagulation factors (Rehberg et al, 2012(b)), important safety consideration in septic shock patients that often have a positive fluid balance and coagulation disturbances.

1.2 Scientific Justification for Conducting the Trial

Septic shock is characterised by hypotension and decreased tissue perfusion due to vasodilation and capillary leakage. In this setting, vasopressors such as norepinephrine / noradrenaline, epinephrine / adrenaline, and dopamine are considered to be critical life-support necessary to quickly restore perfusion pressure. However, at the same time these drugs may cause serious adverse effects such as arrhythmias, myocardial, mesenteric, cerebral, or digital ischaemia/infarction, and acute kidney injury. Such complications may prevent or prolong the recovery from septic shock and may cause long-term sequelae. Therefore, interventions that decrease the duration of the need for vasopressors are expected to result in reduced risk of complications and possibly improved patient outcome.

In the first clinical trial with selepressin in septic shock patients (FE 202158 CS02), selepressin reduced the need for norepinephrine/noradrenaline in a dose-dependent manner (Ferring report, CS02). Furthermore, selepressin significantly reduced the duration of mechanical ventilation compared to placebo during the first 7 days. Also, cumulative fluid balance and fluid overload tended to be reduced compared to placebo. However, the FE 202158 CS02 trial was not powered to assess clinical outcomes and therefore, a large pivotal trial is needed to fully assess the effects and clinical outcomes associated with the use of selepressin.

The present trial (trial code: 000133) is an adequately powered Phase 2b/3 clinical trial designed to assess and confirm the efficacy and safety of selepressin as a treatment for patients with vasopressor-dependent septic shock. The trial design has been reviewed by both the United States Food and Drug Administration and the European Medicines Agency.

1.3 Benefit / Risk Aspects

Selepressin is a potent vasoconstrictor and expected pharmacological effects include correction of hypotension which makes treatment potentially beneficial to the septic shock patients participating in the trial. The vasoconstrictor effect of selepressin has been investigated in a number of studies in vitro and in vivo in healthy animals, in sheep models of severe sepsis and septic shock, in isolated human resistance arteries, and in septic shock patients. In animal models of severe sepsis and septic shock, selepressin was shown to limit fluid accumulation and pulmonary oedema formation (Maybauer et al, 2014; Su et al, 2012). In patients with septic shock, this may provide clinical benefit by reducing mortality and/or the total duration of time that the patient requires life support with vasopressors and mechanical ventilation, speeding up the recovery, and reducing the risk of short- and long-term sequelae from organ dysfunction.

In healthy adult men and women, the safety profile of selepressin was consistent with the pharmacological effects of a V_{1a} agonist, mainly related to its vasoconstrictor properties, and did not cause any safety concerns (Ferring report, FE 202158 CS01). The dose-limiting effect was reduction in cardiac output. The infusion of selepressin was interrupted in 7 out of 10 subjects in the highest dose group (13.1 µg/h [i.e. 3.1 ng/kg/min assuming 70 kg body weight]) due to a decrease in cardiac output by more than 25%, which was a pre-defined criterion for termination of infusion. No negative renal or hepatic effects were observed. Some subjects reported signs of gastrointestinal discomfort, pallor, and feeling of body temperature change (i.e. signs of effects on the peripheral blood flow). The ECG, blood gases, lactate, and the other safety laboratory parameters were not affected to any measurable extent by the infusion of selepressin. QTcF values of >450 msec, or an increase of >30 msec, were sporadically observed in all dose groups at all time-points, including 7 subjects that received placebo. V_{1a}-induced mesenteric vasoconstriction is a general safety concern. However, no signs of mesenteric or myocardial ischaemia were observed in the healthy subjects. The lack of signs of myocardial ischaemia is consistent with previous findings that selepressin did not demonstrate specific coronary vasoconstrictive properties in anaesthetised dogs.

Two clinical trials in septic shock patients have been conducted. In the first trial (trial code: FE 202158 CS02), selepressin was safe and well tolerated up to an infusion rate of 2.5 ng/kg/min. Too few patients were enrolled in the highest dose group (i.e. 3.75 ng/kg/min), and hence it was not possible to conclude on the safety of this dose level. In approximately 50% of the patients receiving 2.5 ng/kg/min of selepressin, norepinephrine/noradrenaline could be completely weaned within 12 hours whereas all patients receiving 1.25 ng/kg/min required norepinephrine/noradrenaline at 12 hours (Ferring report, FE 202158 CS02). There were neither apparent differences between treatment with selepressin and placebo with respect to adverse event reporting nor apparent shifts or trends in the means of the ECG parameters during the treatment period that could be attributed to the treatment. A number of patients in all treatment groups, including >75% of the placebo patients, had occasional QTcF values >450 ms, several of whom already had this finding at baseline, or an increase of >30 ms. These observations were regarded as caused by the underlying disease.

The second trial in septic shock patients (trial code: 000025) investigated the dose-range from 2.5-7.5 ng/kg/min and allowed free up- as well as down-titration of selepressin within a certain range. Data from this trial showed that: 1) it was possible to wean norepinephrine/noradrenaline completely within few hours in most of the patients when the selepressin dose was increased to 5-7.5 ng/kg/min, 2) it was possible to maintain mean arterial pressure (MAP) within a target range by adjusting the dose, and 3) in the majority of patients, doses above 3.75 ng/kg/min were only needed during the first 6 hours. Five patients reported a total of eight treatment-emergent serious adverse events which were regarded as related to the treatment. Four of these events occurred in the same patient including one with fatal outcome. Although it cannot be ruled out that the infusion of selepressin, which was stopped after 9.5 hours, approximately 17 hours before the fatal outcome, contributed to the development of the adverse event, it should be recognised that the condition of the patient at enrolment was very serious with a very high baseline infusion rate of noradrenaline (1 µg/kg/min) and a high lactate level (5.4 mmol/L).

The pharmacokinetic profile of selepressin in septic shock patients is based on data from the FE 202158 CS02 and the 000025 trials. As the infusion rate of selepressin was adjusted according to the need of the patient, the pharmacokinetic parameters were calculated by means of population pharmacokinetic modelling. Clearance was on average estimated to 11.28 L/h in a typical patient with a body weight of 70 kg, with inter-individual variability of 22 (CV%). The time to steady state concentration was approximately 7 hours and the steady-state concentrations were proportional to the initial infusion rate. The terminal half-life was approximately 1.4 hours irrespective of dose. However, the initial distribution phase half-life was short, approximately 10 minutes.

The evaluation of benefits and risks indicate that participation in this trial is associated with a favourable benefit-risk ratio. The trial is justified by the potential clinical benefit of a selective V_{1a} receptor agonist treatment in patients with vasopressor-dependent septic shock and the risk posed to the patients participating in the trial is deemed low as the standard septic shock treatment will be in line with the Surviving Sepsis Campaign guidelines (Dellinger et al, 2013). Patients participating in this trial will be closely monitored and they will have either the same or more frequent contacts with treating clinicians compared to routine treatment, depending on local clinical practice.

For further information regarding selepressin, please refer to the current investigator's brochure.

2 TRIAL OBJECTIVES AND ENDPOINTS

2.1 Objectives

Primary Objective

- To demonstrate superiority of selepressin plus standard care versus placebo plus standard care in the number of vasopressor- and mechanical ventilator-free days (with penalty for mortality) in patients with vasopressor-dependent septic shock

Secondary Objectives

- To determine the efficacy of selepressin on:
  - Organ dysfunction
  - Morbidity and mortality
  - Fluid balance
  - Health-related quality of life
- To determine the safety profile of selepressin
- To determine the pharmacokinetics of selepressin
- To determine the health economics of selepressin
- To further evaluate a range of biomarkers in relation to the mode of action of selepressin

2.2 Endpoints

Primary Endpoint

- Vasopressor- and mechanical ventilator-free days (P&VFDs) up to Day 30

This composite endpoint is defined as the number of days (reported to one decimal place [0.0 to 30.0 days]) from start of treatment with the investigational medicinal product (IMP) [selepressin or placebo] to 30.0 days thereafter during which the patient is: 1) alive; 2) free of treatment with intravenous vasopressors; and 3) free of any invasive mechanical ventilation (see definition below).

Any patient that dies within this 30-day period is assigned zero P&VFDs, even if there is a period during which the patient is free of both vasopressor treatment and mechanical ventilation. If vasopressors need to be restarted or mechanical ventilation needs to be initiated or restarted, and the use of either is greater than 60 minutes within a 24-hour period, then the clock is reset and the patient is not considered free of vasopressors and/or mechanical ventilation until after those therapies are again finally discontinued. Vasopressor use or mechanical ventilation during - and up to three hours after - surgery / procedure (including bedside) is exempt from this rule (i.e. does not reset the calculation of P&VFDs). The intent is for the endpoint to reflect the speed of recovery from septic shock and respiratory failure, with appropriate penalties for recurrent shock, new or recurrent respiratory failure, and death.

Vasopressor use is defined as any intravenous dose of norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, and IMP (i.e. selepressin and placebo).

Mechanical ventilation is defined as use of endotracheal or tracheostomy tube assisted ventilation (>5 cm H_2O continuous positive airway pressure and >5 cm H_2O of pressure support from the ventilator in tracheostomy patients). End of mechanical ventilation is defined as: 1) extubation of intubated patients or 2) ≤5 cm H_2O continuous positive airway pressure and ≤5 cm H_2O of pressure support from the ventilator in tracheostomy patients. If non-invasive ventilation by mask or bag (>5 cm H_2O of pressure support) is deployed to avoid (re)intubation, it also counts as mechanical ventilation. However, all other uses of non-invasive ventilation such as chronic night-time use of positive airway pressure for chronic obstructive pulmonary disease (COPD) or sleep apnea does not count as mechanical ventilation (regardless of pressure).

Key Secondary Endpoints

The following key secondary endpoints have been selected for the purpose of a possible label inclusion (see Section 9.6.3).

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Day 90
- Renal replacement therapy (RRT)-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)

- Intensive care unit (ICU)-free days up to Day 30

Secondary Efficacy Endpoints
Other secondary endpoints are listed below. All ssessments related to the endpoints are described in Section 7.1.

Organ dysfunction
- Vasopressor-free days up to Day 30
- Mechanical ventilator-free days up to Day 30
- Duration of septic shock (i.e. vasopressor use) up to Day 30
- Duration of mechanical ventilation up to Day 30
- Incidence of RRT up to Day 30 (counting patients who die as on RRT and excluding patients on RRT for chronic renal failure at time of randomisation)
- Duration of RRT up to Day 90 (excluding patients on RRT for chronic failure at time of randomisation)
- Daily overall and individual organ (cardiovascular, respiratory, renal, hepatic, coagulation) scores using a modified version of the Sequential Organ Failure Assessment (SOFA) until ICU discharge
- Incidence of new organ dysfunction and new organ failure (based on the SOFA score) up to Days 7 and 30

Morbidity and mortality
- ICU length of stay up to Day 30
- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Days 30 and 180

Fluid balance
- Daily and cumulative fluid balance until ICU discharge (for a maximum of 7 days)
- Daily and cumulative urine output until ICU discharge (for a maximum of 7 days)

Health-related quality of life
- Change in utility, based on the EuroQol group's 5-dimension 5-level (EQ-5D-5L) questionnaire, up to Day 180

Safety Endpoints
- Incidence of adverse events (type, frequency, and intensity) with specific emphasis on:
    - Ischaemic events (e.g. myocardial, skin, cerebral, mesenteric, and limb ischaemia)
    - Changes in vital signs assessed as unanticipated in the setting of septic shock
    - Changes in safety laboratory variables assessed as unanticipated in the setting of septic shock
- Changes in vital signs and safety laboratory variables

- Episodes of hypotension (MAP <60 mmHg for longer than one hour)

Additional Endpoints

- Hospital-free days up to Day 90
- Hospital length of stay up to Day 90
- Patient residence at Day 30, Day 60, Day 90, and Day 180
- Health economic evaluation - to be reported separately according to a pre-specified health economic analytical plan
- Mean arterial pressure (MAP), until ICU discharge (for a maximum of 7 days)
- Norepinephrine/noradrenaline and other vasopressor doses
- Pharmacokinetic response (in a subset of approximately 200 patients) - to be reported separately according to a pre-specified pharmacokinetic analysis plan (including plasma level of selepressin at the first attempt to wean the IMP infusion)
- Creatinine clearance
- Ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2 ratio)
- Extravascular lung water and pulmonary permeability index (in a subset of 100-350 patients)
- Cardiac output (in a subset of 100-350 patients)
- Cytokines (in a subset of 100-350 patients)
- Angiopoietin-1 and -2 (in a subset of 100-350 patients)

3 INVESTIGATIONAL PLAN

3.1 Overall Trial Design

The overall trial design is a multi-centre, double-blind, randomised, placebo-controlled, two-part adaptive clinical trial. The trial is designed to investigate the efficacy and safety of multiple dosing regimens of selepressin and to confirm the efficacy and safety of one dosing regimen in treatment of adult patients with septic shock requiring vasopressor treatment.

Up to four dosing regimens of selepressin, as described in Table 1, will be investigated in the first part of the trial and the best-performing dosing regimen will be selected for the second part of the trial.

Table 1 Dosing Regimens

	Starting Dose (ng/kg/min)	Maximum Dose (ng/kg/min)	Range (ng/kg/min)
Arm 1	1.7	2.5	0-2.5
Arm 2	2.5	3.75	0-3.75
Arm 3	3.5	5.25	0-5.25
Arm 4	5.0	7.5	0-7.5

3.1.1 Trial Design Diagram

The overall trial design is illustrated in Figure 1. The entire trial duration for an individual patient is up to 6 months (see Section 6).

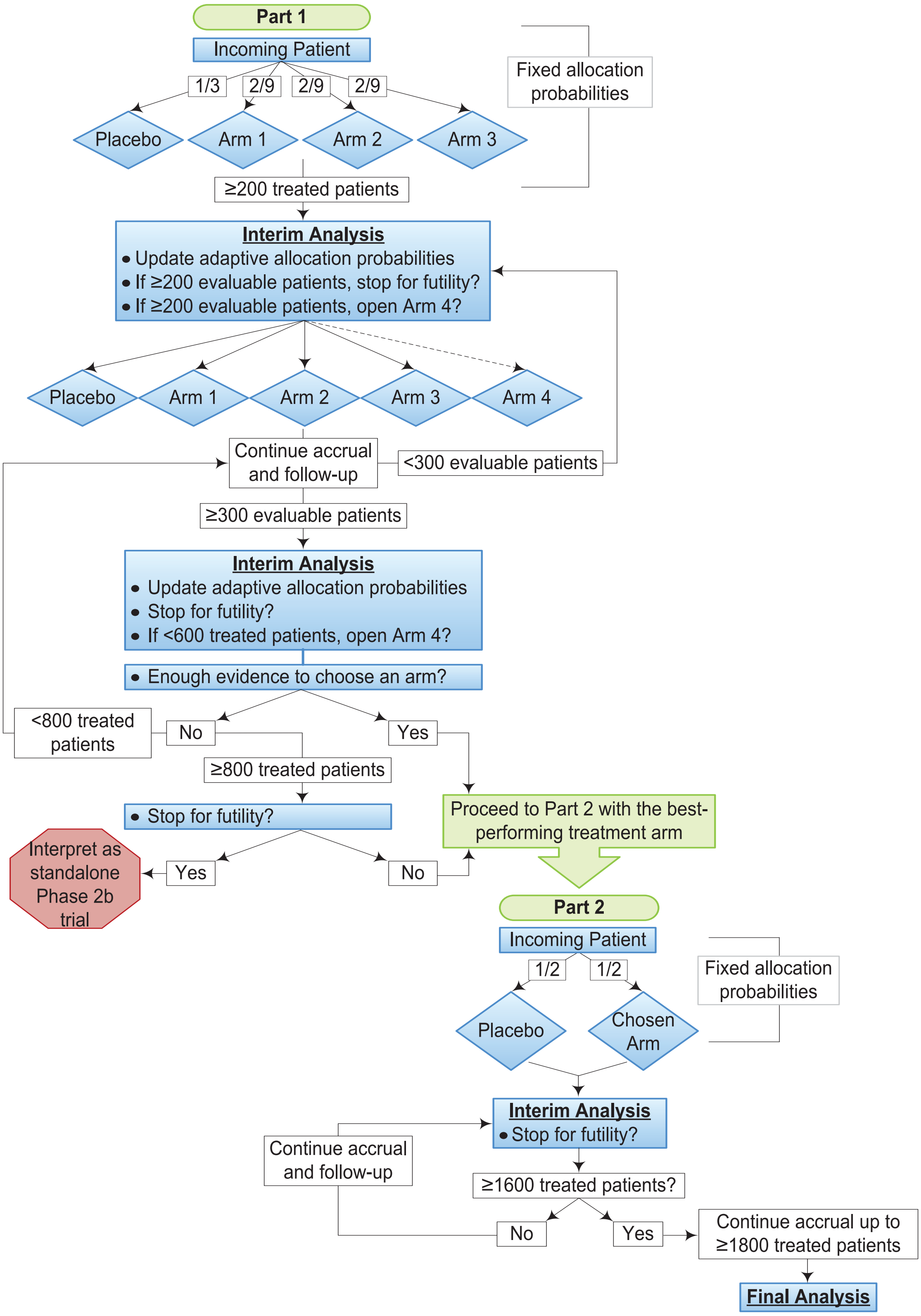


Figure 1 Trial Design

3.1.2 Overall Design and Control Methods

This is a Phase 2b/3 adaptive clinical trial with two parts: Part 1 - Phase 2b and Part 2 - Phase 3 (Figure 1). The entire trial, combining both parts, represents an adequate and well-controlled comparison of selepressin versus placebo. Part 1 comprises 300 evaluable – 800 treated patients. The size of Part 2 will include enough patients to bring the total number of patients in Part 1 and Part 2 to 1800 evaluable patients, ensuring a minimum sample size of 1000 patients in Part 2. The final analysis uses all patients from both parts of the trial.

Part 1 will begin with a 200-patient ‘burn-in’ period during which fixed randomisation across Arms 1, 2, and 3 will be used (the arms are described in Table 1) with:

- One-third of the patients randomised to placebo
- Two-third of the patients randomised to selepressin (two-ninths of the patients to each of the Arms 1, 2, and 3).

After completion of the ‘burn-in’ period, Part 1 will utilise response-adaptive randomisation to preferentially allocate patients to the treatment arms that appear to have the maximum benefit with respect to the primary endpoint (i.e. vasopressor- and mechanical ventilator-free days [P&VFD]). A fixed fraction (one-third) of patients will be randomised to placebo throughout Part 1 to ensure contemporaneous control patients are enrolled throughout the trial. Arm 4 will only be opened between 200 evaluable and 600 treated patients and if there is at least a 50% posterior probability that Arm 3 has a higher expected P&VFD than Arm 2 and data from the lower dosing regimens do not suggest any significant safety concerns.

If Part 1 results in the decision to run Part 2, Part 2 will utilise a fixed 1:1 randomisation proportion between placebo and the best-performing dosing regimen of selepressin. The best-performing dosing regimen will be identified at the end of Part 1.

Pre-defined interim analyses will be conducted as described in Section 3.3 and Section 9.9. Patient enrolment will continue without any stop at the interim analyses and a seamless transition to Part 2 can occur after any interim analysis after 300 evaluable - 800 treated patients in Part 1.

3.1.3 Trial Schedule

The estimated timelines are:

- First patient first visit (FPFV): Q3 2015
- Last patient at Day 30 (primary database lock): Q2 2018
- Last patient last visit (LPLV) / end-of-trial: Q4 2018

It is expected that all patients will be recruited within a period of up to 3 years. Hence, the duration of the entire trial is expected to be no longer than 3.5 years.

The primary database will be locked when all ongoing patients have passed Day 30.

3.2 Planned Number of Trial Sites and Patients

Enough patients will be randomised to bring the total number of evaluable patients up to 1800 in the entire trial programme (including both parts of the trial). Patient recruitment will be competitive between trial sites. To achieve the requested number of patients within the given timelines in Section 3.1.3, approximately 60 - 100 trial sites will actively participate in the trial, with additional sites identified as 'back-up' sites to replace non-performing sites.

3.3 Interim Analysis

There will be no interim analyses with the potential to stop the trial early for success. The trial will either stop for futility or run to 1800 evaluable patients.

Once the 'burn-in' period in Part 1 is completed, interim analyses will be conducted regularly during Part 1 to improve the efficiency of dosing regimen selection and to allow early termination for futility or for successful dosing regimen selection.

During Part 2, regular interim analyses for futility will be performed until 1600 patients have been treated.

The interim analyses reports will be prepared by an external company who is not otherwise involved in the conduct of the trial, and who will not divulge these data to Ferring personnel. The measures taken to protect the overall blinding during the interim analyses are illustrated in Figure 2 in Section 5.5.1.

The statistical considerations of the interim analyses are further described in Section 9.9.

3.4 Data and Safety Monitoring Board

A data and safety monitoring board (DSMB) will be established for this trial. The DSMB will be an independent group of critical care and emergency medicine experts not otherwise involved in the trial and a statistician with expertise in adaptive designs. The DSMB will oversee safety and ensure appropriate trial conduct, which includes overseeing that the adaptive design is implemented and performing as intended.

The DSMB will have access to unblinded interim efficacy and safety data as well as recruitment data so that they are able to monitor the enrolment of the intended patient population. The DSMB will keep the interim results confidential from any individuals involved in the trial conduct. A trial-specific DSMB charter specifies the composition of the DSMB and its responsibilities and working procedures.

3.5 Discussion of Overall Trial Design and Choice of Control Groups

3.5.1 Trial Design

This is a multi-centre, randomised, placebo-controlled, double-blind Phase 2b/3 adaptive clinical trial, which will be conducted in accordance with the protocol, good clinical practice, and applicable regulatory requirements.

The trial will be conducted at multiple trial sites predominantly across Europe and North America. High-quality trial sites with shared standards of practice and values will be selected; all trial sites follow the globally accepted Surviving Sepsis Campaign guidelines (Dellinger et al, 2013) which provides recommendations for the best care of patients with severe sepsis and septic shock.

Patients will be enrolled in the trial and the IMP infusion will be initiated as early as possible. To ensure, as far as possible, that only eligible patients enter the trial (see Section 4.1), all potential patients will be discussed with one of the assigned clinical coordinating centres (CCCs) prior to randomisation. The CCCs will be available 24 hours a day throughout the trial to answer investigators' medical questions (such as assessment of eligibility and medical support).

As described in Section 3.4, the trial will be overseen by an independent DSMB. In addition, a trial steering committee (TSC) will oversee the overall conduct of the trial in a blinded manner. The TSC includes representatives of Ferring and the CCCs, trial investigators, and experts in the fields of critical care, emergency medicine and clinical trial methodology. The TSC will make recommendations to Ferring regarding all trial-related decisions including those based on recommendations from the DSMB. A TSC charter specifies the composition of the TSC and its responsibilities and working procedures.

The trial design is robust with clear, prospectively determined clinical and statistical analytic criteria. The design achieves control of type 1 error through analytical means. While the trial can stop early for futility, a successful Phase 3 trial can only be achieved with at least 1800 evaluable patients. At the end of the trial, a single test statistic will be calculated to compare two populations that are defined before the trial begins, namely patients allocated to placebo compared to patients allocated to any dosing regimen of selepressin.

3.5.2 Selection of Endpoints

Mortality is indisputably a critical endpoint to consider in septic shock patients; however, as more and more patients survive septic shock it becomes increasingly important to focus on patient-centred outcomes in survivors such as improving the speed of recovery, the ability to limit the need for life support, and long-term sequelae of septic shock. Therefore in this trial, we will not only assess mortality, but also compare survival free of both vasopressor and mechanical ventilation – the two primary forms of life-support provided to septic shock patients in the ICUs. The primary endpoint is constructed to capture selepressin's ability to hasten the resolution of septic shock and to reduce the time the patient is dependent on life-support. A faster resolution of septic shock is expected to lead to a reduction in irreversible morbidities and serious outcomes resulting from fluid overload and ischaemic damage to organs.

The primary endpoint is a composite endpoint capturing the need for vasopressor, the need for mechanical ventilation, and mortality. All three components on their own represent important beneficial effects for septic shock patients and will also be captured separately as secondary endpoints. This combined endpoint (vasopressor- and mechanical ventilator-free days [P&VFDs])

is well aligned with the currently understood beneficial actions of selepressin and supported by the effects of selepressin found in previous non-clinical and clinical trials as discussed in Section 1.

Septic shock is associated with a wide array of serious and troublesome sequelae that may impact long-term outcomes. The secondary efficacy and safety endpoints (as listed in Section 2.2) were selected to focus on key effects related to the primary mode of action of selepressin (i.e. vasopressor and anti-leakage effects) and at the same time to capture other clinically meaningful outcomes such as incidence of organ dysfunction, mortality, quality of life, and general safety of selepressin. Hence, the secondary endpoints will capture both efficacy and safety aspects of the treatment. Furthermore, the length of stay at the ICU as well as health-related quality of life represent society and life impact focused endpoints.

Patients with septic shock represent a notoriously heterogeneous patient population. To account for potential differences in outcome based on baseline characteristics, patients will be randomised in a stratified manner as described in Section 4.2.2.

3.5.3 Choice of Control Group

In this trial, selepressin plus standard of care will be compared to placebo plus standard of care with norepinephrine/noradrenaline as primary vasopressor. Use of placebo allows provision of usual standard of care in both the selepressin-treated group and the placebo-treated group. The investigators can continue the use of norepinephrine/noradrenaline, continue to add (or not) antibiotics, fluids, inotropic agents (such as dobutamine), oxygen, mechanical ventilation, corticosteroids, feeding, and other supportive care in accordance with standard clinical practice following the Surviving Sepsis Campaign guidelines (Dellinger et al, 2013). Thus, this background of ethical usual clinical care would occur in both the selepressin-treated group and the placebo-treated group.

3.5.4 Selection of Doses in the Trial

Four dosing regimens of selepressin ranging from 1.7 ng/kg/min to 7.5 ng/kg/min (as detailed in Table 1) have been selected for this trial. These dosing regimens are chosen to cover the relevant dose range based on dosing experience from the previous clinical trials (i.e. FE 202158 CS02 and 000025).

In FE 202158 CS02, which was the first clinical trial in septic shock patients, selepressin was safe and well tolerated up to 2.5 ng/kg/min. In approximately 50% of the patients receiving 2.5 ng/kg/min of selepressin, norepinephrine/noradrenaline could be completely weaned within 12 hours whereas all patients receiving 1.25 ng/kg/min of selepressin required norepinephrine/ noradrenaline at 12 hours (Ferring report FE 202158 CS02). This suggests that some patients may require more than 2.5 ng/kg/min of selepressin in order to wean norepinephrine/noradrenaline completely.

The second clinical trial (000025) was initiated to assess whether use of higher doses of selepressin would allow for a faster and more complete weaning of norepinephrine/noradrenaline. In that trial, the dose-range from 2.5 to 7.5 ng/kg/min with free up- and down-titration of selepressin within a certain range was investigated. The flexible dose-adjusting was designed to mimic the current clinical practice for the use of vasopressors. The 000025 trial showed that it was possible to wean norepinephrine/noradrenaline completely within a few hours in most of the patients when the selepressin dose was increased to 5 to 7.5 ng/kg/min. Despite the pharmacokinetic characteristics of selepressin (i.e. approximately 7 hours to reach steady state plasma levels and a half-life of approximately 1.4 hours), it was possible to maintain MAP within the target range by adjusting the dose. In the majority of patients, doses above 3.75 ng/kg/min were only needed in the first 24 hours. However, as the 000025 trial was not designed (or powered) to provide data on efficacy and safety, it is not known whether allowing the use of higher doses of selepressin is associated with an increased benefit or safety risk; hence, four dosing regimens (see Table 1) have been selected for investigation in the 000133 trial.

The lowest dosing regimen selected for the 000133 trial is expected to allow partial weaning of norepinephrine/noradrenaline and will address the question whether low doses of selepressin or co-treatment of selepressin and norepinephrine/noradrenaline is superior to full substitution of norepinephrine/noradrenaline with selepressin. The higher dosing regimens will allow progressively faster and more complete weaning of norepinephrine/noradrenaline and will answer the question whether higher doses of selepressin are associated with increased clinical benefit. The high granularity in the dose-selection is chosen to increase the ability to detect the dosing regimen that provides the optimal risk-benefit ratio.

3.5.5 Selection and Timing of Dose for Each Patient

Treatment with the IMP (selepressin or placebo) has to start as early as possible following fulfilment of the eligibility criteria and no later than 12 hours after initiation of the required continuous infusion of vasopressor treatment for septic shock. All patients will be on norepinephrine/noradrenaline treatment as part of standard of care at the time when the IMP infusion is initiated.

During the course of treatment, the IMP will be continuously administered and adjusted within pre-defined infusion rates. An administration guide will be provided with recommendations on how to adjust the IMP infusion rate.

As the vasopressor-need varies significantly from patient to patient and over time in any given patient, it is necessary to allow dose-adjustments. A lower starting infusion rate of the IMP has been included to minimise the risk of overshooting when IMP is added on top of the norepinephrine/noradrenaline infusion the patient is receiving to maintain the target MAP. The infusion rate of the IMP will be increased if the starting rate is insufficient to wean norepinephrine/noradrenaline completely. If the MAP is increased above the target, norepinephrine/noradrenaline will be weaned first while aiming to keep MAP at the target. Norepinephrine/noradrenaline must be completely weaned prior to weaning of the IMP. If the IMP alone increases the MAP above the target, the IMP will be weaned step-wise while aiming to keep MAP at the target. If the maximum allowed infusion rate of the IMP is not sufficient to maintain the MAP at the target, norepinephrine/

noradrenaline will be added to achieve the targeted MAP. If target MAP cannot be maintained despite maximum allowed infusion rate of IMP and ≥1 µg/kg/min norepinephrine/noradrenaline base (or a total catecholamine equivalent of 1 µg/kg/min norepinephrine/noradrenaline base), vasopressin may be added.

The IMP infusion will continue as long as blood pressure support is deemed necessary with a maximum of 30 days. After complete weaning, IMP infusion may be re-started during this 30-day period if necessary. Patients who still need vasopressor treatment after 30 days will be switched to other vasopressors at the discretion of the investigator.

4 SELECTION OF TRIAL POPULATION

4.1 Trial Population

The intention is to enrol a typical sample of patients presenting with septic shock and commence treatment with the IMP during the initial hours of resuscitation, within 12 hours from the onset of vasopressor treatment, targeting those who do not respond rapidly to fluids and whose vasopressor-need persists for at least one hour.

4.1.1 Inclusion Criteria

1. 18 years of age or older.

2. Proven or suspected infection.

3. Septic shock defined as hypotension (systolic blood pressure less than 90 mmHg OR MAP less than 65 mmHg) requiring vasopressor treatment (i.e. any dose of norepinephrine / noradrenaline base greater than 5 µg/min) despite adequate fluid resuscitation (at least one litre for hypotension).

   The MAP threshold for inclusion in the trial is a MAP below 65 mmHg before vasopressor support is started. However, it is not a requirement that patients are under the target MAP during vasopressor treatment with norepinephrine/noradrenaline. The patients must require vasopressor treatment to stay on the target MAP i.e. a patient can be at the target of 65 mmHg or higher while on at least 5 µg/min of norepinephrine/noradrenaline base at least for one hour before inclusion and when IMP is started.

   A patient can also be included based on systolic blood pressure less than 90 mmHg even if the MAP is above 65 mmHg if the patient is judged in need of vasopressor treatment based on evidence of poor organ perfusion.

   The requirement of at least one litre of fluid for hypotension to start the randomisation process balances the need to ensure that patients have been properly fluid resuscitated while still allowing for early enrolment before there is marked endothelial injury and increased permeability so that the proposed permeability-protection of selepressin can be assessed. Fluid resuscitation should continue according to the recommendations in the Surviving Sepsis Campaign guidelines (Dellinger et al, 2013) and therefore the patients should have received the recommended 30 mL/kg fluid from the onset of hypotension and to the time IMP infusion is started (unless evidence of fluid replete/overload) (see Section 4.1.3).

4. Informed consent obtained in accordance with local regulations.

4.1.2 Exclusion Criteria

1. Not possible to initiate IMP treatment within 12 hours from onset of vasopressor treatment for septic shock.

   Patients will be excluded if IMP infusion cannot be started within 12 hours from onset of vasopressor treatment. This time limit exclusion is added to ensure that patients are included early in the septic shock state. If the inclusion is left too late, then there is often irreparable organ dysfunction and endothelial injury with increased permeability in septic shock. Thus, even an effective intervention could fail if applied later when there is irreversible injury.

2. Primary cause of hypotension not due to sepsis (e.g. major trauma including traumatic brain injury, haemorrhage, burns, or congestive heart failure/cardiogenic shock).

3. Previous severe sepsis with ICU admission within this hospital stay.

   Patients who have had a prior episode of severe sepsis have a poorer prognosis and may still be recovering from the associated organ dysfunction so patients with previous severe sepsis within this hospital stay are not eligible.

4. Known/suspected acute mesenteric ischaemia.

   Selepressin is a potent V_{1a} agonist and V_{1a}-induced mesenteric vasoconstriction is a safety concern so patients with known or suspected acute mesenteric ischaemia are not allowed for safety reasons.

5. Suspicion of concomitant acute coronary syndrome based on clinical symptoms and/or ECG during this episode of septic shock.

   V_{1a} agonism could also induce coronary vasoconstriction and so, for safety reasons, patients are not allowed in the trial if the investigator believes the ECG and clinical symptoms suggest a concomitant acute coronary syndrome.

6. Chronic mechanical ventilation for any reason OR severe COPD requiring either continuous daily oxygen use during the preceding 30 days or mechanical ventilation (for acute exacerbation of COPD) during the preceding 30 days.

   A potential confounder to interpretation of the efficacy of selepressin on ventilator-free days would be inclusion of patients who have severe COPD requiring chronic oxygen use or mechanical ventilation. Such patients may recover from the acute pulmonary effects of septic shock (such as acute respiratory distress syndrome) because of the proposed beneficial effects of selepressin but then prove difficult and slow to wean from mechanical ventilation because of their significant underlying disease. Accordingly such patients are not allowed in the trial. However, patients with less severe COPD are allowed.

7. Received bone marrow transplant during the preceding 6 months or chemotherapy during the preceding 30 days for lymphoma or leukemia.

   Patients who have had bone marrow transplant during the preceding 6 months or chemotherapy during the preceding 30 days for lymphoma or leukemia are excluded because these patients can have a significantly worse prognosis compared to the average septic shock patient due to their impaired immunity and other effects of their underlying disease and its treatment. Depending on the state of immune dysfunction, the mortality rate of these patients when they get septic shock - even with aggressive intensive care, resuscitation, and appropriate intravenous broad spectrum antibiotics - can exceed 90%. Furthermore, many of these patients have thrombocytopenia secondary to their disease or their therapies and this underlying thrombocytopenia increases the risks of worsening to profound thrombocytopenia during septic shock because septic shock induces thrombocytopenia directly and independent of prior chemotherapy. Finally, many of these patients have other mortality risk factors such as anemia, hepatic and renal dysfunction, all of which would be worsened during septic shock.

8. Known to be pregnant.

9. Decision to limit full care taken before obtaining informed consent.

10. Use of vasopressin in the past 12 hours prior to start of the IMP infusion or use of terlipressin within 7 days prior to start of the IMP infusion.

11. Prior enrolment in the trial.

12. Prior use of an investigational medicinal product within the last month OR planned or concurrent participation in a clinical trial for any investigational drug or investigational device.

    In order to be able to assess the safety and efficacy of selepressin without confounding factors from the use of other investigational drugs or devices, co-enrolment in trials involving investigational products are not allowed. Co-enrolment in a non-investigational trial requires preapproval of the TSC and will be assessed on a case by case basis. In principle, co-enrolment is allowed unless it is expected to impact the outcome of this clinical trial.

4.1.3 Eligibility Criteria – Post-randomisation / Before Start of IMP Infusion

In addition, the following criteria must be met at start of IMP infusion:

1. Received a minimum of 30 mL/kg fluid in total from the onset of hypotension (or less if evidence of fluid replete/overload).
2. Received a continuous infusion of norepinephrine/noradrenaline base greater than 5 µg/min for at least one hour and is still receiving at least 5 µg/min norepinephrine/noradrenaline base.

3. Less than 12 hours since onset of vasopressor treatment for septic shock.

   The requirement of at least one hour duration of vasopressor support is intended to ensure a certain severity of the septic shock while balancing the need to recruit patients early during the initial hours of resuscitation.

4.2 Method of Assigning Patients to Treatment Groups

4.2.1 Recruitment

Both emergency departments (EDs) and ICUs will recruit patients. Each patient considered for the trial will receive a unique screening number. The screening number will be allocated sequentially within each trial site in the order in which the patients are screened. The screening number and the result of each screening will be recorded.

A screening log with patient identification details for consecutive patients with septic shock must be maintained at each trial site to capture how many patients that were screened to include the 1800 evaluable patients in the trial. The main reason for exclusion will be described.

4.2.2 Randomisation

A computer-generated randomisation list will be prepared prior to enrolment of the first patient into the trial and updated during the trial via the response-adaptive randomisation procedure.

To minimise the risk of imbalance between the treatment arms, the randomisation will be stratified by: 1) trial site, 2) need for mechanical ventilation (‘Yes’ or ‘No’), 3) norepinephrine/noradrenaline requirement (< or ≥30 µg/min), and 4) serum creatinine (< or ≥150 µmol/L).

The patient-specific randomisation number will be allocated in the order at which the patients are being randomised into the trial. The actual patient randomisation number will be added on the screening log. No patient can be enrolled twice.

The first 200 patients will be randomised to treatment in a fixed manner. The randomisation is 3:2:2:2:0 for placebo and active treatment arms 1, 2, 3, and 4, respectively. After the first 200 patients and throughout the rest of Part 1, the patients will be randomised with one-third probability to placebo and the remaining probability allocated to active treatment arms in a response-adaptive randomisation manner. A pre-defined algorithm will be used to determine the relative randomisation to each of the treatment arms. Interim analyses will be conducted regularly in Part 1 to adjust the adaptive randomisation probabilities for the active treatment arms. The enrolment of patients will continue without any stop at the interim analyses.

During Part 2, a minimum of 1000 patients will be randomised in a 1:1 fashion to placebo and the active treatment arm selected from Part 1 in order to get the 1800 evaluable patients needed for the final analysis.

4.3 Restrictions

4.3.1 Prior and Concomitant Medications/Procedures

Medicinal products may be administered and concomitant procedures may be conducted for the well-being of the patients at the discretion of the investigators.

Use of vasopressors, mechanical ventilation, RRTs, and fluids must be documented thoroughly as these are related to the endpoints of the trial (see Section 2.2). Concomitant medications/procedures that are likely to influence the outcome from septic shock have to be detailed.

4.3.2 Prohibited Medications/Procedures

The following medications/procedures are prohibited:

- Vasopressin in the past 12 hours prior to start of IMP infusion and during IMP infusion unless the patient remains hypotensive despite the maxium allowed infusion rate of IMP and ≥1 µg/kg/min norepinephrine/noradrenaline base (or a total catecholamine equivalent of 1 µg/kg/min norepinephrine/noradrenaline base).
- Terlipressin (from 7 days prior to start of IMP infusion to end of ICU stay).
- Another investigational medicinal product (from 1 month prior to trial enrolment to 30 days after initiation of IMP infusion).
- Participation in another clinical trial for an investigational drug or investigational device or co-enrolment in a non-investigational trial that is not pre-approved by the TSC (from 1 month prior to trial enrolment to 30 days after initiation of IMP infusion).

4.4 Discontinuation and Withdrawal

For all discontinuations and withdrawals, the investigator will document the date of the termination and the main reason.

Premature discontinuation of IMP infusion

The IMP infusion will continue as long as blood pressure support is deemed necessary (up to a maximum of 30 days). Premature discontinuation of IMP infusion is defined as termination of IMP infusion even though continued intravenous vasopressor treatment for blood pressure support is needed. A premature discontinuation of IMP infusion may occur if the investigator decides that IMP infusion should be discontinued or if the patient, the patient's legal representative, or attending physician requests that IMP infusion be discontinued. If the IMP infusion is prematurely discontinued due to a serious adverse event, an additional blood sample will be collected (see Section 7.2.2).

Patients whose IMP infusion is prematurely discontinued, regardless of reason, are not discontinued from the trial. These patients will continue in the trial and undergo the trial assessments following the trial protocol in order to provide the data needed for the analyses and to determine their survival status.

If a patient who does not meet the eligibility criteria is inadvertently enrolled and the IMP infusion has been started, the investigator will consult with the CCC regarding termination or continuation of the IMP infusion.

Withdrawal from the trial

A patient has the right to withdraw from the trial at any time for any reason, without the need to justify the decision.

In the event that the patient (or the patient's legal representative) withdraws consent or the investigator or Ferring, for any reason, prematurely stops the patient's participation in the trial, the IMP infusion and all scheduled trial-related assessments and laboratory testings will be stopped. Data collected up to withdrawal will remain in the database but data obtained after the patient has withdrawn his/her consent will not be entered into the database. However, results from assessments and blood samples collected prior to the withdrawal of the consent but not analysed at the time of the withdrawal will be entered into the database unless the patient refuses. The patient can request destruction of samples which would otherwise have been kept in storage.

Refer to Section 12.3 for information regarding premature trial termination.

5 TREATMENTS

5.1 Treatments Administered

5.1.1 Investigational Medicinal Product (IMP)

The IMP (selepressin and placebo) will be administered through a central venous catheter as a continuous intravenous infusion using a syringe pump. The administration is discussed in Section 3.5.5. A detailed IMP and vasopressor administration guide will be provided to the trial sites.

5.1.2 Norepinephrine/Noradrenaline

In order to be eligible for the trial, all patients will be on norepinephrine/noradrenaline treatment as part of standard of care (see Section 4.1.1). During the trial, the norepinephrine/noradrenaline treatment will continue as deemed necessary. Norepinephrine/noradrenaline will be from the commercial batches that the trial sites are using as part of usual clinical care and no modification to their commercial state will be made.

5.2 Characteristics and Source of the IMPs

Selepressin

Selepressin 0.3 mg/mL is a concentrate for solution for infusion. It has been manufactured in accordance with the principles of Good Manufacturing Practice (GMP) and will be provided by Ferring in vials in which the drug substance has been dissolved in an isotonic 10 mM acetate buffer of pH 4.0.

The concentrate will be diluted with sterile 0.9% sodium chloride solution to one of four different concentrations as detailed in a dilution protocol. Sterile 0.9% sodium chloride solution will be provided together with the vial with selepressin concentrate. The dilution will be prepared by dedicated and trained personnel at the hospital pharmacy or at another approved facility at the hospital. A dilution log will be provided in which the dilution will be documented.

Placebo

Sterile 0.9% sodium chloride solution will be used as the placebo. It will be provided by Ferring from commercial batches and no modification to their commercial state will be made.

5.3 Packaging and Labelling

Packaging and labelling of the IMPs (selepressin and placebo) will be performed under the responsibility of the IMP department at Ferring in accordance with GMP and national regulatory requirements.

The IMPs (selepressin and placebo) will be labelled with trial-specific labels and the content on the labels will be in accordance with Annex 13, EudraLex, volume 4 and national requirements. The labels will contain a self-adhesive tear-off portion to be affixed to the dispensing log maintained at the trial site.

5.4 Conditions for Storage and Use

The IMPs will be stored in accordance with the label.

The investigator will ensure that the IMPs will be stored in appropriate conditions in a secure location with controlled access. The storage compartment shall be monitored regularly and the temperature shall be documented. Deviations in storage temperature must be reported to Ferring without delay and the IMPs must not be used until further instructions are received.

Diluted IMP dosing solutions must be used within 26 hours after the preparation if prepared in controlled and validated aseptic conditions, otherwise within 24 hours after the preparation.

5.5 Blinding / Unblinding

5.5.1 Blinding

This is a double-blind, placebo-controlled trial in which patients, investigators and other trial site staff, the CCCs, the TSC, the clinical trial team at Ferring and its representatives will be blinded to the treatment assignment. As discussed in Section 3.4, the DSMB will have access to unblinded data during the trial. The personnel preparing the IMPs and the drug accountability monitors will also be unblinded. Figure 2 illustrates the applicable safeguards to maintain the overall blinding during conduct of trial. Three independent parties will be involved in forming the safeguard during the adaptive portion of the trial. One part (the randomisation company) will provide the expertise to run the adaptive algorithm. The second part (the statistical consulting company) will provide expertise in producing the DSMB reports and provide statistical and project management support for the DSMB. The third part (the adaptive design company) will provide expertise in adaptive clinical trial design to oversee the performance of the adaptive algorithm and provide statistical support for the DSMB in relation to the adaptive trial design.

Adequate blinding of the treating investigators and nurses is important to ensure the integrity of the results. Use of placebo will ensure effective blinding due to the significant individual variability in the vasopressor need from patient to patient. Thus, in some patients the vasopressor need declines quickly and it will not be possible to tell whether fast weaning of norepinephrine/noradrenaline is due to the fact that the patient receives active treatment or whether it is just because the patient is improving. Also, the potential side effects of selepressin are similar to side effects that can be seen with norepinephrine/noradrenaline or that may be caused by worsening of septic shock so there are no obvious effects of selepressin that will lead to unblinding.

The IMPs will be prepared in accordance with the trial-specific computer-generated randomisation numbers. The randomisation will be securely kept without access for personnel involved in the conduct of the trial with exception of the personnel preparing the IMPs until the trial database is declared clean and released to the trial team statistician. The prepared IMPs (i.e. the four diluted selepressin dosing solutions and the placebo) will be indistinguishable i.e. identical appearance and smell and will be administered using similar infusion rates.

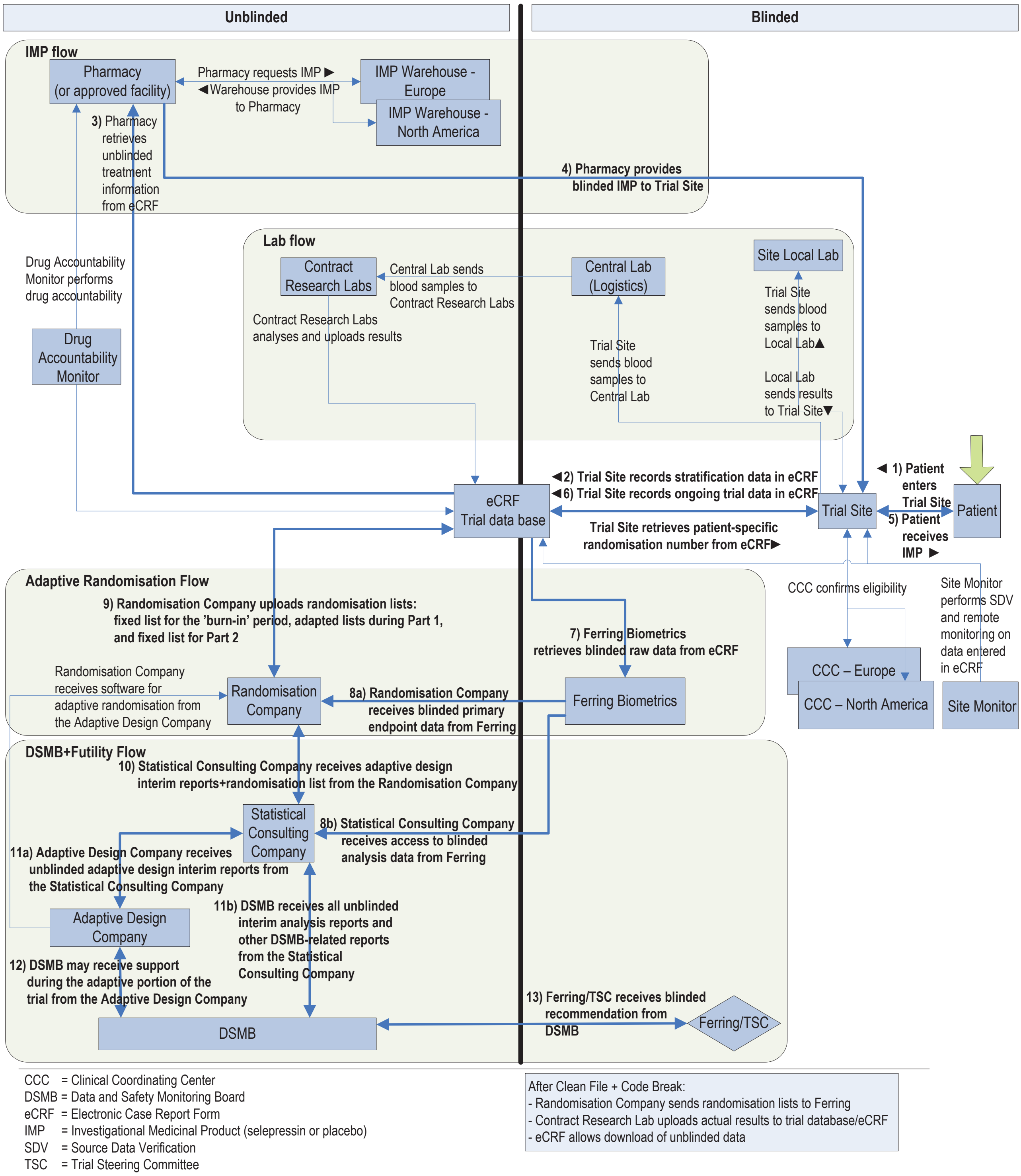


Figure 2 Safeguards to Maintain Blinding during Trial Conduct

5.5.2 Unblinding of Individual Patient Treatment

An emergency decoding possibility (via the eCRF) will be available to the investigator and designated persons at Ferring. Breaking of the blind for individual patients in emergency situations is an investigator responsibility. As far as the emergency permits, the need to break the blind will be discussed with the CCC and communicated to Ferring.

The unblinding in emergency situations is only permitted in case of a suspected unexpected serious adverse reaction (SUSAR) or in case of an important adverse event where the knowledge of the medicinal product in question is required for therapeutic decisions for the management of the patient. It will be documented in the eCRF that the code is broken, when, and by whom. The investigator must record the event of unblinding in the patient's medical record, including the reason for unblinding, but not the treatment allocation if this can be avoided.

In case of accidental unblinding, the same documentation as for emergency unblinding must be obtained.

It may be necessary to unblind an individual patient's treatment for the purposes of expedited reporting to the authorities and/or independent ethics committees (IECs) / institutional review boards (IRBs). In that situation, every effort will be made to maintain blinding of personnel involved in data analysis and interpretation. Other personnel may be unblinded for SUSARs, including trial site staff as well as staff acting on behalf of Ferring.

Information on whether the blind has been broken for any patients must be collected before the database is declared clean and released to the trial team statistician.

5.6 Treatment Compliance

The IMPs will only be administered by authorised staff at the trial sites to patients who meet the eligibility criteria and are randomised to a treatment in the trial. The investigator (or his/her designated personnel, e.g. trial nurse) will maintain a drug dispensing log detailing the dates and quantities of IMP administered to, and used by, each patient, as well as the unique batch identifier used in the trial.

The tear-off portion of the labels will be affixed to the drug accountability form. The monitors will verify the drug accountability during the trial.

5.7 Return and Destruction of IMP

All used IMP will be destructed at the trial site in accordance with local requirements after the drug accountability has been finalised, verified by the monitor, and signed off by the investigator. Any material used for preparation of the infusion solution and for the infusion will be destroyed at the trial site immediately after usage according to standard procedures at the trial site.

All unused IMP will be accounted for and must be destructed in a certified way in accordance with trial-specific instructions.

6 TRIAL PROCEDURES

Participating sites should consider all septic shock patients for inclusion in the trial; the main reason for not being eligible will be documented. Refer to Section 4.1 for inclusion, exclusion, and eligibility criteria. One of the assigned CCCs must be contacted to confirm the eligibility of each patient.

The trial consists of a pre-IMP treatment period, an IMP treatment period, and a follow-up period (see Section 6.1).

The pre-IMP treatment period is defined as the time from onset of vasopressor treatment for septic shock to the start of IMP infusion. To ensure start of IMP treatment without delay, informed consent will be obtained, in compliance with local regulations, as early as possible. During this period, baseline evaluations will be made.

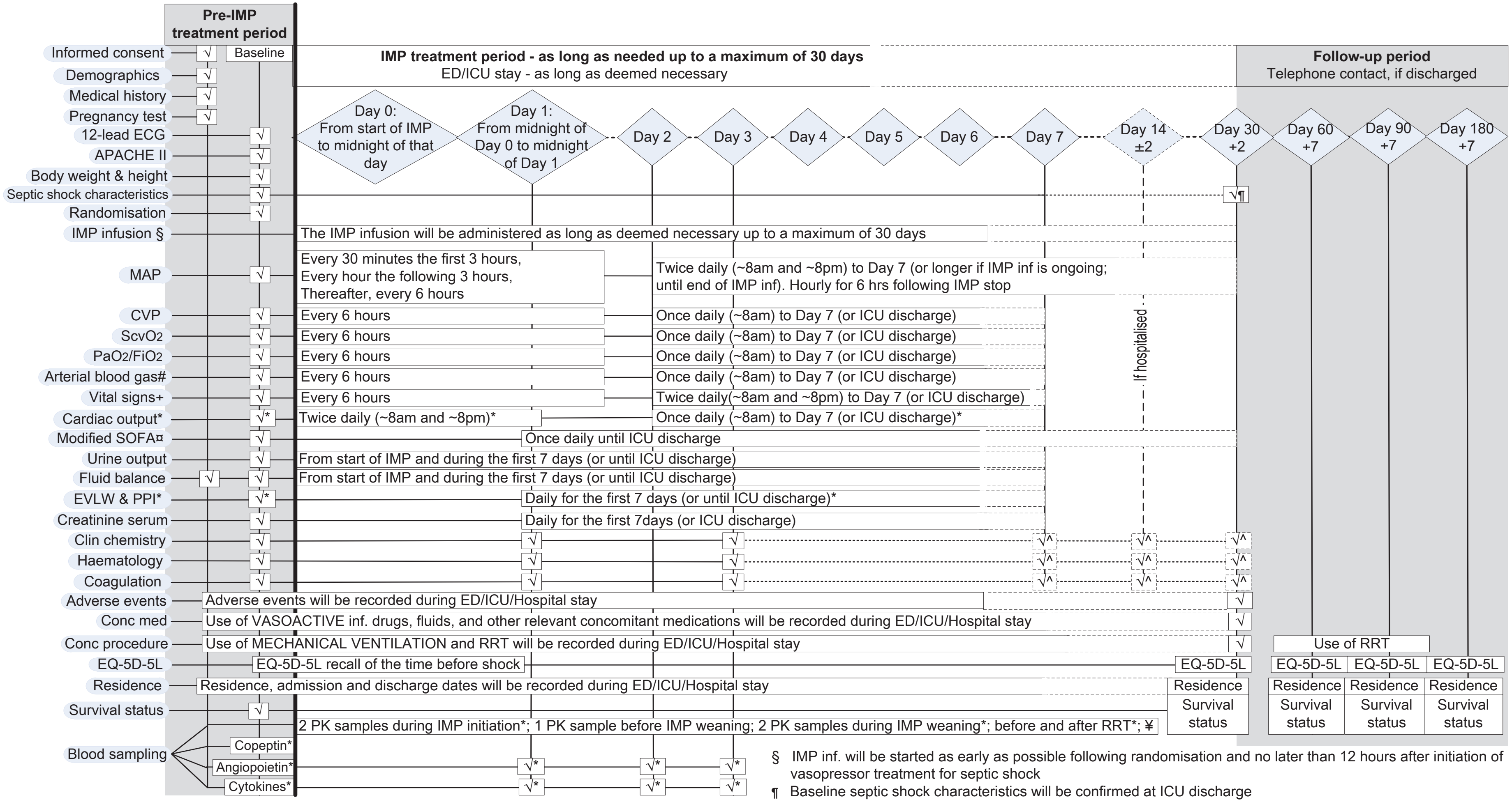
The IMP treatment period begins with Day 0 (which is defined as time from onset of IMP infusion to midnight of that day). The infusion of the IMP (selepressin or placebo) will be started as early as possible following randomisation and no later than 12 hours after initiation of continuous infusion of vasopressor treatment for septic shock. During the course of treatment, the IMP infusion rate will be adjusted as within pre-defined infusion rates to keep the MAP at the target. The IMP administration is described in Section 3.5.5 and the target MAP is defined in Section 7.1.11. A detailed IMP and vasopressor administration guideline will be provided to trial sites. The IMP infusion will continue as long as blood pressure support is deemed necessary with a maximum period of 30 days. A confirmation of the septic shock characteristics recorded at time of enrolment will be recorded in the eCRF at ICU discharge.

The follow-up period includes Days 30, 60, 90, and 180. If a patient has been discharged from the trial hospital, trial site personnel will contact the patient, the patient's legal representative, or the patient's health care professional to collect required trial information.

All assessments to be performed and all data to be collected during the trial are summarised in Section 6.1 and further described in Section 7. The majority of the assessments are widely and routinely used clinically and generally regarded as reliable, accurate, and relevant. Collection of data requiring invasive equipment or mechanical ventilation is applicable only if it is measured as part of local clinical practice. Samples collected and assessments performed for clinical purposes in accordance with standard of care after start of fluid resuscitation for hypotension and before start of IMP may be used as baseline values if deemed appropriate. In case several values of the same assessment are available, the recordings obtained closest to the start of IMP treatment will be used as baseline and the recordings obtained closest to a given time-point will be used as post-baseline values (if not otherwise specified).

At pre-selected trial sites, additional data collection will be performed to further evaluate the effect of selepressin.

6.1 Trial Flow Chart



§ IMP inf. will be started as early as possible following randomisation and no later than 12 hours after initiation of vasopressor treatment for septic shock
¶ Baseline septic shock characteristics will be confirmed at ICU discharge
* Only applicable for patients at pre-selected trial sites
^ Only applicable in hospitalised patients
\# PaO_2, $PaCO_2$, SaO_2, HCO_3, pH, base excess, and lactate
\+ Diastolic and systolic blood pressure, heart rate, respiratory rate, body temperature
¤ Based on respiratory, cardiovascular, renal, hepatic, and coagulation
¥ A blood sample will be collected if the IMP infusion is prematurely stopped due to a serious adverse event

7 TRIAL ASSESSMENTS

7.1 Assessments Related to Endpoints

Vasopressor treatment, mechanical ventilation, and mortality are all part of the primary endpoint and it is extremely important to document these variables thoroughly and without delay. If a patient has been discharged from the trial hospital before Day 30, trial site personnel will contact the patient, the patient's legal representative, or the patient's health care professional to collect required information. If the trial site personnel are unable to collect the required information, Ferring should be notified. Completeness of data collection for the primary endpoint will be closely monitored during the conduct of the trial and issues of missing data collection will result in re-training (or termination) of trial sites.

7.1.1 Vasopressors

The integrity of the primary endpoint depends on accurate start and stop times of vasopressor treatment and therefore start and stop time of all vasopressor periods must be recorded in the eCRF. It is key to ensure that vasopressor treatment is neither prolonged longer than necessary nor prematurely stopped leading to episodes of hypotension.

All vasopressor treatment (any intravenous dose of norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, and IMP [selepressin and placebo]) must be thoroughly documented up to Day 30. The infusion rate of IMP will be recorded at baseline and at 1, 3, 6, 12, and 24 hours after start of IMP infusion. After 24 hours, the infusion rate of IMP will be recorded twice daily (around 8:00 a.m. and 8:00 p.m. when the MAP is recorded) until end of IMP treatment. In addition, the IMP infusion rate at the time of pharmacokinetic blood sampling will be recorded in the eCRF. In approximately 200 patients at pre-selected trial sites, the IMP infusion rate at 30 minutes after start IMP infusion will also be recorded (see Section 7.2.2). Furthermore, the total volume of infused IMP within the following time periods will be recorded: 0-12 hours, 12-24 hours, and from 24 hours to regular schedule according to clinical practice at each trial site. Thereafter, daily according to local clinical practice. For all other vasopressors, the dose of each vasopressor will be recorded at baseline and at 1, 3, 6, 12, and 24 hours after start of IMP infusion. After 24 hours, the dose of the individual vasopressors will be recorded twice daily (around 8:00 a.m. and 8:00 p.m. when the MAP is recorded) until end of vasopressor treatment.

7.1.2 Mechanical Ventilation

Respiratory failure, requiring mechanical ventilation, is a common complication of septic shock. In septic shock patients, it is a key goal of management of ventilation to minimise the duration of ventilation because increased duration of mechanical ventilation increases the risk of nosocomial pneumonia, neuromuscular weakness, and death.

The use of mechanical ventilation (as defined in Section 2.2) must be thoroughly documented up to Day 30. Start and stop time of all mechanical ventilation periodsmust be recorded in the eCRF.

The weaning of mechanical ventilation will be based on daily spontaneous breathing trials as described in a trial-specific weaning guide. The time and outcome (i.e. success or failure) of each spontaneous breathing trial will be documented in patient's medical records to monitor if a successful spontaneous breathing trial is followed by extubation.

7.1.3 Renal Replacement Therapy (RRT) and Renal Function

Acute kidney injury is a common complication of septic shock. Treatment of acute kidney injury is primarily supportive including RRT (continuous RRT, intermittent haemodialysis, or peritoneal dialysis). The decision to initiate RRT in a patient enrolled in this trial will be based on local clinical practice. The use of RRT must be thoroughly documented up to Day 90. The reason for and type of RRT as well as start and stop time will be recorded in the eCRF. If a patient is shifted from continuous RRT to intermittent haemodialysis, the patient is still on RRT until the last hemodialysis has been performed.

Renal function will be assessed, using urine output, serum creatinine, and creatinine clearance, at baseline and the first 7 days after initiation of IMP infusion [or until ICU discharge if the patient leaves the ICU before Day 7]). Creatinine clearance will be determined by estimated glomerular filtration rate (using serum creatinine, age, and gender as per Cockcroft-Gault equation).

7.1.4 Modified Sequential Organ Failure Assessment (SOFA) Score

SOFA is a scoring system used to track the patient's organ function status during episodes of critical illness (Vincent et al, 1996).

In this trial, a modified version of the SOFA will be used (i.e. SOFA except the Glasgow Coma Scale). As many patients are sedated due to mechanical ventilation a meaningful assessment of the neurological function using the Glasgow Coma Scale cannot be performed.

The worst value for each individual organ system (i.e. respiratory, cardiovascular, renal, coagulation, and hepatic) components within the past 24 hours (at baseline and once daily until ICU discharge) will be recorded in the eCRF and the eCRF will calculate the overall modified SOFA score.

7.1.5 Mortality Rate and Hospitalisation

The actual date and time for ED, ICU, and hospital admission and discharge as well as time of death (if applicable) will be recorded. Trial site personnel will visit or contact each patient who is still hospitalised and contact each patient or healthcare professional for patients who has left the hospital to determine survival status and current residence.

Functional status and residence of each patient before this episode of septic shock (baseline) as well as functional/survival status and residence throughout the trial will be recorded by using one of the following alternatives:

- Home, receiving no support
- Home, receiving paid professional support

- Home, receiving unpaid support
- Rehabilitation site/skilled (or unskilled) nursing facility
- Other acute care hospital (including long-term acute care)
- Still in trial hospital
- Unknown
- Dead

7.1.6 Health-related Quality of Life

The EuroQoL-5-Dimensions (EQ-5D™) will be used to assess patient's overall health. EQ-5D™ is a standardised instrument in two parts for use as a measure of health outcome; it provides a simple, generic measure of health for clinical and economic appraisal (The EuroQol Group; http://www.euroqol.org). The first part includes five dimensions where the patient will indicate which given statements best describe the health state on the day of questionnaire completion. The second part contains a visual analogue scale (VAS) where the patient will indicate how good or bad his or her own health is on the day of questionnaire completion. The VAS scores range from 0 (worst health state) to 100 (best health state).

The five level version of the instrument will be used in this trial (i.e. EQ-5D-5L).

The baseline value refers to a recall of the time before the septic shock episode and the EQ-5D-5L will be completed as soon as possible after the shock state. A telephone interview will be adequate at Days 30, 60, 90, and 180 if the patient has been discharged. The responses will be entered into the eCRF.

The investigator and/or delegated personnel will receive training and instruction in completion of the questionnaire before enrolment of patients.

Each patient must receive proper training and instruction before use. The investigator or a delegated trial team member will instruct the patient to respond to each question without influence from trial team members or accompanying family or friends and explain that there are no right or wrong answers. Nobody will be allowed to answer or interpret questions for the patient.

7.1.7 Fluid balance, Fluids, and Urine Output

In order to investigate selepressin's effect on fluid balance, it is important to document all fluid administered during this episode of severe sepsis/septic shock including the time (i.e. up to 18 hours) before initiation of the IMP infusion (baseline) and during the first 7 days after initiation of IMP infusion [or until ICU discharge if the patient leaves the ICU before Day 7]). The period of Day 0 is from the start of IMP infusion to the time of the first regular daily fluid recording according to local clinical practice. Thereafter, daily fluid recordings according to local clinical practice.

The fluid balance (as calculated in accordance with local clinical practice) and the total amount of intravenous fluid and urine output will be recorded in the eCRF. Urine output will be used for assessing renal function (see Section 7.1.3).

7.1.8 Adverse Events

The procedure for collecting and reporting adverse events is described in Section 8.

7.1.9 Safety Laboratory Variables (Clinical Chemistry, Haematology, and Coagulation)

Standard safety laboratory variables will be analysed using standard equipment in accordance with local clinical practice. Accreditation/certification of the laboratories and reference ranges of the laboratory variables will be kept in the trial files. The investigator must document their review of the laboratory results. Any laboratory abnormality should be assessed by the investigator as to whether it constitutes an adverse event (see Section 8.1).

The baseline levels and the results, of the variables as listed in Table 2, obtained 1 and 3 days after initiation of the IMP infusion will be recorded. The results obtained at Days 7, 14, 30 will also be recorded if the patient is still hospitalised.

Daily creatinine, bilirubin, and platelets values will be used for the SOFA scores (see Section 7.1.4). Serum creatinine level will also be used for assessing renal function and calculation of creatinine clearance (see Section 7.1.3).

Table 2 Safety Laboratory Variables

Clinical Chemistry	Haematology	Coagulation
Alanine aminotransferase	Haematocrit	Activated partial thromboplastin time
Albumin	Haemoglobin	
Alkaline phosphatase	Platelet count	Prothrombin time / international normalised ratio
Aspartate aminotransferase	Red blood cell count	
Calcium	White blood cell count	
Chloride		
C-reactive protein		
Creatine phosphokinase		
Creatinine (serum)		
Lactate dehydrogenase		
Phosphate		
Potassium		
Sodium		
Total bilirubin		
Troponin		
Urea (blood urea nitrogen)		
Uric acid		

7.1.10 Diastolic and Systolic Blood Pressure, Heart Rate, Respiratory Rate, and Body Temperature

Diastolic and systolic blood pressure, heart rate, respiratory rate, and body temperature will be monitored up to Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) using standard equipment in accordance with local clinical practice. The baseline values, the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and twice daily (around 8:00 a.m. and 8:00 p.m.) the following days will be recorded in the eCRF. Any abnormality should be assessed by the investigator as to whether it constitutes an adverse event (see Section 8.1).

7.1.11 Mean Arterial Pressure

The MAP will be measured intra-arterially (or non-invasively if an arterial line is not available) using standard equipment in accordance with local clinical practice. The method (intra-arterially or non-invasively) used will be documented in the eCRF.

The infusion rate of the IMP will be adjusted to keep the MAP at the target of 65 mmHg. However, a different target MAP will be accepted, if pre-specified in the eCRF and if appropriate, as judged by the investigator, for the clinical management e.g. in patients with previous hypotension or hypertension (if deemed necessary to maintain adequate organ perfusion). In such case, the target MAP and the reason for why a different target MAP has been chosen will be recorded in the eCRF.

The following MAP values will be recorded in the eCRF:

- Baseline.
- Every 30 minutes during the first 3 hours after initiation of IMP infusion.
- Every hour the following 3 hours.
- Thereafter, every 6 hours up to midnight of Day 1.
- Twice daily (around 8:00 a.m. and 8:00 p.m.) until Day 7 or longer if IMP infusion is ongoing (until end of IMP infusion).
- Every hour for 6 hours following discontinuation of the IMP infusion.

In order to assess that vasopressor treatment is not prematurely stopped leading to episodes of hypotension, the MAP will be recorded hourly for the first 6 hours after complete weaning of IMP and episodes of clinically relevant hypotension (i.e. any period recorded in the hospital/medical source records through routine clinical care where the MAP drops below 60 mmHg for more than one hour) will be recorded in the eCRF detailing the total duration of the episode, the lowest MAP measured during the episode, and the actions taken to correct the hypotension.

MAP is also needed for the SOFA score (see Section 7.1.4).

In addition, in the first patients at each trial site, the MAP should be noted in the patient's medical record at the time when the infusion rate of the IMP or the norepinephrine/noradrenaline is adjusted in order to monitor compliance with the IMP and vasopressor administration guide.

7.1.12 PaO_2/FiO_2 Ratio

PaO_2/FiO_2 ratio will be monitored up to Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) in accordance with local clinical practice. The baseline value, the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and daily (around 8:00 a.m.) the following days will be recorded in the eCRF.

The PaO_2 and FiO_2 are also needed for the SOFA score (see Section 7.1.4).

7.1.13 Extravascular Lung Water and Pulmonary Permeability Index

The extravascular lung water (EVLW) is the amount of water that is contained in the lungs outside the pulmonary vasculature, that is, in the interstitial and alveolar spaces (Jozwiak et al, 2013). The ratio between the EVLW and the pulmonary blood volume is called the pulmonary permeability index (PPI) which is believed to reflect the permeability of the alveolo-capillary barrier (Monnet et al, 2007). Both EVLW and PPI are useful tools to characterise pulmonary oedema.

EVLW and PPI will be collected at pre-selected trial sites who measure these variables as part of clinical practice. The baseline values and the daily (around 8:00 a.m.) values for the first 7 days after initiation of IMP infusion (or until ICU discharge if the patient leaves the ICU before Day 7), as measured using standard equipment in accordance with local clinical practice, will be recorded if available.

7.1.14 Cardiac Output

Cardiac output will be monitored up to Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) at pre-selected trial sites who measure cardiac output as part of clinical practice. The baseline value and the values obtained twice daily (around 8:00 a.m. and 8:00 p.m.) for the first 2 days after initiation of IMP infusion and daily (around 8:00 a.m.) for the following days, as measured using standard equipment in accordance with local clinical practice, will be recorded if available.

7.2 Trial-specific Blood Sampling

7.2.1 Copeptin Levels

In patients at pre-selected trial sites, a blood sample will be collected before initiation of the IMP infusion for measurement of baseline levels of copeptin.

7.2.2 Pharmacokinetics

A blood sample for measurement of plasma concentration of selepressin will be collected from all patients right before first attempt to wean the IMP infusion.

The IMP infusion rate at the time of the blood sampling will be recorded in the eCRF.

In approximately 200 patients at pre-selected trial sites, additional blood sampling for analysis of pharmacokinetic parameters will be conducted twice during the initiation of the IMP infusion (at approximately 1-3 hours and 6-9 hours after start of infusion) and twice during the weaning of the IMP infusion (at approximately 1-2 hours and 2-3 hours after stop of infusion). If these patients are on RRT, additional blood sampling will also be performed before and after the RRT. The IMP infusion rate at 30 minutes after start of IMP infusion will be recorded in the eCRF.

In addition, if the IMP infusion is prematurely discontinued due to a serious adverse event, a blood sample will be collected at the time of IMP discontinuation for measurement of plasma concentration of selepressin.

7.2.3 Cytokines

The development of septic shock is associated with elevated levels of proinflammatory cytokines (Fjell et al, 2013). Exposure to inflammatory mediators and interaction with leukocytes causes endothelial activation and damage (Aird et al, 2003). Vasopressin has effects on immunity (Russell et al, 2010) and decreases cytokines more than does norepinephrine/noradrenaline (Russell et al, 2013). Selepressin is a potent $V1_a$ agonist that could also have effects on cytokines. Furthermore, cytokine levels in the blood may be predictive of the response to selepressin compared to placebo in septic shock.

In patients at pre-selected trial sites, a blood sample will be collected at baseline (before initiation of IMP infusion) and at the first 3 days after initiation of IMP infusion for analysis of several cytokines e.g. VEGFA (vascular endothelial growth factor), interleukin (IL)-6, IL-2, CCL (CC chemokine ligand)-22, CCL11, LTA (lymphotoxin alpha), CSF (colony-stimulating factor)-2.

7.2.4 Angiopoietins

Angiopoietin-1 and angiopoietin-2 are circulating proteins with opposing roles on the vascular endothelium, i.e. angiopoietin-1 protects against vascular leakage whereas angiopoietin-2 promotes increased vascular permeability.

In patients at pre-selected trial sites, a blood sample will be collected at baseline (before initiation of IMP infusion) and at the first 3 days after initiation of IMP infusion for measurement of angiopoietin-1 and -2 levels.

7.3 Other Assessments

7.3.1 Central Venous Pressure

If central venous pressure (CVP) is measured for clinical purposes (via a central venous catheter using standard equipment in accordance with local clinical practice), the baseline value and the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and a daily (around 8:00 a.m.) value the following days until Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) will be recorded, if available.

7.3.2 Central Venous Oxygen Saturation

If central venous oxygen saturation ($ScvO_2$) is measured for clinical purposes (via a central venous catheter using standard equipment in accordance with local clinical practice), the baseline value and the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and a daily (around 8:00 a.m.) value the following days until Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) will be recorded, if available.

7.3.3 Arterial Blood Gases and Lactate Levels

If arterial blood gases and acid/base status (arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$), arterial oxygen saturation (SaO_2), arterial pH, bicarbonate (HCO_3), base excess) and lactate levels are available, as measured using standard equipment in accordance with local clinical practice, the baseline values and the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and a daily (around 8:00 am) result the following days until Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) will be recorded.

PaO_2 will be used for the SOFA (see Section 7.1.4) and the PaO_2/FiO_2 ratio (see Section 7.1.12).

7.4 Demographics and Other Baseline Assessments

7.4.1 Demographics

Demographic data including date of birth, race, and ethnic origin will be recorded.

7.4.2 Septic Shock Characteristics

Baseline septic shock characteristics (including site of infection, cause of infection, and whether the infection was proven or suspected) will be recorded. In addition, a confirmation of the septic shock characteristics recorded at time of enrolment and whether initial antibiotic treatment and source control was adequate will be recorded at ICU discharge.

7.4.3 APACHE II

APACHE II is a classification system which uses a point score based upon initial values of routine physiologic measurements, age, and previous health status to provide a general measure of severity of disease (Knaus et al, 1985). In this trial, the baseline (within the preceding 24 hours before initiation of the IMP infusion) APACHE II score will be recorded to assess the similarity of illness severity at baseline between treatment arms.

7.4.4 Electrocardiography

A 12-lead baseline ECG obtained prior to start of IMP infusion must be available. ECGs obtained for clinical purposes within 48 hours of starting IMP may be used as baseline ECG, provided there is no indication of any change in the cardiac condition. If there is any reason to suspect a change in cardiac condition, a new 12-lead ECG should be obtained prior to initiation of IMP. The purpose of the baseline ECG is to have a status at entry to which later ECGs may be compared, should this become relevant. The ECG should be obtained in accordance with local clinical practice and ECG recordings should capture at least four QRS complexes, i.e. three evaluable RR intervals.

Continuous ECG monitoring will be performed as medically required in accordance with local clinical practice.

7.4.5 Body Weight and Height

The baseline (usual) body weight and height will be assessed in accordance with local clinical practice. The baseline weight will be used for calculation of the infusion rate of IMP and the norepinephrine/noradrenaline dosing.

7.4.6 Medical History

Information about relevant medical history will be collected. The Charlson Comorbidity Index will be used.

7.4.7 Prior and Concomitant Medication/Procedure

Information about relevant concomitant medications/procedures will be collected (see Section 4.3).

7.4.8 Pregnancy Test

Women of child bearing potential will be tested for pregnancy (in accordance with local clinical practice) before initiation of IMP infusion. Urine dipstick pregnancy tests will be provided to the trial sites.

7.5 Handling of Biological Samples

Copeptin, cytokines, angiopoietin-1, angiopoietin-2, and selepressin levels will be measured by contract research laboratories. A detailed description of the sample collection and shipment procedures will be included in a trial-specific laboratory manual. These blood samples will be maintained in storage after the end of the trial. Destruction will take place within one year after last visit/trial-related contact with the last ongoing patient. Bio bank/data protection will be handled in compliance with the national/local regulations.

Handling of all other biological samples will be in accordance with local clinical practice.

8 ADVERSE EVENTS

8.1 Adverse Event Definition

An adverse event is any untoward medical occurrence in a patient participating in a clinical trial. It includes:

- Any unfavourable and unintended sign, symptom or disease temporally associated with the use of the IMP, whether or not considered to be caused by the IMP.
- Adverse events commonly observed and adverse events anticipated based on the pharmacological effect of the IMP.
- Any laboratory abnormality, vital sign, or finding from physical examination assessed as clinically significant and unanticipated in a setting of septic shock by the investigator. Findings from assessments and examinations done during screening are not adverse events, but are recorded as medical history.
- Accidental injuries, reasons for any change in medication (drug and/or dose), reasons for any medical, nursing or pharmacy consultation, or reasons for admission to hospital or surgical procedures.
- Overdoses and medication errors with and without clinical consequences.

8.2 Collection and Recording of Adverse Events

8.2.1 Collection of Adverse Events

The investigator must monitor the condition of the patient throughout the trial from the time of obtaining informed consent until the last visit/trial-related contact.

The sources of adverse events cover:

- Investigations and examinations where the findings are assessed by the investigator to be clinically significant changes or abnormalities which are unanticipated in the setting of septic shock.
- The patient’s response to questions about his/her health (a standard non-leading question such as "How have you been feeling since your last visit?" is asked at each visit).
- Symptoms spontaneously reported by the patient.
- Other information relating to the patient’s health becoming known to the investigator (e.g. hospitalisation in the follow-up period).

8.2.2 Recording of Adverse Events

The investigator must record all adverse events in the adverse event log provided in each patient's eCRF with information about:

- Adverse event

- Date and time of onset (time can be omitted, if applicable)
- Intensity
- Causal relationship to the IMP
- Action taken to the IMP
- Other action taken
- Date and time of outcome (time can be omitted, if applicable)
- Outcome
- Seriousness

Each of the items in the adverse event log is described in detail in the following sections.

Adverse Event

Adverse events should be recorded as diagnoses, if available. If not, separate signs and symptoms should be recorded. One diagnosis/symptom should be entered per record.

If a patient suffers from the same adverse event more than once and the patient recovers in between the events, the adverse events should be recorded separately.

If an adverse event changes (decreases or increases) in intensity, a worst-case approach should be used when recording the event, i.e. the highest intensity and the longest duration of the event. Exception: if an adverse event with onset before the first administration of the IMP (i.e. a pre-treatment adverse event) changes in intensity after the administration of the IMP, this must be recorded as two separate events. The initial adverse event should be recorded with outcome “not yet recovered” and the date and time of outcome is when the intensity changed. The second adverse event should be recorded with date and time of onset when the intensity changed.

Pre-existing conditions not related to the patient’s current clinical setting of vasopressor-dependent septic shock are not adverse events, but become adverse events if worsening occurs after administration of the IMP during the trial. Pre-existing clinically significant conditions diagnosed or observed as a result of the screening procedures must be recorded as medical history.

Note the following: A procedure is not an adverse event; the reason for conducting the procedure is. However, a procedure may be captured along with the reason for conducting the procedure if the investigator finds it adds value to emphasise the procedure. Hospitalisation is not an adverse event; the reason for hospitalisation is. Death is not an adverse event, but the cause of death is (an exception is sudden death of unknown cause, which is an adverse event).

Overdoses and medication errors with or without clinical consequences are recorded as adverse events. The medication error must be specified. Any clinical consequence must be reported as “xxxx due to overdose/medication error”. In the absence of a clinical consequence this must specified e.g. “overdose with no clinical consequence”.

Date and Time of Onset

The date of onset is the date when the first sign(s) or symptom(s) were first noted. If the adverse event is an abnormal clinically significant laboratory test or outcome of an examination, the onset date is the date the sample was taken or the examination was performed. For pre-existing clinically significant conditions (diagnosed or observed as a result of the screening procedures) becoming worse after administration of the IMP, the date of onset is the date the worsening began. Time is to be recorded if relevant for the adverse event.

Intensity

The intensity of an adverse event must be classified using the following 3-point scale:

- Mild: Awareness of signs or symptoms, but no disruption of usual activity.
- Moderate: Event sufficient to affect usual activity (disturbing).
- Severe: Inability to work or perform usual activities (unacceptable).

Causal Relationship to IMP

The possibility of whether the IMP caused the adverse event must be classified as one of the following:

Reasonable possibility

There is evidence or argument to suggest a causal relationship between the IMP and the adverse event. The adverse event may occur as part of the pharmacological action of the IMP or may be unpredictable in its occurrence. Examples:

- Adverse events that are uncommon but are known to be strongly associated with exposure of the IMP.
- Adverse events that are not commonly associated with exposure of the IMP, but the event occurs in association with other factors strongly suggesting causation, such as a strong temporal association or the event recurs on rechallenge.

No reasonable possibility

There is no reasonable evidence or argument to suggest a causal relationship between the IMP and the adverse event. Examples:

- Known consequences of the underlying disease or condition under investigation.
- Adverse events common in the trial population, which are also anticipated to occur with some frequency during the course of the trial, regardless of exposure of the IMP.

Action Taken to IMP

The action taken to the IMP in response to an adverse event must be classified as one of the following:

- No change (medication schedule maintained or no action taken).

- Withdrawn (medication schedule modified through termination of prescribed regimen of medication).
- Interrupted (medication schedule was modified by temporarily terminating a prescribed regimen of medication).

Other Action Taken

Adverse events requiring therapy must be treated with recognised standards of medical care to protect the health and well-being of the patient. Appropriate resuscitation equipment and medicines must be available to ensure the best possible treatment of an emergency situation.

If medication is administered to treat the adverse event, this medication will be entered in the concomitant medication log provided in each patient's eCRF.

Date and Time of Outcome

The date and time (if applicable) the patient recovered or died.

Outcome

The outcome of an adverse event must be classified as one of the following:

- Recovering (the event is improving).
- Recovered (the event has improved i.e. fully recovered or the condition has returned to the level observed at initiation of trial treatment).
- Recovered with sequelae (patient recuperated but retained pathological conditions resulting from the prior disease or injury e.g. resulted in persistent or significant disability / incapacity).
- Not recovered (the event has not improved).
- Fatal (termination of life as result of an adverse event).

8.3 Adverse Events of Special Interest

Some event types are considered especially important to record during this trial as they are considered a crucial part of both septic shock and the safety profile of the IMP. Event types that should always be reported as adverse events are:

- Episodes of atrial fibrillation and other cardiac arrhythmias that require treatment intervention, specifying the type of arrhythmia, treatment and/or intervention, severity and outcome.
- Stroke and other cerebrovascular events.
- Ischaemic events (myocardial ischaemia, peripheral ischaemia or mesenteric ischaemia).

8.4 Pregnancy

Known pregnancy is an exclusion criteria and women of child bearing potential will be tested for pregnancy and excluded if pregnant.

8.5 Serious Adverse Events

8.5.1 Serious Adverse Event Definition

An event is defined as being a serious adverse event if it:	**Guidance:**
Results in **death**	Any event resulting in a fatal outcome must be fully documented and reported, including deaths occurring within four weeks after the treatment ends and irrespective of the causal relationship to the IMP. The death of a patient enrolled in a trial is *per se* not an event, but an outcome (except in case of sudden death from unknown cause).
Is **life-threatening**	The term life-threatening refers to an adverse event in which the patient was at immediate risk of death at the time of the event. It does not refer to an event, which may have caused death if it were more severe.
Requires in-patient **hospitalisation** or prolongation of existing hospitalisation	The term hospitalisation means that the patient was admitted to hospital or that existing hospitalisation was extended as a result of an event. Hospitalisation describes a period of at least 24 hours. Over-night stay for observation, stay at emergency room or treatment on an out-patient basis do not constitute a hospitalisation. However, medical judgement must always be exercised and when in doubt the case should be considered serious (i.e. if case fulfils the criterion for a medically important event). Hospitalisations for administrative or social purposes do not constitute a serious adverse event. Hospital admissions and/or surgical operations planned before trial inclusion are not considered adverse events, if the illness or disease existed before the patient was enrolled in the trial, provided that the condition did not deteriorate during the trial.
Results in persistent or significant **disability/incapacity**	Disability/incapacity means a substantial disruption of a person's ability to conduct normal life functions. In doubt, the decision should be left to medical judgement by the investigator.
Is a **congenital anomaly/birth defect**	Congenital anomaly/birth defect observed in any offspring of the patient conceived during treatment with the IMP.
Is an **important medical event**	Important medical events are events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of important medical events include adverse events that suggest a significant hazard, contraindication or precaution, occurrence of malignancy or development of drug dependency or drug abuse. Medical and scientific judgement should be exercised in deciding whether events qualify as medically important. Important medical events include any suspected transmission of an infectious agent via a medicinal product. Any organism virus or infectious particle (e.g. prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings indicating an infection in a patient exposed to a medicinal product.

8.5.2 Collection, Recording and Reporting of Serious Adverse Events

Serious Adverse Event Reporting by the Investigator

All serious adverse events must be reported **immediately** to Global Pharmacovigilance at Ferring as soon as it becomes known to the investigator and not later than within 24 hours of their knowledge of the occurrence of a serious adverse event.

The investigator is responsible for submitting the completed serious adverse event report form with the fullest possible details **within 3 calendar days** of his/her knowledge of the serious adverse event.

The serious adverse event report form is included in the eCRF system, and must be completed and submitted according to the instructions provided. In case the eCRF cannot be accessed and hence the serious adverse event report form cannot be filled in within the eCRF system, a paper serious adverse event report form should be used and sent to Global Pharmacovigilance at Ferring using the contact details below.

Global Pharmacovigilance, Ferring Pharmaceuticals A/S
E-mail: [redacted]
Fax: [redacted]

Completion of the demographics, adverse event log, medical history log, and concomitant medication log are mandatory for initial reports and for follow-up reports if any relevant changes have been made since the initial report. Data entries must have been made in the eCRF for Ferring Global Pharmacovigilance to access the information.

Additional information relevant to the serious adverse event such as hospital records, results from investigations, e.g. laboratory parameters (that are not already uploaded in the eCRF), invasive procedures, scans and x-rays, and autopsy results can be faxed or scanned and e-mailed to Ferring Global Pharmacovigilance using the contact details in the section above. In any case this information must be supplied by the investigator upon request from Ferring. On any copies provided, details such as patient's name, address, and hospital identification number should be concealed and instead patient number should be provided.

The investigator will supply Ferring and the IEC/IRB with any additional requested information such as results of post-mortem examinations and hospital records.

Expedited Reporting by Ferring

Ferring will report all adverse events that are **serious, unexpected and with a reasonable possible causality to the IMP** as judged by either the investigator or Ferring to the relevant parties within the stipulated timelines.

Taking into consideration the patient population, fatal outcome of certain events occurring in the trial will be considered expected; a mortality rate of 20-25% has been assumed. Consequently the fatal outcome is not considered to be a reportable event, whereas the investigator must still report the events to Ferring as outlined above. If the cause of death is one of the below MedDRA terms or those medically judged to be similar, the event will not qualify for expedited reporting:

Circulatory collapse, distributive shock, endotoxic shock, septic shock, shock, toxic shock syndrome, acute respiratory failure, cerebral hypoperfusion, hypoperfusion, multi-organ failure.

The expectedness is assessed by Ferring according to the investigator’s brochure for selepressin.

Serious adverse events will be considered reportable regardless of whether or not the IMP was used in accordance with the provisions in the protocol and investigator’s brochure.

8.6 Follow-up of Adverse Events and Serious Adverse Events

8.6.1 Follow-up of Adverse Events with Onset during the Trial

During the trial, the investigator must follow-up on each adverse event until it is resolved or until the medical condition of the patient is stable.

After the patient’s last visit, the investigator must follow-up on any adverse event that occurred during the trial and are classified as serious or considered to have a reasonable possible causality to the IMP with the outcome ‘not recovered’ until it is resolved or until the medical condition of the patient is stable. All such relevant follow-up information must be reported to Ferring. If the event is a chronic condition, the investigator and Ferring may agree that further follow-up is not required.

8.6.2 Collection of Serious Adverse Events with Onset after End of Trial

If an investigator becomes aware of a serious adverse event after the end of the trial, and he/she assesses the serious adverse event to have a reasonable possible causality to the IMP, the case will have to be reported to Ferring, regardless how long after the end of the trial this takes place.

9 STATISTICAL METHODS

The Global Biometrics department at Ferring will be responsible for the final statistical analyses. External statistical consultancies will be responsible for all interim analyses during the trial. All analyses will be detailed in a separate statistical analysis plan.

The Health Economics & Outcome Research department at Ferring will evaluate the cost-effectiveness of selepressin utilising relevant data recorded in this trial. Details of these analyses will be described in a separate health economic analytical plan.

The department of Experimental Medicine at Ferring will perform an analysis of pharmacokinetic parameters in a subset of patients. Details of this analysis will be described in a separate pharmacokinetic analysis plan.

9.1 Determination of Sample Size

At least 1800 evaluable patients combined for Part 1 and Part 2 are needed for the final analysis. The overall power of obtaining statistical significance based on combined evidence from Part 1 and Part 2 is 91% in situations where all four arms have a true underlying 1.5% lower mortality rate and a 1.5-day higher expected number of P&VFDs for survivors (corresponding to an overall treatment effect of 1.5 P&VFDs) as compared to placebo. If the effect sizes are 2% on mortality and 2 days for P&VFDs in survivors for all four arms (corresponding to an overall treatment effect of 2 P&VFDs) then the overall power is 98%. In this latter case the probability of engaging into Part 2 is ~99%. The four arms are described in Table 1.

9.2 Patient Disposition

A summary table will present, for each part of the trial and overall, the number of patients in the population sets: ‘Screened’, ‘Intention to treat’, Full analysis set’, ‘Per protocol’, ‘Safety’, ‘Completed trial’, ‘Withdrawals/IMP discontinuations (whichever comes first)’ with a breakdown of reasons/categories for and trial withdrawals/IMP discontinuations.

The patient disposition table will be broken down by each of the stratification variables [the need for mechanical ventilation (Yes/No), norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and serum creatinine (< or ≥150 µmol/L)] and broken down chronologically displaying number of patients ‘completed’ and ‘withdrawn from trial/discontinued from IMP’ at Day 30, Day 90, and Day 180.

The number of patients screened but not randomised/allocated to treatment will be presented with the reason(s) for screen failure in a data listing.

All major protocol deviations (including misrandomisation), based on the full analysis set (FAS), will be summarised for each part of the trial.

Furthermore, 1-Kaplan Meier (KM) plots, based on the intention-to-treat (ITT), will be presented for the time to trial withdrawals/IMP discontinuations (whichever comes first) differentiated by reason for trial withdrawal/IMP discontinuation using cumulative incidence functions. Dropout rates between treatment groups will be evaluated by the log-rank test.

9.3 Protocol Deviations

Protocol deviations will be classified as 'minor' or 'major' by the Ferring clinical trial team on the basis of a blinded review of data before declaration of clean file and lock of database.

Major protocol deviations, such as significant non-compliance or other serious unforeseen violations deemed to invalidate the data and affect the conclusions of the trial, will lead to exclusion of data from the per protocol (PP) analysis set, while data will not be excluded because of minor protocol deviations. All major protocol deviations will be detailed and documented in the clean file document prior to database release. All protocol deviations (minor and major) will be listed in patient data listings.

9.4 Analysis Sets

9.4.1 Intention-to-Treat Analysis Dataset

The ITT analysis set comprises all randomised (as planned) patients.

9.4.2 Full Analysis Set

The FAS comprises data from all randomised (as planned) and dosed patients.

9.4.3 Per Protocol Dataset

Patients in the FAS will be excluded from the PP analysis set if they meet any major protocol deviations as defined in the statistical analysis plan. Data will be used up to the point of protocol deviation.

9.4.4 Safety Dataset

The safety analysis set comprises all treated patients and is analysed according to the actual treatment received.

9.5 Trial Population

9.5.1 Demographics and other Baseline Characteristics

Descriptive statistics of demographics and other baseline characteristics (including vital signs) will be presented for the FAS population by treatment arm and total.

Categorical data will be summarised using numbers and percentages. The percentages are based on the total number of patients with a corresponding assessment. Continuous data will be presented, for example, using the number of patients, mean and standard deviation, median, interquartile range, minimum and maximum. All baseline characteristics will be listed.

9.5.2 Medical History and Prior/Concomitant Medication

Medical history will be summarised by treatment arm and total and presented in patient data listings.

Prior and concomitant medication will be summarised by treatment arm and total and presented in patient data listings.

9.6 Endpoint Assessments

9.6.1 General Considerations

All statistical tests will be performed using a two-sided test at a 5% significance level.

If the trial is stopped prematurely due to e.g. futility, the data will be analysed as planned in accordance with the statistical analysis plan.

The efficacy endpoints will be analysed for the FAS and the PP analysis set, with the FAS being considered as primary and the PP analyses as supportive.

Categorical data will be summarised using counts and percentages, while continuous data will be presented using the number of patients, mean, standard deviation, median, interquartile range, minimum and maximum.

All assessments will be listed in patient data listings.

9.6.2 Primary Endpoint

The primary endpoint of this trial is "vasopressor and mechanical ventilator-free days (P&VFDs) up to Day 30."

This composite endpoint is defined as the number of days (reported to one decimal place (0.0 to 30.0)) from start of treatment with the investigational medicinal product (IMP) [selepressin or placebo] to 30.0 days thereafter during which the patient is: 1) alive; 2) free of treatment with intravenous vasopressors; and 3) free of any invasive mechanical ventilation (see definition below).

Patient Death

By definition, any patient that dies within this 30-day period will be assigned zero P&VFDs, even if there is a period during which the patient is alive and free of both vasopressor treatment and mechanical ventilation.

Definition of "Free of Vasopressors"

Free of vasopressors means less than 60 minutes during any contiguous 24-hour period (regardless of calendar day). If a patient requires periods of vasopressors longer than 60 minutes in total during any 24-hour period, the intervening intervals during which they are free of vasopressors will not be included in the period free of vasopressors in the determination of the number of P&VFDs. Thus, the period free of vasopressors begins at the end of the last use of vasopressors that was either: 1) longer than 60 minutes in duration; or 2) part of greater than 60 minutes of use within a contiguous 24-hour period.

Norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, and IMP (i.e. selepressin and placebo) all constitute a vasopressor for the purpose of the primary analysis.

Vasopressor use due to anaesthesia or procedure-induced hypotension during and up to three hours after a surgery or procedure (including bedside) is exempt from this rule (i.e. such use of vasopressors would not affect the calculation of P&VFDs).

Definition of "Free of Mechanical Ventilation"

Mechanical ventilation is defined as use of endotracheal or tracheostomy tube assisted ventilation (>5 cm H_2O continuous positive airway pressure and >5 cm H_2O of pressure support from the ventilator in tracheostomy patients). End of mechanical ventilation is defined as: 1) extubation of intubated patients or 2) ≤5 cm H_2O continuous positive airway pressure and ≤5 cm H_2O of pressure support from the ventilator in tracheostomy patients. If non-invasive ventilation by mask or bag (>5 cm H_2O of pressure support) is deployed to avoid (re)intubation, it also counts as mechanical ventilation. However, all other uses of non-invasive ventilation such as chronic night-time use of positive airway pressure for COPD or sleep apnea does not count as mechanical ventilation (regardless of pressure). Free of mechanical ventilation means less than 60 minutes during any contiguous 24-hour period (regardless of calendar day). If a patient requires mechanical ventilation for periods longer than 60 minutes in total during any 24-hour period, the intervening intervals during which they are not receiving mechanical ventilation will not be included in the period free of mechanical ventilation in the determination of the number of P&VFDs. Thus, the period free of mechanical ventilation begins at the end of the last use of mechanical ventilation that was either: 1) longer than 60 minutes in duration; or 2) part of greater than 60 minutes of use within a contiguous 24-hour period.

The use of mechanical ventilation associated with anaesthesia or procedural sedation during and up to three hours after a surgery or procedure (including bedside) is exempt from this rule (i.e. such use of mechanical ventilation would not affect the calculation of P&VFDs).

It is important to note that the determination of freedom from vasopressors and freedom from mechanical ventilation are made separately; in other words, periods of vasopressor use and mechanical ventilation are not combined when determining whether 60 minutes of use has occurred within a 24-hour period.

Missing data during the time of hospitalisation will be imputed using a worst case approach taking into account previous and subsequent starting and stopping times of vasopressor administration and mechanical ventilation. If only the stop date but not time is given, the imputed time will be midnight of that date, unless a subsequent starting time was recorded prior to midnight in which case the imputed time would be the start time of the subsequent record. If neither stop date nor time is given, the imputed stop time will be the start date and time of the subsequent recording. Likewise, missing start dates and times would be imputed as worst case scenarios, i.e. is the patient found to be on mechanical ventilation with a date but no time for intubation, the imputed start time would be recorded as 00:01 of that day or the stop date of a preceding recording on that same date, whichever occurs last. If both start date and time is missing, the imputed start time would be the date and time of the preceding stop time recorded. In case of data being completely missing from a certain time point and onwards (i.e. patient lost to follow up), the "last status carried forward" imputation will be applied. If a patient was last seen on either ventilator or vasopressors, it is assumed that the patient remained so, and is imputed to a value of 0 (zero) P&VFDs. If the patient was last seen off ventilator and vasopressors, it is also assumed that the patient remained so in the remaining 30-day period. If the patient was last seen (alive) on e.g. day 10 and at that point had been off both ventilator and vasopressors for three days, a value of 23 P&VFDs is imputed.

The primary endpoint, P&VFDs, will be analysed using a van Elteren test, stratified by need for ventilation (Yes/No), time from onset of shock (onset of vasopressor (i.e. norepinephrine/ noradrenaline)) to start of IMP treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 μg/min).

The primary analysis will compare all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) to all patients on the placebo arm from both parts of the trial. The primary analysis will be a test of superiority using a two-sided 5% significance level test. This test, within the trial, controls the type 1 error at a two-sided 5% level. Further details are provided in the statistical analysis plan.

Treatment effects will be estimated assuming a negative binomial distribution (to allow for possible overdispersion in a Poisson distribution) for the quantity (30 minus P&VFDs) for survivors, and a binomial distribution to model the probability of surviving. Both models adjusted for need for ventilation (Yes/No), time from onset of shock to start of IMP treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 μg/min). Further details are provided in the statistical analysis plan.

Furthermore, P&VFDs will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically by histograms and cumulative distributions functions.

The success (statistical/clinical significance) of the trial will be based upon the comparison of the analysis above (all patients on all selepressin dosing regimens from both parts of the trial [pooled together and treated as a single arm] compared to all patients on the placebo arm from both parts of the trial).

9.6.2.1 Sensitivity Analyses of the Primary Endpoint

As the adaptations of the trial provide a conservative estimate of the p-value, sensitivity p-values will be provided using post-simulation bootstrap calculations.

In order to check for consistency, the primary endpoint treatment differences will, as a minimum, be estimated and presented by forest plots for the following subgroups:

- Region
- Age
- Gender
- Race/ethnicity

Furthermore, the primary endpoint will be stratified by severity of the patients, with risk of dying as indicator of severity (see Figure 3). Mortality (the risk of dying) will be analysed by a logistic regression model, with relevant baseline characteristics as covariates (e.g. the individual SOFA scores and age). The model used to generate the predicted risk (for all patients) will be based on patients in the placebo arm only, as the risk of dying should reflect the severity in the absence of selepressin. Stratified by the risk of dying (intervals of 20% if suitable, based on the mortality rates in the covariate categories in the model), the treatment effect of the primary endpoint will be presented graphically, in order to visually inspect whether the average treatment effect is distributed evenly across the severity of patients.

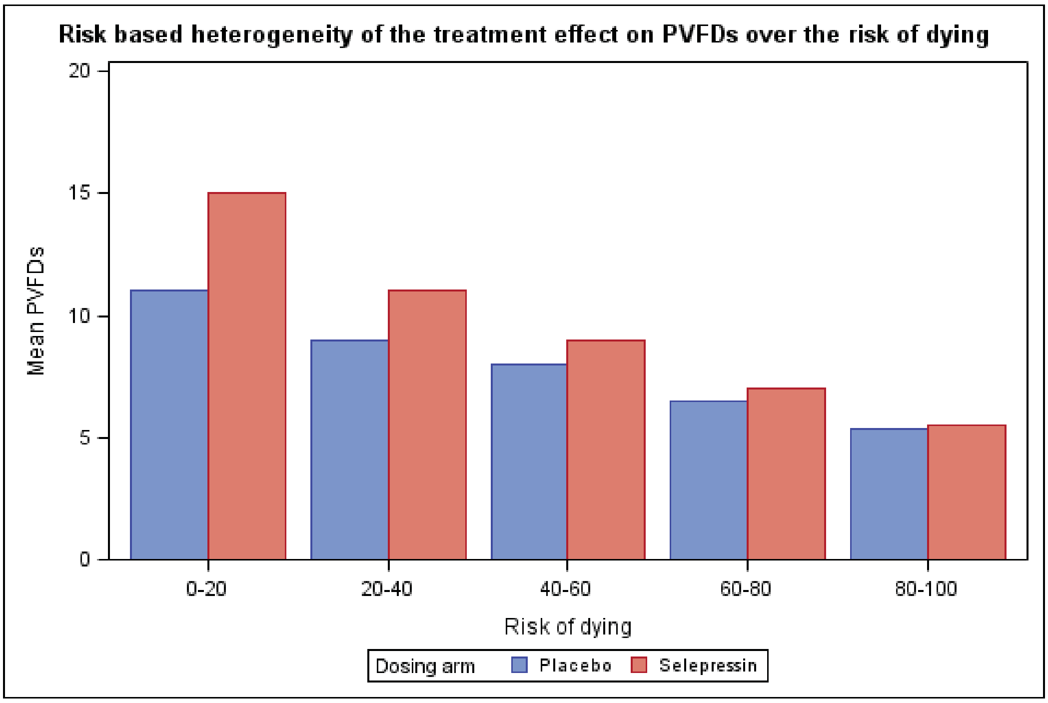


Figure 3 Risk-based Heterogeneity of the Treatment Effect on P&VFDs over the Risk of Dying (an example)

The impact and robustness of the imputation of missing data will be checked by analysing data in the following ways

- excluding all patients with missing/imputed data
- imputing the 30-day P&VFD status for patients lost to follow up using the observed ratio of P&VFDs at time of lost to follow up, to the same proportion for a 30-day status

For this analysis the 30-day P&VFD status for patients lost to follow up will be imputed so that the 30-day ratio of P&VFDs is equal to the ratio of P&VFDs at time of lost to follow up. E.g. a patient being lost to follow up at Day 15 with 4 P&VFDs (a ratio of 4/15 P&VFDs per days observed) will be imputed to 8 P&VFDs at Day 30 (equivalent ratio 8/30 = 4/15). Patients having zero P&VFDs at time of lost to follow up will be imputed to a value of zero P&VFDs.

- tipping point analysis

The tipping point analysis will compare all possible combinations of 'best case' and 'worst case' scenarios between placebo and selepressin. Best case being an imputation assuming the remaining days off ventilator and vasopressors, and worst case being an imputation of zero P&VFDs. N_P and N_S will be the number of patients in the placebo and selepressin arms with missing data. The tipping point analysis will compare all combinations (from 0 to N_P) of X patients on placebo imputed best case and N_P – X imputed worst case, to Y patients on selepressin imputed best case and N_S – Y imputed worst case. In other words, all N_P +1 times N_S +1 combinations will be analysed for the primary endpoint. Since the 'best case' is not the same for all patients (depending on when they were last seen off both ventilator and vasopressors), there are multiple outcomes within each combination. For each combination, the average p-value of the multiple outcomes will be plotted in the tipping point analysis.

Below is an example of a tipping point analysis of 25 placebo versus 40 selepressin patients with imputed values. The x- and y-axis displays the number of patients with the 'best case' imputed. In the example in Figure 4, the red area displays the non-significant p-values, indicating that one would have to impute almost all placebo patients to a 'best case' and almost all selepressin to a 'worst case' in order to get non-significant p-values, and hence 'proving' the robustness of the imputation method.

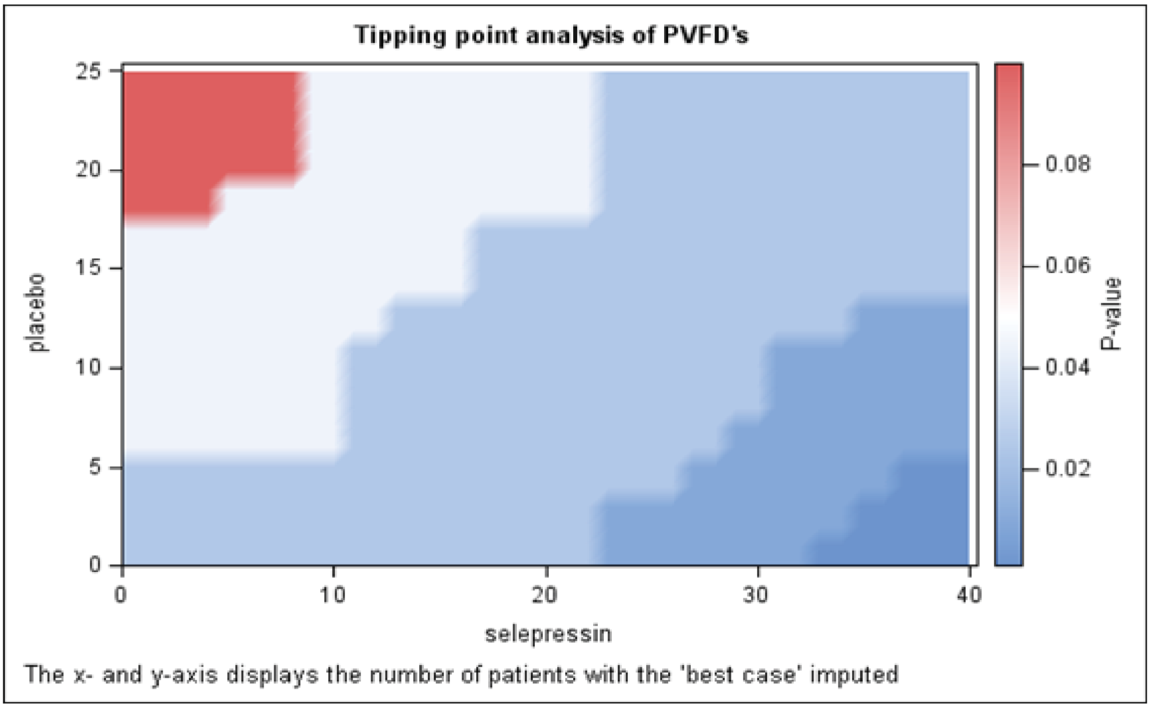


Figure 4 Tipping Point Analysis of P&VFDs (an example)

Also, to make sure that the use of vasopressor in each group is not simply being replaced by an increased use of inotropic agents, there will be a sensitivity analysis of the primary endpoint in which the use of inotropic agents will count as vasopressor use.

9.6.2.2 Additional Analyses

The primary analysis will be repeated for:

- The selected dose only, i.e. comparing all patients on the selected dose (from Part 1) from both parts of the trial (pooled), to all patients on the placebo arm from both parts of the trial.
- Data from Part 2 only, i.e. comparing the selected dose to placebo on data from Part 2 only.

9.6.3 Secondary Endpoints

For the purpose of a possible label inclusion, the Hochberg procedure for adjustment on multiplicity will be implemented to selected key secondary endpoints. Only if the primary efficacy analysis leads to a statistically significant result at the (one-sided) 2.5% level, then the Hochberg procedure, which is described below, is applied to the selected key secondary analyses. If the primary efficacy analysis does not result in statistical significance at the (one-sided) 2.5% level, then statistical significance (for the purpose of a possible label inclusion only) will not be declared for any of the key secondary analyses, regardless of their p-values.

The selected key secondary endpoints aimed at further demonstrating treatment effect are:

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Day 90
- RRT-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)
- ICU-free days up to Day 30

In this application of the Hochberg procedure there are three hypothesis tests of superiority for each of the selected secondary endpoints. The target alpha level is (one-sided) 2.5%. The Hochberg procedure is as follows:

- Order the p-values from the smallest to the largest value, p(1) < p(2) < p(3), with corresponding null hypothesis $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$.
- Start with the highest p-value. If p(3) < 2.5% (one-sided), then stop and declare all three comparisons significant at the 2.5% (one-sided) level (i.e. reject $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$). Otherwise, accept $H_{(3)}$ for the endpoint related to p(3), and go to p(2) the second highest p-value.
- If p(2) < 2.5/2 = 1.25% (one-sided), then stop and declare significance for $H_{(1)}$ and $H_{(2)}$. Otherwise, accept $H_{(2)}$, for the endpoint related to p(2), and go to p(1) the lowest p-value.
- If p(1) < 2.5/3 = 0.833% (one-sided), then stop and declare significance for $H_{(1)}$. Otherwise, accept $H_{(1)}$, for the endpoint related to p(1).

Regardless of the statistical significance declared according to the Hochberg procedure, all analyses will be included and presented in the statistical report.

As for the primary analysis, the primary comparison (which determines the success, i.e. statistical and clinical significance) for the secondary efficacy endpoints is between all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) and all patients on the placebo arm from both parts of the trial.

As an additional analysis, all secondary efficacy analyses will, as for the primary, be repeated for:

- The selected dose only, i.e. comparing all patients on the selected dose (from Part 1) from both parts of the trial (pooled), to all patients on the placebo arm from both parts of the trial.
- Data from Part 2 only, i.e. comparing the selected dose to placebo on data from Part 2 only.

All free-days endpoints will be reported to one decimal place.

The following secondary endpoints will be defined and analysed in a similar manner as the primary endpoint:

- ***Vasopressor-free Days up to Day 30***
- ***Mechanical ventilator-free Days up to Day 30***

- ***RRT-free Days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)***
- ***ICU-free Days up to Day 30***

Incidence of RRT up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation) is defined as any form of renal replacement therapy defined as continuous renal replacement therapy, intermittent haemodialysis, or peritoneal dialysis. In order to ensure that any reduction in incidence of RRT is not caused by an increase in mortality, all patients dying within the 30-day period will be counted as on RRT. For patients withdrawn prior to Day 30, incidence of RRT will be based on the data available up until the time of withdrawal.

Incidence of RRT will be analysed by a logistic regression model with time from onset of shock to start of treatment, baseline creatinine and norepinephrine/noradrenaline requirement at baseline, as covariates, and treatment, and need for ventilation as factors. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method. Patients already on RRT at time of inclusion will be excluded from the analysis of incidence of RRT.

Non-inferiority will be claimed if the upper limit of the two-sided 95% CI of the adjusted difference in proportions is less than 20% of the estimated incidence of RRT in the placebo group. Superiority can be claimed if the upper limit is less than 0.

I.e., let $\widehat{p_S}$ and $\widehat{p_P}$ be the estimated incidences of RRT in the combined selepressin groups and the placebo group respectively. Non-inferiority will then be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0.2 * \widehat{p_P}$$

and superiority will be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0$$

Furthermore, incidence of RRT will be tabulated by treatment arm (including pooled active treatment arms).

A subgroup analysis will be performed on patients without acute RRT at baseline.

Duration of septic shock up to Day 30. Shock is defined as the cumulative periods (>1 hour) from start of IMP until Day 30, on IMP or vasopressors. Vasopressor use due to anaesthesia / procedure-induced hypotension during - and up to three hours after - surgery / procedure (including bedside) is exempt from this rule. For patients withdrawn (in the survivors analysis) or dying (in the non-survivors analysis) while still in septic shock, the duration will be based on the data available up until the time of withdrawal or death.

Duration of septic shock will be analysed separately for survivors, non-survivors (within the first 30 days) and overall, comparing treatment arms by an analysis of covariance (ANCOVA) model with time from onset of shock to start of treatment and norepinephrine/noradrenaline requirement at baseline as covariates, and treatment and need for ventilation (Yes/No) as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented.

Some patients will get out of shock prior to Day 30 (and stay alive until Day 30), some will get out of shock and die later on (prior to Day 30), others will die while still in shock (prior to Day 30), and the remaining few will not get out of shock prior to Day 30. This means that if mortality rates vary between treatment arms, the results of the analysis for the overall population will be influenced by the skewed mortality rates. Hence, for the overall population, the distribution of duration of shock (time to out of shock), will be presented graphically as competing risks between 'time to out of shock' and 'dying while in shock'. Further, a KM (sub)-graph on 'time to death' will be presented for those getting out of shock (for which some will die later on, prior to Day 30). This is done in order to elucidate any skewness in mortality rates, influencing the results of the analysis. The duration of septic shock will also be tabulated by treatment arm (including pooled active treatment arms).

The following secondary endpoints will be defined and analysed in a similar manner as for duration of septic shock:

- ***Duration of mechanical ventilation up to Day 30***
- ***Duration of RRT up to Day 90 (excluding patients on RRT for chronic renal failure at time of randomisation)***
- ***ICU length of stay up to Day 30***

Daily overall (modified) and individual organ scores of the SOFA will be compared between treatment arms up until Day 7 using a repeated measures ANCOVA model with baseline SOFA score as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented.

Last observation carried forward (LOCF) will be used for missing SOFA scores on Days 2-7. No LOCF for Day 1 (as previous value is baseline). Patients dying will be imputed with a worst possible outcome, i.e. a value of 4 for each individual SOFA score.

Daily overall (modified) and individual SOFA scores will be tabulated by treatment arm (including pooled active treatment arms).

Incidence of new organ dysfunction and new organ failure. New organ failure is defined as an increase (i.e. worsening) in any of the individual SOFA scores from (0, 1, 2) at baseline to (3, 4) post baseline up until the end of the period (Days 7 or 30) (if the SOFA scores goes from [0, 1, 2] to [3, 4] and back to [0, 1, 2] again within the period, that will still count as a new organ failure). If a patient dies within the period, he/she is considered to fail on all organs, and the number of new organ failures will be all organs except those already failed at baseline. Patients discontinued within the period will be evaluated based on the data available at time of discontinuation.

Incidence of new organ dysfunction is defined as an increase ≥1 from baseline to post baseline up until the end of the period (e.g. going from 1 to 2) in any of the individual SOFA scores. Patients with an individual SOFA score of 4 at baseline can per default not have a new organ dysfunction. If a patient dies within the period, he/she is considered to have dysfunction on all organs, and the number of new organ dysfunctions will be all organs except those already having a score of 4 at baseline. Patients withdrawn within the period will be evaluated based on the data available at time of withdrawal.

Incidence of at least one new organ failure will be analysed for any new organ failure (across all organ systems) and by individual organ systems, and compared between treatment arms using a logistic regression model with age, modified SOFA score, and norepinephrine/noradrenaline requirement at baseline as covariates and gender and treatment arm as factors, presenting odds ratios with 95% confidence intervals.

Incidence of at least one new organ dysfunction will be analysed for any new organ dysfunction (across all organ systems) and by individual organ systems, and will be analysed as above for new organ failures.

The number of new organ dysfunctions and new organ failures will be compared between treatment arms using a negative binominal model with age, modified SOFA score, and norepinephrine/ noradrenaline requirement at baseline as covariates, and gender and treatment as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method.

Incidence of any new organ dysfunction and any new organ failure and the number of new organ dysfunctions and new organ failures will be tabulated by treatment arm (including pooled active treatment arms).

All-cause mortality is defined as the fraction of patients that have died, regardless of cause, by the end of Day 30, Day 90, and Day 180 and will be analysed and compared between treatment arms using a logistic regression model with the individual SOFA scores and age as covariates and treatment arm as factor. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method as for incidence of RRT. There will be no imputations for mortality.

Non-inferiority will be claimed if the upper limit of the two-sided 95% CI of the adjusted difference in proportions is less than 30% of the estimated incidence of mortality in the placebo group. Superiority can be claimed if the upper limit is less than 0.

Assuming an observed 30-day mortality rate of 20-25% in the placebo group, a non-inferiority limit of 30% corresponds to a maximum observed mortality rate of 2-3% in the combined selepressin groups in order for selepressin to be non-inferior to placebo.

Furthermore, mortality will be tabulated by treatment arm (including pooled active treatment arms), and the time to death presented graphically by a Kaplan-Meier plot.

EQ-5D-5L will be analysed by the index value, the overall quality-adjusted life years (QALY) at Day 30 and 180, and the VAS score. The QALY scores will NOT be adjusted to e.g. a half yearly time scale at Day 180. As the QALY is not defined for patients with all remaining values missing, and hence also not defined for those who die, the analyses will automatically only be analysed for those surviving up until Day 30 and Day 180, respectively.

For patients with missing baseline index value, the QALY score will also be set to missing. For robustness, a sensitivity analyses will be performed, imputing the missing baseline scores with the overall mean of the baseline health index. Baseline is the timing prior to acute admission.

The QALY at Day 30 and Day 180 will be compared between treatment arms using an ANCOVA model with baseline health index as covariate, and treatment as factor. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

The index value and VAS scores will be analysed separately for survivors and non-survivors at Day 180 (since all non-survivors will have non-random missing values, and hence would artificially inflate the mean estimates if survivors and non-survivors were analysed together) and will be compared between treatment arms using a repeated measures ANCOVA model with baseline health index/VAS score as covariate, treatment, time and treatment by time interaction as factors, and subject as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented for Day 30, Day 60, Day 90, and Day 180. There will be no imputations for missing values.

The QALY, index value, and VAS scores will be tabulated by treatment arm (including pooled active treatment arms), and the index value and VAS scores will be presented graphically.

Daily and cumulative fluid balance (for 7 days or until ICU discharge). Fluid overload is defined as fluid balance as a percentage of baseline weight (e.g. if a patient weighs 90 kg at baseline and has a fluid balance of 9 litres, fluid overload is then 100% * 9 litres / 90 kg = 10%.

Fluid balance and cumulative fluid balance will be presented both unadjusted and adjusted for weight.

Daily and cumulative fluid balance as well as daily and cumulative fluid overload will all be compared between treatment arms using a repeated measures ANCOVA model with baseline (baseline fluid balance or baseline fluid overload) as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

The absolute values and change from baseline will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

Daily and cumulative Urine Output (for 7 days or until ICU discharge) will be analysed as for fluid balance.

9.6.4 Other Efficacy Endpoints

Other assessments include:

- Hospital-free Days up to Day 90

Will be defined and analysed in a similar manner as for the primary endpoint.

- Hospital length of stay up to Day 90

Will be defined and analysed in a similar manner as for duration of septic shock.

- Norepinephrine/noradrenaline and other vasopressor doses

The dose of norepinephrine/noradrenaline administered (adjusted for baseline weight) will be compared between treatment arms using a repeated measures ANCOVA model with baseline dose of norepinephrine/noradrenaline as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented. There will be no imputations of missing values.

The mean dose administered will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

The dose of other vasopressors is defined as the cumulative dose at any given time of epinephrine/adrenaline, dopamine, phenylephrine and vasopressin.

The dose of other vasopressors will be will be analysed as for doses of norepinephrine/ noradrenaline with baseline dose of other vasopressors as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit.

- Patient residence at Day 30, Day 60, Day 90, and Day 180
- MAP
- Arterial blood gases and acid/base status (PaO_2, $PaCO_2$, SaO_2, pH, bicarbonate, base excess) and lactate levels
- $ScvO_2$
- PaO_2/FiO_2
- EVLW and PPI (in a subset of patients)

- Cardiac output (in a subset of patients)
- Cytokines, angiopoietin-1 and -2 levels (in a subset of patients)

These assessments will be presented by descriptive statistics, i.e. the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point. Baseline value will be the value obtained at the last assessment prior to the infusion start of the IMP.

- Creatinine clearance

Creatinine clearance will be analysed as for fluid balance with baseline creatinine as a covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit.

9.7 Extent of Exposure and Treatment Compliance

The total amount (adjusted by weight [µg/kg]) of selepressin administered and the number of (decimal) days treated with selepressin will be summarised by (active) treatment arm and total (active treatment arms).

Furthermore, the mean cumulative amount administered and the mean infusion rate will be tabulated by treatment arm and presented graphically (also by treatment arm and total).

9.8 Safety

9.8.1 General Considerations

Safety parameters will be evaluated for the safety analysis data set. All safety summaries will be tabulated by treatment arm (including pooled active treatment arms).

9.8.2 Adverse Events

Adverse events will be classified according to the medical dictionary for regulatory activities (MedDRA). The MedDRA version will be documented.

A pre-treatment adverse event is any adverse event occurring after signed informed consent and before administration of the IMP.

A treatment-emergent adverse event is any adverse event occurring after the administration of the IMP and within the time of residual drug effect (i.e. 12 hours), or a pre-treatment adverse event or pre-existing medical condition that worsens in intensity after start of IMP and within the time of residual drug effect. The time of residual drug effect is the estimated time after the end of the administration of the IMP, where the effect of the product is still considered to be present based on pharmacokinetic, pharmacodynamic, or other substance characteristics. A generally accepted time for residual drug effect is 5 half-lives. The terminal half-life of selepressin is expected to be not more than 1.8 hours, and hence, a treatment-emergent adverse event is defined as any adverse event occurring after the start of IMP infusion and within 12 hours after the IMP infusion is stopped.

A post-treatment adverse event is any adverse event occurring after the residual drug effect period.

Missing values will be treated as missing, except for causality, intensity, seriousness, and outcome of adverse events. A "worst case" approach will be used: if causality is missing, the adverse event will be regarded as related to the IMP; if the intensity of an adverse event is missing, the adverse event will be regarded as severe; if seriousness is missing the adverse event will be regarded as serious; if start date is missing or incomplete, worst case will be assumed and the adverse event will be regarded as treatment-emergent (only if the incomplete start date is not compromised). If start date is completely missing, start date will be set as same day as start of treatment. If start date is incomplete, the date closest to start of treatment will be assumed, without compromising the incomplete data available on the start date; if outcome is missing and no date of outcome is present the outcome is regarded as 'not recovered'.

An overview of treatment-emergent adverse events will be provided in a summary table including the number of patients reporting a treatment-emergent adverse event, the percentage of patients with a treatment-emergent adverse event, and the number of events reported, for the following categories:

- Adverse events
- Deaths
- Serious adverse events
- Adverse events leading to discontinuation of IMP
- Adverse events leading to withdrawal from the trial
- Severe and life-threatening adverse events
- Adverse drug reactions

Treatment-emergent adverse events will be summarised in a table by SOC (sorted alphabetically) and PT (sorted in decreasing frequency of occurrence) using MedDRA. The table will display the total number of patients reporting an adverse event, the percentage of patients with an adverse event, and the number of events reported.

Summary tables will be prepared for:

- All adverse events
- Adverse events with an incidence ≥5% of patients in any treatment arm
- Non-serious adverse events with an incidence ≥5% of patients in any treatment arm
- Critical adverse events, details are provided in the statistical analysis plan
- Adverse events based on changes in vital signs assessed as unanticipated in the setting of septic shock
- Adverse events based on changes in safety laboratory variables assessed as unanticipated in the setting of septic shock

- Adverse events by causality (related/unrelated)
- Adverse events leading to death
- Adverse events by intensity
- Serious adverse events
- Adverse events leading to discontinuation of IMP (related/unrelated)
- Adverse events leading to withdrawal from trial (related/unrelated)

Supporting data listings will be provided for:

- All adverse events sorted by trial site and patient number
- All adverse events sorted by MedDRA PT
- Serious adverse events
- Adverse events leading to death
- Adverse events leading to discontinuation of IMP (related/unrelated)
- Adverse events leading to withdrawal from trial (related/unrelated)
- Post-treatment adverse events

9.8.3 Safety Laboratory Variables

Safety laboratory variables will be grouped under ‘Clinical Chemistry’, ‘Haematology’, and ‘Coagulation’.

Baseline for all safety laboratory variables will be the values obtained at the last assessment prior to the infusion start of the IMP. End of treatment period will include the last post-baseline observation during the trial up until Day 30.

Mean change and mean percentage change from baseline at end of treatment period will be presented for each laboratory variable. In addition, descriptive statistics, i.e. the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each laboratory variable.

Furthermore, a summary table will be prepared for selected laboratory variables that display the number and percentage of patients in each treatment arm with X% increments (increase or decrease) from baseline at each time-point. The selected laboratory variables and categories are detailed in the statistical analysis plan.

9.8.4 Vital Signs and Central Venous Pressure

Baseline for all vital signs variables and CVP will be the values obtained at the last assessment prior to the IMP infusion start. End of treatment period will include the last post-baseline observation during the trial up until Day 30.

Mean change and mean percentage change from baseline at end of treatment period will be presented for each variable. In addition, descriptive statistics, i.e. the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each variable.

Furthermore, a summary table will be prepared for each variable that display the number and percentage of patients in each treatment arm with X% increments (increase or decrease) from baseline at each time-point and end of treatment period. The variables and categories are detailed in the statistical analysis plan.

9.8.5 Episodes of Hypotension

Descriptive statistics of number of patients with episodes of hypotension and the total length of periods with hypotension will be summarised by treatment arm.

The total length of periods with hypotension will be summarised for both all patients, and patients having one or more episodes of hypotension.

9.9 Interim Analyses

There will be no interim analyses with the potential to stop the trial early for treatment efficacy. However, once the "burn-in" period in Part 1 is completed (first 200 treated patients), interim analyses will be conducted regularly to improve the efficiency of dose selection and to allow early termination of the part or the trial for futility or for successful dose selection. The following steps will be considered at each interim analysis:

- When 200 patients are treated, the allocation probabilities for the active treatment arms are changed using response-adaptive randomisation (with placebo still 1/3). For the two-thirds of patients assigned to the active arms, the probability that a given active arm is assigned to a patient is proportional to the probability that that arm is the arm with the largest expected number of P&VFD.
- Potentially stopping the trial for futility during Part 1. This occurs if no active arm has better than a 5% predictive probability of a significant result in Part 2 if it were to start immediately. This decision can occur at any interim during Part 1 after 200 evaluable patients.
- Potentially ending Part 1 and selecting an active treatment arm to continue to Part 2. This decision can occur at any interim analysis between 300 evaluable and 800 treated patients, and it occurs if some arm has a predictive probability of a successful trial of at least 90% before 800 treated patients, and the threshold drops to 25% for the final Part 1 interim analysis at 800 treated patients. The selected arm is the arm with the largest posterior predictive probability of trial success. This will generally be the best-performing active arm, but if multiple arms are performing equally well, it will be the arm with the lowest dosing level. If Part 1 ends after *N* patients, then Part 2 will consist of up to 1800 - *N* evaluable patients.

- If the trial is not stopped for futility or proceeding to Part 2 and active treatment Arm 4 has not yet been approved for assignment of patients, the decision can be made to open up Arm 4. Arm 4 is only opened between 200 evaluable and 600 treated patients and if there is at least a 50% probability that Arm 3 has a higher expected P&VFD than Arm 2 and if data from the lower dosing levels do not suggest any significant safety signals.
- If Part 1 reaches its maximum of 800 treated patients and no arm has a predictive probability of Part 2 success of more than 25%, the trial stops with an inconclusive result and will be interpreted as a standalone Phase 2b trial.

During Part 2, interim analyses will be conducted regularly (until 1600 patients have been treated) to allow early termination of the trial for futility. This occurs if the predictive probability of an overall significant result is less than 5%. In addition, if the predictive probability of observing a more than 2% higher mortality in the active arms compared to placebo is greater than 90% then the trial will stop for futility.

10 DATA HANDLING

10.1 Source Data and Source Documents

Source Data – ICH Definition

Source data are defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

Source Documents - ICH Definition

Source documents are defined as original documents, data, and records (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, patient files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial).

Trial-specific Source Data Requirements – Ferring

The investigators must maintain patient records. For each randomised patient, the investigators will indicate in the hospital/medical source records that the patient participates in this trial and the date of obtaining the informed consent. The records will include data on the condition of the patient at the time the patient is enrolled in the trial in order to document (and enable verification of) eligibility. Signed and dated informed consent forms will be stored and archived in accordance with local requirements.

The following information, as a minimum, has to be recorded in the hospital/medical source records for each patient:

- Documentation of informed consent obtained
- Trial identification
- Screening/patient (randomisation) number
- Patient's name
- Demographic data including date of birth, race, and ethnic origin
- Diagnosis, septic shock characteristics
- Relevant medical history
- Relevant concomitant medications/procedures
- Body weight and height
- Eligibility for participation in the trial (documenting all inclusion/exclusion/eligibility criteria)

- Details of the administration of IMP
- Details of the administration of norepinephrine/noradrenaline and other vasopressors
- Details of the MAP during the IMP infusion
- Details of mechanical ventilation (including spontaneous breathing trials) and RRT
- Functional/survival status of the patient throughout the trial
- ED/ICU/hospital admission and discharge dates and times
- Details and results of all other examinations and tests performed
- Date of each visit/contact
- Details of adverse events
- Reason for discontinuation/withdrawal, if applicable

Information included in the patient's hospital records may be subject to local regulations. If there is a discrepancy between local requirements and the trial protocol, local regulations should be followed. The identification of source data for each variable may then be described in a separate document.

Documents collected during the trial (e.g. health-related quality of life questionnaires, laboratory reports, print-outs of MAP and ECG) should be stored and archived together with the patient's hospital/medical records or in the investigator file as agreed upon prior to the trial start at each trial site.

No specific protocol data can be recorded directly in the eCRF without prior written or electronic record.

10.2 Electronic Case Report Form (eCRF)

An eCRF system provided by an independent third-party contract research organisation will be used for data capture. Contact details of the contract research organisation are provided in a trial-specific contact list. The system is validated and access at all levels to the system is granted/revoked following Ferring and vendor procedures, in accordance with regulatory and system requirements.

Data should be entered into the system within a reasonable time after source data is collected.

The investigator will approve/authorise the eCRF entries for each patient with an electronic signature which is equivalent to a handwritten signature.

The eCRF system and the database will be hosted at and administered by the independent third party contract research organisation. After the trial database is declared clean and released to the statistician, a final copy of the database will be stored at Ferring. The investigator will also receive a copy of the trial site's final and locked data (including audit trail, electronic signature and queries) as write-protected PDF-files produced and distributed by the independent third party contract research organisation. The PDF-files will be stored on a CD and will be provided to the investigator before read access to the eCRF is revoked.

Modification of data entered into the eCRF will be captured in an electronic audit trail detailing the date and time of the correction and the user name of the person making the correction. Only site coordinator and investigator have privileges to modify data.

10.3 Data Management

A data management plan will be created under the responsibility of the Global Biometrics department at Ferring. The data management plan will be issued before data collection begins and will describe all functions, processes, and specifications for data collection, cleaning, and validation.

The data management plan will describe capture methods, who is authorised to enter the data, decisions about ownership of data, source data storage, which data will be transferred (including timing of transfers), the origin and destination of the data, and who will have access to the data at all times.

10.4 Provision of Additional Information

On request, the investigators will provide Ferring with additional data relating to the trial, duly anonymised and protected in accordance with applicable requirements.

11 MONITORING PROCEDURES

11.1 Periodic Monitoring

The monitors will contact and visit the investigators periodically to ensure adherence to the protocol, International Conference of Harmonisation-Good Clinical Practice (ICH-GCP), standard operating procedures and applicable regulatory requirements, maintenance of trial-related source records, completeness, accuracy and verifiability of eCRF entries compared to source data, verification of drug accountability, and compliance to safety reporting instructions. The investigators will permit the monitors direct access to all source data, including electronic medical records, and/or documents in order to facilitate data verification. The investigators will co-operate with the monitors to ensure that any discrepancies that may be identified are resolved. The investigators are expected to be able to meet the monitors during these visits. The first on-site monitoring visit will take place shortly after randomisation of the first patient. The frequency of the on-site monitoring visits is dependent on the number of enrolled patients at the trial site.

The source data verification process and definition of key variables to be monitored will be described in the trial-specific monitoring plan.

11.2 Audit and Inspection

The investigators will make all the trial-related source data and records available at any time to quality assurance auditor(s) mandated by Ferring, or to domestic/foreign regulatory inspector(s) or representative(s) from IECs/IRBs who may audit/inspect the trial.

The main purposes of an audit or inspection are to assess compliance with the trial protocol and the principles of ICH-GCP including the Declaration of Helsinki and all other relevant regulations.

The patients must be informed by the investigators and in the informed consent documents that authorised Ferring representatives and representatives from regulatory authorities and IECs/IRBs may wish to inspect their medical records. During audits/inspections the auditors/inspectors may copy relevant parts of the medical records. No personal identification apart from the screening/ randomisation number will appear on these copies.

The investigators should notify Ferring without any delay of any inspection by a regulatory authority or IEC/IRB.

11.3 Confidentiality of Patient Data

The investigators will ensure that the confidentiality of the patients' data will be preserved. In the eCRF or any other documents submitted to Ferring, the patients will not be identified by their names, but by an identification system, which consists of an assigned number in the trial. Documents that are not for submission to Ferring, e.g. the confidential patient identification code and the signed informed consent documents, will be maintained by the investigators in strict confidence.

12 CHANGES IN THE CONDUCT OF THE TRIAL

12.1 Protocol Amendments

Any change to this protocol will be documented in a protocol amendment, issued by Ferring, and agreed upon by the TSC, investigators, and Ferring prior to its implementation. Amendments may be submitted for consideration to the approving IECs/IRBs and regulatory authorities, in accordance with local regulations. Changes to the protocol to eliminate immediate hazard(s) to trial patients may be implemented prior to IECs/IRBs approval/favourable opinion.

12.2 Deviations from the Protocol

The investigators must inform the monitor if deviations from the protocol occur and the implications of the deviation must be reviewed and discussed. Any deviation must be documented, either as an answer to a query in the eCRF, in a protocol deviation report, or a combination of both. A log of protocol deviation reports will be maintained by Ferring. Protocol deviation reports and supporting documentation must be kept in the investigator’s file and the trial master file.

12.3 Premature Trial Termination

Both the investigators (with regard to his/her participation) and Ferring reserve the right to terminate the trial at any time. Should this become necessary, the procedures will be agreed upon after consultation between the two parties. In terminating the trial, Ferring and the investigators will ensure that adequate consideration is given to the protection of the best interests of the patients. Regulatory authorities and IECs/IRBs will be informed.

In addition, Ferring reserves the right to terminate the participation of individual trial sites. Conditions that may warrant termination include, but are not limited to, insufficient adherence to protocol requirements and failure to enter patients at an acceptable rate.

13 REPORTING AND PUBLICATION

13.1 Clinical Trial Report

The data and information collected during this trial will be reported in a clinical trial report prepared by Ferring and submitted for comments and signature to the signatory investigators.

13.2 Confidentiality and Ownership of Trial Data

Any confidential information relating to the IMP or the trial, including any data and results from the trial will be the exclusive property of Ferring. The investigators and any other persons involved in the trial will protect the confidentiality of this proprietary information belonging to Ferring.

13.3 Publications and Public Disclosure

13.3.1 Publication Policy

Sponsor recognises and accepts that investigators may have a meaningful right to publish research results of the trial. Investigators must agree that the first publication of trial results is to be a joint publication covering all trial sites, and that subsequent publications will reference that primary publication. At the end of the trial, one or more manuscripts (including manuscripts, presentation, abstracts, posters etc.) for joint publication may be prepared in collaboration between the investigators and the TSC and Ferring, and the criteria for such publication shall be coordinated through the TSC. As the trial is a multi-centre trial, all publications shall be joint publications covering all trial sites unless specific written permission is obtained in advance from the TSC. However, if a joint manuscript has not been submitted for publication within 18 months of completion or termination of the trial, the investigators shall be free to publish separately. Any publication of results must acknowledge all trial sites.

Under the coordination of the TSC, authorship is granted based on the International Committee of Medicinal Journal Editors (ICMJE) criteria (see current official version: http/www.ICMJE.org).

Any external contract research organisation or laboratory involved in the conduct of this trial has no publication rights regarding this trial.

Any publication, whether joint or independent, on the results of the trial must be submitted in writing to the TSC and Ferring for comment prior to submission at least 60 days in advance of the submission of such proposed publication to the applicable journal or other forum in which the publication or presentation may be published or presented. At Ferring’s request, the respective institution and/or investigator shall arrange for an additional delay in publication or presentation, not to exceed an additional 60 days, to enable Ferring to request deletion of Ferring Confidential Information and to arrange for filing of patent applications or other intellectual property protection. This statement does not give Ferring editorial rights over the content of a publication, other than to restrict the disclosure of Ferring’s intellectual property. If the matter considered for publication is deemed patentable by Ferring, scientific publication will not be allowed until after a filed patent application is published. Under such conditions the publication will be modified or delayed to allow sufficient time for Ferring to seek patent protection of the invention.

13.3.2 Public Disclosure Policy

ICMJE member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public, clinical trials registry. Thus, it is the responsibility of Ferring to register the trial in an appropriate public registry, i.e. www.ClinicalTrials.gov which is a website maintained by the National Library of Medicine at the U.S. National Institutes of Health.

14 ETHICAL AND REGULATORY ASPECTS

14.1 Independent Ethics Committees or Institutional Review Boards

Independent ethics committees/institutional review boards will review the protocol and any amendments. The IECs/IRBs will review the patient information sheet and the informed consent form, their updates (if any), and any written materials given to the patients. A list of all IECs/IRBs to which the protocol has been submitted will be included in the clinical trial report.

14.2 Regulatory Authorities Authorisation / Approval / Notification

The regulatory permission to perform the trial will be obtained in accordance with applicable regulatory requirements. All ethical and regulatory approvals must be available before a patient is exposed to any trial-related procedure, including screening tests for eligibility.

14.3 End-of-Trial and End-of-Trial Notification

End of-trial is defined as the date of the last trial-related contact with the last patient ongoing in the trial. At the end-of-trial, Ferring shall notify the regulatory authorities and the IECs/IRBs in the participating countries about the completion of the clinical trial in accordance with national/local regulations.

14.4 Ethical Conduct of the Trial

This trial will be conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki (World Medical Association, 2013), in compliance with the approved protocol, ICH-GCP, and applicable regulatory requirements.

14.5 Patient Information and Consent

Critically ill patients receiving care in the EDs/ICUs represent a highly vulnerable population with regard to informed consent as these patients are often not capable of participating in the consent process. Because of this, proxies are often required to provide consent and other health decisions for impaired patients.

The informed consent process in this trial will be obtained in accordance with national/local regulations.

The investigator (or the person delegated by the investigator) will obtain a freely given written consent from each patient or his/her legally acceptable representative after an appropriate explanation of the aims, methods, anticipated benefits, potential hazards, and any other aspects of the trial which are relevant to the patient’s decision to participate. The patient or the legal representative should be given ample time to consider participation in the trial, before the consent is obtained. The patient (and the legal representative, if applicable) will receive a copy of the patient information and the signed informed consent form.

The investigator (or the person delegated by the investigator) will explain that it is completely free to refuse to enter the trial or to withdraw from trial at any time, without any consequences for the patient's further care and without the need to justify the decision.

The investigator (or the person delegated by the investigator) will inform that the monitor(s) and quality assurance auditor(s) mandated by Ferring, IRB/IEC representatives, or regulatory authority inspector(s), in accordance with applicable regulatory requirements, may review the patient's source records and data. Data protection will be handled in compliance with the national/local regulations.

If new information becomes available that may be relevant to the willingness to continue participation in the trial, a new patient information and informed consent form will be forwarded to the IECs/IRBs and the regulatory authorities, if required. The trial patients (and the legal representatives, if applicable) will be informed about this new information and re-consent will be obtained.

14.6 Patient Information Card

If required by local regulations, the patient will be provided with a patient information card bearing required trial-related information.

Each patient's primary care physician will be notified of their participation in the trial by the investigator, if the patient agrees.

14.7 Compliance Reference Documents

The Helsinki Declaration, the consolidated ICH-GCP, the European Union Clinical Trials Directive, 21 CFR Part 312, and other national laws in the countries where the trial takes place shall constitute the main reference guidelines for ethical and regulatory conduct.

15 LIABILITIES AND INSURANCE

15.1 ICH-GCP Responsibilities

The responsibilities of Ferring, the monitors, and the investigators are defined in the ICH-GCP consolidated guideline, and applicable regulatory requirements in the country where the trial takes place. The investigators are responsible for adhering to the ICH-GCP responsibilities of investigators, for dispensing the IMP in accordance with the approved protocol or an approved amendment, and for its secure storage and safe handling throughout the trial.

15.2 Liabilities and Insurance

In case of any damage or injury occurring to a patient in association with the IMP or the participation in the trial, Ferring has contracted an insurance which covers the liability of Ferring, the investigators, and other persons involved in the trial in compliance with the laws in the countries involved.

16 ARCHIVING

16.1 Investigator File

The investigator is responsible for maintaining all the records, which enable the conduct of the trial at the site to be fully understood, in compliance with ICH-GCP. The trial documentation including all the relevant correspondence should be kept by the investigator for at least 15 years (or longer if so required by local law) after the completion or discontinuation of the trial, if no further instructions are given by Ferring.

The investigator is responsible for the completion and maintenance of the confidential patient identification code which provides the sole link between named patient source records and anonymous eCRF data for Ferring. The investigator must arrange for the retention of this patient identification log and signed informed consent documents for at least 15 years (or longer if so required by local law) after the completion or discontinuation of the trial.

No trial site document may be destroyed without prior written agreement between the investigator and Ferring. Should the investigator elect to assign the trial documents to another party, or move them to another location, Ferring must be notified. If the investigator retires and the documents can no longer be archived by the site, Ferring can arrange having the investigator file archived at an external archive.

16.2 Trial Master File

Ferring will archive the trial master file in accordance with ICH-GCP and applicable regulatory requirements.

17 REFERENCES

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CLINICAL TRIAL PROTOCOL AMENDMENT

A Double-blind, Randomised, Placebo-controlled, Phase 2b/3 Adaptive Clinical Trial Investigating the Efficacy and Safety of Selepressin as Treatment for Patients with Vasopressor-dependent Septic Shock

Trial Code: 000133

SEPSIS-ACT
Selepressin Evaluation Programme for Sepsis-Induced Shock–Adaptive Clinical Trial

EudraCT Number:	2014-003973-41
IND Number:	77246
Investigational Medicinal Products:	Selepressin; concentrate for solution for infusion Placebo; sterile 0.9% sodium chloride solution
Indication:	Vasopressor-dependent septic shock
Phase:	2b/3
Name and Address of Sponsor:	Ferring Pharmaceuticals A/S Clinical Research and Development Kay Fiskers Plads 11 2300 Copenhagen S, Denmark Telephone number: +45 88 33 88 34
Amendment Number:	02
Type of Amendment:	Substantial
Sites where Effective:	All sites
Date of Original Protocol:	24 Mar 2015

TABLE OF CONTENTS

AMENDMENT TYPE:

This is a substantial amendment applicable to all sites.

SUMMARY OF AMENDMENT:

The main reasons for this protocol amendment are to implement the following **changes to the protocol**:

1. To allow use of infusion pumps for administration of the IMP.
2. To allow for calcium (free or total), creatinine (plasma or serum), and troponin (I or T) measurements according to local clinical practice. As uric acid is not routinely measured in clinical practice and organ function is sufficiently covered by the other variables, uric acid will no longer be collected.
3. To introduce the recording of the highest lactate level obtained in accordance with local clinical practice in the pre-IMP treatment period following start of vasopressor treatment and to clarify that venous lactate can be recorded if arterial lactate has not been measured.

This protocol amendment also includes the following **clarifications to the statistical analyses**:

4. To clarify that if terlipressin is used, even though it is a prohibited medication, it will be counted as a vasopressor when analysing the primary endpoint.
5. To clarify that PaO_2/FiO_2 will be analysed in a subset of patients.
6. To clarify how the adverse events will be analysed.
7. To allow for imputing the 30-day vasopressor- and mechanical ventilation free-day status for patients withdrawn from the trial by the same method as for patients lost to follow up.
8. To have the analysis of other vasopressors divided into several components and to add imputation rules for norepinephrine/noradrenaline and other vasopressors.
9. To clarify how the fluid balance will be analysed.

Futhermore, the following **clarifications to the protocol** are included:

10. To clarify the timing and need for obtaining blood samples for analysis of selepressin concentration in plasma.
11. To further clarify when the investigational medicinal product (IMP) infusion may be re-started during the 30-day IMP treatment period.
12. To clarify that the pre-IMP treatment period includes the time from sepsis-induced hypotension to the start of IMP infusion.
13. To clarify that IMP, other vasopressors, and positive inotropes will be included in the calculation of the cardiovascular component in the Sequential Organ Failure Assessment (SOFA).
14. To clarify the baseline time for functional status, residence, and health-related quality of life. To clarify how to document the residence for a patient who have been discharged from trial hospital but readmitted at a given time-point.

15. To clarify how adverse events will be recorded.
16. To further clarify that the informed consent process will be conducted in accordance with national/local regulations.
17. To clarify that the trial will be publicly available at the EU Clinical Trials Register and other registries in accordance with local regulatory requirements.
18. Various editorial changes.

IMPLICATIONS OF AMENDMENT:

The administration guide for IMP and vasopressors and the master informed consent documents are affected by this amendment and have been updated accordingly. If deemed necessary, the local informed consent documents should be updated in accordance with local regulations. Reconsent is not required.

AMENDED TEXT:

New wording is shown in underlined italics; superseded wording is marked with strike-through.

CHANGES TO THE PROTOCOL:

1 Investigational Medicinal Product

1.1 Amended Section

1.1.1 Section 5.1.1 Investigational Medicinal Product (IMP)

Superseded Prior Wording

The IMP (selepressin and placebo) will be administered through a central venous catheter as a continuous intravenous infusion using a syringe pump. The administration is discussed in Section 3.5.5. A detailed IMP and vasopressor administration guide will be provided to the trial sites.

New Protocol Wording

The IMP (selepressin and placebo) will be administered through a central venous catheter as a continuous intravenous infusion *at a controlled flow rate* using a syringe pump *or an infusion pump suited for vasopressor administration*. The administration is discussed in Section 3.5.5. A detailed IMP and vasopressor administration guide will be provided to the trial sites.

1.2 Reason for Change

To allow use of infusion pumps for administration of the IMP as some sites do not use syringe pumps for adults in their clinical practice.

2 Creatinine and other Safety Laboratory Variables

2.1 Amended Sections

2.1.1 Section 4.2.2 Randomisation (2nd paragraph)

Superseded Prior Wording

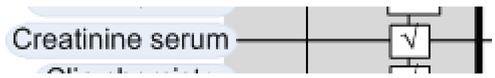
To minimise the risk of imbalance between the treatment arms, the randomisation will be stratified by: 1) trial site, 2) need for mechanical ventilation (‘Yes’ or ‘No’), 3) norepinephrine/noradrenaline requirement (< or ≥30 µg/min), and 4) serum creatinine (< or ≥150 µmol/L).

New Protocol Wording

To minimise the risk of imbalance between the treatment arms, the randomisation will be stratified by: 1) trial site, 2) need for mechanical ventilation (‘Yes’ or ‘No’), 3) norepinephrine/noradrenaline requirement (< or ≥30 µg/min), and 4) *plasma/*serum creatinine (< or ≥150 µmol/L).

2.1.2 Section 6.1 Trial Flow Chart

Superseded Prior Wording



New Protocol Wording

2.1.3 Section 7.1.3 Renal Replacement Therapy (RRT) and Renal Function (2nd paragraph)

Superseded Prior Wording

Renal function will be assessed, using urine output, serum creatinine, and creatinine clearance, at baseline and the first ~~7~~ days after initiation of IMP infusion [or until ICU discharge if the patient leaves the ICU before Day 7]). Creatinine clearance will be determined by estimated glomerular filtration rate (using serum creatinine, age, and gender as per Cockcroft-Gault equation).

New Protocol Wording

Renal function will be assessed, using urine output, *plasma/*serum creatinine, and creatinine clearance, at baseline and the first *3* days after initiation of IMP infusion *and up to 7 days if collected for clinical purposes* [or until ICU discharge if the patient leaves the ICU before Day 7]). Creatinine clearance will be determined by estimated glomerular filtration rate (using *plasma/*serum creatinine, age, and gender as per Cockcroft-Gault equation).

2.1.4 Section 7.1.9 Safety Laboratory Variables (Clinical Chemistry, Haematology, and Coagulation) (2nd and 3rd paragraph and Table 2)

Superseded Prior Wording

The baseline levels and the results, of the variables as listed in Table 2, obtained 1 and 3 days after initiation of the IMP infusion will be recorded. The results obtained at Days 7, 14, 30 will also be recorded if the patient is still hospital~~ised~~.

Daily creatinine, bilirubin, and platelets values will be used for the SOFA scores (see Section 7.1.4). ~~Serum c~~reatinine level will also be used for assessing renal function and calculation of creatinine clearance (see Section 7.1.3).

Table 2 Safety Laboratory Variables

Clinical Chemistry	Haematology	Coagulation
Alanine aminotransferase	Haematocrit	Activated partial thromboplastin time
Albumin	Haemoglobin	
Alkaline phosphatase	Platelet count	Prothrombin time / international normalised ratio
Aspartate aminotransferase	Red blood cell count	
Calcium	White blood cell count	
Chloride		
C-reactive protein		
Creatine phosphokinase		
Creatinine (serum)		
Lactate dehydrogenase		
Phosphate		
Potassium		
Sodium		
Total bilirubin		
Troponin		
Urea (blood urea nitrogen)		
~~Uric acid~~		

New Protocol Wording

The baseline levels and the results, of the variables as listed in Table 2, obtained 1 and 3 days after initiation of the IMP infusion will be recorded. The results obtained at Days 7, 14, 30 will also be recorded if the patient is still *in trial* hospital.

Daily creatinine, bilirubin, and platelets values will be used for the SOFA scores (see Section 7.1.4). *C*reatinine level will also be used for assessing renal function and calculation of creatinine clearance (see Section 7.1.3).

Table 2 Safety Laboratory Variables

Clinical Chemistry	Haematology	Coagulation
Alanine aminotransferase	Haematocrit	Activated partial thromboplastin time
Albumin	Haemoglobin	
Alkaline phosphatase	Platelet count	Prothrombin time / international normalised ratio
Aspartate aminotransferase	Red blood cell count	
Calcium *(free or total)*	White blood cell count	
Chloride		
C-reactive protein		
Creatine phosphokinase		
Creatinine (*plasma or* serum)		
Lactate dehydrogenase		
Phosphate		
Potassium		
Sodium		
Total bilirubin		
Troponin *(I or T)*		
Urea (blood urea nitrogen *or blood urea*)		

2.2 Reason for Change

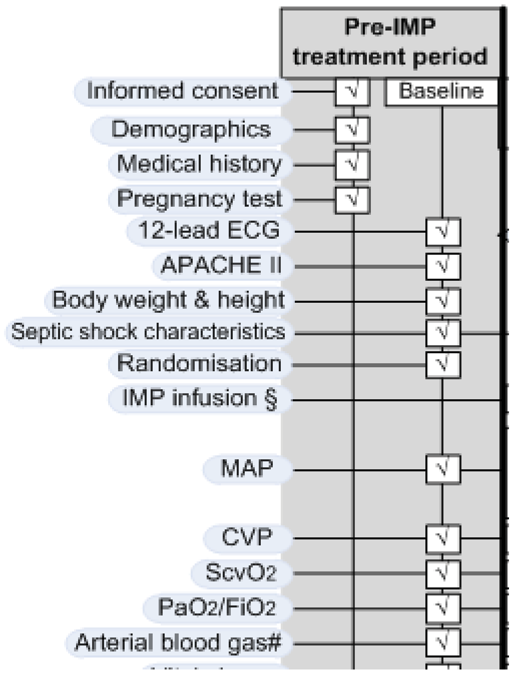
To allow for calcium (free or total), creatinine (plasma or serum), and troponin (I or T) measurements according to local clinical practice. As uric acid is not routinely measured in clinical practice and organ function is sufficiently covered by the other variables, uric acid will no longer be collected.

3 Arterial Blood Gases and Lactate Levels

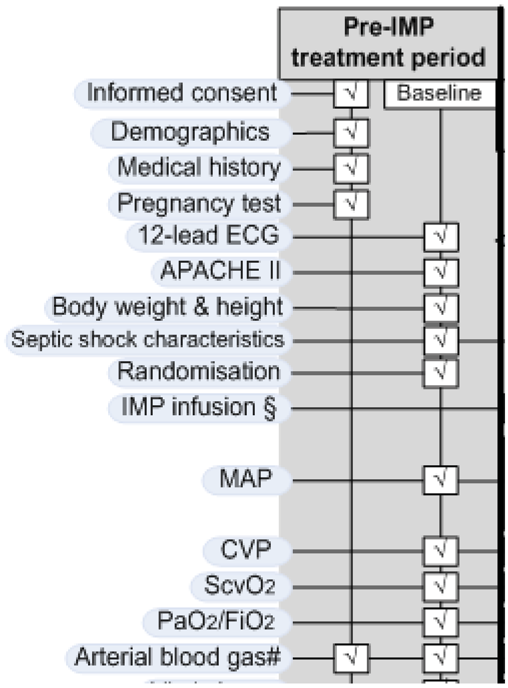
3.1 Amended Sections

3.1.1 Section 6.1 Trial Flow Chart

Superseded Prior Wording



New Protocol Wording



3.1.2 Section 7.3.3 Arterial Blood Gases and Lactate Levels

Superseded Prior Wording

If arterial blood gases and acid/base status (arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$), arterial oxygen saturation (SaO_2), arterial pH, bicarbonate (HCO_3), base excess) and lactate levels are available, as measured using standard equipment in accordance with local clinical practice, the baseline -values and the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and a daily (around 8:00 am) result the following days until Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) will be recorded.

PaO_2 will be used for the SOFA (see Section 7.1.4) and the PaO_2/FiO_2 ratio (see Section 7.1.12).

New Protocol Wording

If arterial blood gases and acid/base status (arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$), arterial oxygen saturation (SaO_2), arterial pH, bicarbonate (HCO_3), base excess) and lactate levels are available, as measured using standard equipment in accordance with local clinical practice, the baseline values and the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and a daily (around 8:00 am) result the following days until Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) will be recorded. *In addition, the highest lactate level obtained after start of vasopressor treatment but before start of IMP will be recorded. If arterial lactate level has not been measured, the venous value can be recorded if available.*

PaO_2 will be used for the SOFA (see Section 7.1.4) and the PaO_2/FiO_2 ratio (see Section 7.1.12).

3.2 Reason for Change

To introduce the recording of the highest lactate level obtained in accordance with local clinical practice in the pre-IMP treatment period following start of vasopressor treatment and to clarify that venous lactate can be recorded if arterial lactate has not been measured.

CLARIFICATIONS TO THE STATISTICAL ANALYSES:

4 Terlipressin is Counted as a Vasopressor

4.1 Amended Sections

4.1.1 Synopsis - Primary Endpoint (1st bullet point, 3rd paragraph) and Section 2.2 Endpoints, Primary Endpoint (1st bullet point, 3rd paragraph)

Superseded Prior Wording

Vasopressor use is defined as any intravenous dose of norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, and IMP (i.e. selepressin and placebo).

New Protocol Wording

Vasopressor use is defined as any intravenous dose of norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, *terlipressin,* and IMP (i.e. selepressin and placebo).

4.1.2 Section 7.1.1 Vasopressors (2nd paragraph, 1st sentence)

Superseded Prior Wording

All vasopressor treatment (any intravenous dose of norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, and IMP [selepressin and placebo]) must be thoroughly documented up to Day 30.

New Protocol Wording

All vasopressor treatment (any intravenous dose of norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, *terlipressin,* and IMP [selepressin and placebo]) must be thoroughly documented up to Day 30.

4.1.3 Section 9.6.2 Primary Endpoint (5th paragraph)

Superseded Prior Wording

Norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, and IMP (i.e. selepressin and placebo) all constitute a vasopressor for the purpose of the primary analysis.

New Protocol Wording

Norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, *terlipressin,* and IMP (i.e. selepressin and placebo) all constitute a vasopressor for the purpose of the primary analysis.

4.2 Reason for Change

To clarify that if terlipressin is used, even though it is a prohibited medication, it will be counted as a vasopressor when analysing the primary endpoint.

5 PaO_2/FiO_2

5.1 Amended Sections

5.1.1 Synopsis - Additional Endpoints (9th bullet point) and Section 2.2 Endpoints, Additional Endpoints (9th bullet point)

Superseded Prior Wording

- Ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2 ratio)

New Protocol Wording

- Ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2 ratio) *(in a subset of 100-350 patients)*

5.1.2 Section 9.6.4 Other Efficacy Endpoints (8th bullet point)

Superseded Prior Wording

- PaO_2/FiO_2 ratio

New Protocol Wording

- PaO_2/FiO_2 ratio *(in a subset of patients)*

5.2 Reason for Change

To clarify that the PaO_2/FiO_2 ratio will be analysed in a subset of patients.

6 Adverse Events Analyses

6.1 Amended Sections

6.1.1 Synopsis - Safety Endpoints (1th bullet point) and Section 2.2 Safety Endpoints (1th bullet point)

Superseded Prior Wording

Safety endpoints

- Incidence of adverse events (type, frequency, and intensity) with specific emphasis on:
  - Ischaemic events (e.g. myocardial, skin, cerebral, mesenteric, and limb ischaemia)
  - ~~Changes in vital signs assessed as unanticipated in the setting of septic shock~~
  - ~~Changes in safety laboratory variables assessed as unanticipated in the setting of septic shock~~
- Changes in vital signs and safety laboratory variables
- Episodes of hypotension (mean arterial pressure <60 mmHg for longer than one hour)

New Protocol Wording

Safety endpoints

- Incidence of adverse events (type, frequency, and intensity) with specific emphasis on:
  - Ischaemic events (e.g. myocardial, skin, cerebral, mesenteric, and limb ischaemia)
- Changes in vital signs and safety laboratory variables*, including:*
  - *Number of clinically significant results assessed as unanticipated in the setting of septic shock*
- Episodes of hypotension (mean arterial pressure <60 mmHg for longer than one hour)

6.1.2 Section 9.8.2 Adverse Events (6th – 9th paragraph)

Superseded Prior Wording

An overview of ~~treatment-emergent adverse events~~ will be ~~provided in a summary table~~ including the number of patients reporting an adverse event, the percentage of patients with a ~~treatment-emergent~~ adverse event, and the number of events reported, for the following categories:

- Adverse events
- Deaths
- Serious adverse events
- Adverse events leading to discontinuation of IMP
- ~~Adverse events leading to withdrawal from the trial~~
- Severe and life-threatening adverse events
- Adverse drug reactions

~~Treatment-emergent a~~dverse events will be summarised in a table by SOC (sorted alphabetically) and PT (sorted in decreasing frequency of occurrence) using MedDRA. The table will display the total number of patients reporting an adverse event, the percentage of patients with an adverse event, and the number of events reported.

~~S~~ummary tables will be prepared for:

- All adverse events
- Adverse events with an incidence ≥5% of patients in any treatment arm
- Non-serious adverse events with an incidence ≥5% of patients in any treatment arm
- Critical adverse events, details are provided in the statistical analysis plan
- ~~Adverse events based on changes in vital signs assessed as unanticipated in the setting of septic shock~~
- ~~Adverse events based on changes in safety laboratory variables assessed as unanticipated in the setting of septic shock~~
- Adverse events by causality (related/unrelated)

- Adverse events leading to death
- Adverse events by intensity
- Serious adverse events
- Adverse events leading to discontinuation of IMP (related/unrelated)
- ~~Adverse events leading to withdrawal from trial (related/unrelated)~~

Supporting data listings will be provided for:

- All adverse events sorted by trial site and patient number
- All adverse events sorted by MedDRA PT
- Serious adverse events
- Adverse events leading to death
- Adverse events leading to discontinuation of IMP (related/unrelated)
- ~~Adverse events leading to withdrawal from trial (related/unrelated)~~
- Post-treatment adverse events

New Protocol Wording

A*dverse event* overview *summary tables* will be *prepared for treatment-emergent AEs and all AEs (treatment-emergent and non treatment-emergent)* including the number of patients reporting a*n* adverse event, the percentage of patients with a*n* adverse event, and the number of events reported, for the following categories:

- Adverse events
- Deaths
- Serious adverse events
- Adverse events leading to discontinuation of IMP
- Severe and life-threatening adverse events
- Adverse drug reactions

*A*dverse events will be summarised in a table by SOC (sorted alphabetically) and PT (sorted in decreasing frequency of occurrence) using MedDRA. The table will display the total number of patients reporting an adverse event, the percentage of patients with an adverse event, and the number of events reported.

*For both treatment-emergent AEs and all AEs (treatment-emergent and non treatment-emergent) during the treatment period, s*ummary tables will be prepared for:

- All adverse events
- Adverse events with an incidence ≥5% of patients in any treatment arm
- Non-serious adverse events with an incidence ≥5% of patients in any treatment arm
- Critical adverse events, details are provided in the statistical analysis plan

- Adverse events by causality (related/unrelated)
- Adverse events leading to death
- Adverse events by intensity
- Serious adverse events
- Adverse events leading to discontinuation of IMP (related/unrelated)

Supporting data listings will be provided for:

- All adverse events sorted by trial site and patient number
- All adverse events sorted by MedDRA PT
- Serious adverse events
- Adverse events leading to death
- Adverse events leading to discontinuation of IMP (related/unrelated)
- Post-treatment adverse events

6.1.3 Section 9.8.3 Safety Laboratory Variables (3rd paragraph)

Superseded Prior Wording

Mean change and mean percentage change from baseline at end of treatment period will be presented for each laboratory variable. In addition, descriptive statistics, i.e. the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each laboratory variable.

New Protocol Wording

Mean change and mean percentage change from baseline at end of treatment period will be presented for each laboratory variable. In addition, descriptive statistics, i.e. the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each laboratory variable.

Also, summary tables will be presented, displaying by time and laboratory parameter, the number of patients with a clinically significant result which is unanticipated in the setting of septic shock.

6.1.4 Section 9.8.4 Vital Signs and Central Venous Pressure (2nd paragraph)

Superseded Prior Wording

Mean change and mean percentage change from baseline at end of treatment period will be presented for each variable. In addition, descriptive statistics, i.e. the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each variable.

New Protocol Wording

Mean change and mean percentage change from baseline at end of treatment period will be presented for each variable. In addition, descriptive statistics, i.e. the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each variable.

Also, summary tables will be presented, displaying by time and vital signs parameter, the number of patients with a clinically significant result which is unanticipated in the setting of septic shock.

6.2 Reason for Change

To clarify how adverse events will be recorded and analysed. There will be no tables on adverse events leading to withdrawal as there is no option to withdraw a patient from trial due to adverse events.

7 Primary Endpoint Imputation

7.1 Amended Sections

7.1.1 Section 9.2 Patient Disposition (1st and 2nd paragraph)

Superseded Prior Wording

A summary table will present, for each part of the trial and overall, the number of patients in the population sets: 'Screened', 'Intention to treat', Full analysis set', 'Per protocol', 'Safety', 'Completed trial', 'Withdrawals~~/~~IMP discontinuations~~ (whichever comes first)~~' with a breakdown of reasons/categories for and trial withdrawals~~/~~IMP discontinuations.

The patient disposition table will be broken down by each of the stratification variables [the need for mechanical ventilation (Yes/No), norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and serum creatinine (< or ≥150 µmol/L)] and broken down chronologically displaying number of patients 'completed' and 'withdrawn from trial~~/discontinued from IMP~~' at Day 30, Day 90, and Day 180.

New Protocol Wording

A summary table will present, for each part of the trial and overall, the number of patients in the population sets: 'Screened', 'Intention to treat', *'*Full analysis set', 'Per protocol', 'Safety', 'Completed trial', 'Withdrawals*', and '*IMP discontinuations' with a breakdown of reasons/categories for and trial withdrawals *and* IMP discontinuations.

The patient disposition table will be broken down by each of the stratification variables [the need for mechanical ventilation (Yes/No), norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and *plasma/*serum creatinine (< or ≥150 µmol/L)] and broken down chronologically displaying number of patients 'completed' and 'withdrawn from trial at Day 30, Day 90, and Day 180.

7.1.2 Section 9.6.2 Primary Endpoint (10th and 11th paragraph)

Superseded Prior Wording

Missing data during the time of hospitalisation will be imputed using a worst case approach taking into account previous and subsequent starting and stopping times of vasopressor administration and mechanical ventilation. If only the stop date but not time is given, the imputed time will be midnight of that date, unless a subsequent starting time was recorded prior to midnight in which case the imputed time would be the start time of the subsequent record. If neither stop date nor time is given, the imputed stop time will be the start date and time of the subsequent recording. Likewise, missing start dates and times would be imputed as worst case scenarios, i.e. is the patient found to be on mechanical ventilation with a date but no time for intubation, the imputed start time would be recorded as 00:01 of that day or the stop date of a preceding recording on that same date, whichever occurs last. If both start date and time is missing, the imputed start time would be the date and time of the preceding stop time recorded. In case of data being completely missing from a certain time point and onwards ~~(i.e. patient lost to follow up)~~, the "last status carried forward" imputation will be applied. If a patient was last seen on either ventilator or vasopressors, it is assumed that the patient remained so, and is imputed to a value of 0 (zero) P&VFDs. If the patient was last seen off ventilator and vasopressors, it is also assumed that the patient remained so in the remaining 30-day period. If the patient was last seen (alive) on e.g. day 10 and at that point had been off both ventilator and vasopressors for three days, a value of 23 P&VFDs is imputed.

The primary endpoint, P&VFDs, will be analysed using a van Elteren test, stratified by need for ventilation (Yes/No), time from onset of shock (onset of vasopressor ~~(i.e. norepinephrine/noradrenaline)~~) to start of IMP treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min).

New Protocol Wording

Missing data during the time of hospitalisation will be imputed using a worst case approach taking into account previous and subsequent starting and stopping times of vasopressor administration and mechanical ventilation. If only the stop date but not time is given, the imputed time will be midnight of that date, unless a subsequent starting time was recorded prior to midnight in which case the imputed time would be the start time of the subsequent record. If neither stop date nor time is given, the imputed stop time will be the start date and time of the subsequent recording. Likewise, missing start dates and times would be imputed as worst case scenarios, i.e. is the patient found to be on mechanical ventilation with a date but no time for intubation, the imputed start time would be recorded as 00:01 of that day or the stop date of a preceding recording on that same date, whichever occurs last. If both start date and time is missing, the imputed start time would be the date and time of the preceding stop time recorded. In case of data being completely missing from a certain time point and onwards, the "last status carried forward" imputation will be applied. If a patient was last seen on either ventilator or vasopressors, it is assumed that the patient remained so, and is imputed to a value of 0 (zero) P&VFDs. If the patient was last seen off ventilator and vasopressors, it is also assumed that the patient remained so in the remaining 30-day period. If the patient was last seen (alive) on e.g. day 10 and at that point had been off both ventilator and vasopressors for three days, a value of 23 P&VFDs is imputed.

The primary endpoint, P&VFDs, will be analysed using a van Elteren test, stratified by need for ventilation (Yes/No), time from onset of shock (onset of *any* vasopressor) to start of IMP treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min).

7.1.3 Section 9.6.2.1 Sensitivity Analyses of the Primary Endpoint (4th, 5th, and 6th paragraph)

Superseded Prior Wording

The impact and robustness of the imputation of missing data will be checked by analysing data in the following ways

- excluding all patients with missing/imputed data
- imputing the 30-day P&VFD status for patients lost to follow up using the observed ratio of P&VFDs at time of lost to follow up, to the same proportion for a 30-day status

For this analysis the 30-day P&VFD status for patients lost to follow up will be imputed so that the 30-day ratio of P&VFDs is equal to the ratio of P&VFDs at time of lost to follow up. E.g. a patient being lost to follow up at Day 15 with 4 P&VFDs (a ratio of 4/15 P&VFDs per days observed) will be imputed to 8 P&VFDs at Day 30 (equivalent ratio 8/30 = 4/15). Patients having zero P&VFDs at time of lost to follow up will be imputed to a value of zero P&VFDs.

- tipping point analysis

The tipping point analysis will compare all possible combinations of 'best case' and 'worst case' scenarios between placebo and selepressin. …

New Protocol Wording

The impact and robustness of the imputation of missing data will be checked by analysing data in the following ways

- excluding all patients with missing/imputed data
- imputing the 30-day P&VFD status for patients lost to follow up *or otherwise withdrawn from trial* using the observed ratio of P&VFDs at time of lost to follow up *or time of withdrawal*, to the same proportion for a 30-day status

For this analysis the 30-day P&VFD status for patients lost to follow up *or otherwise withdrawn from trial* will be imputed so that the 30-day ratio of P&VFDs is equal to the ratio of P&VFDs at time of lost to follow up *or time of withdrawal*. E.g. a patient being lost to follow up at Day 15 with 4 P&VFDs (a ratio of 4/15 P&VFDs per days observed) will be imputed to 8 P&VFDs at Day 30 (equivalent ratio 8/30 = 4/15). Patients having zero P&VFDs at time of lost to follow up will be imputed to a value of zero P&VFDs.

- tipping point analysis

The tipping point analysis will compare all possible combinations of 'best case' and 'worst case' scenarios between placebo and selepressin *for patients lost to follow up or otherwise withdrawn from trial*. …

7.2 Reason for Change

To allow for imputing the 30-day vasopressor- and mechanical ventilation free-day status for patients withdrawn from the trial by the same method as for patients lost to follow up.

The definition of onset of shock has been expanded to include any vasopressors.

8 Norepinephrine/noradrenaline and Other Vasopressor Doses

8.1.1 Section 9.6.4 Other Efficacy Endpoints (3rd bullet point)

Superseded Prior Wording

- Norepinephrine/noradrenaline and other vasopressor doses

The dose of norepinephrine/noradrenaline administered (adjusted for baseline weight) will be compared between treatment arms using a repeated measures ANCOVA model with baseline dose of norepinephrine/noradrenaline as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented. ~~There will be no imputations of missing values.~~

The mean dose administered will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

The ~~dose of other vasopressors is defined as the cumulative dose at any given time of epinephrine/adrenaline, dopamine, phenylephrine and vasopressin.~~

~~The dose of other vasopressors will be will be analysed as for doses of norepinephrine/ noradrenaline with baseline dose of other vasopressors as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit.~~

New Protocol Wording

- Norepinephrine/noradrenaline and other vasopressor doses

The dose of norepinephrine/noradrenaline administered (adjusted for baseline weight) will be compared between treatment arms using a repeated measures ANCOVA model with baseline dose of norepinephrine/noradrenaline as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented. *If a patient has missing values and the patient is still in the trial (not dead or withdrawn) it will be assumed that the specific vasopressor was not given and a value of zero will be imputed, unless there is an interval in the timing log covering the exact time point (8 AM and 8 PM is the assumed time point for missing morning and evening collection time points). In that case LOCF will be used, but only within the time interval*.

The mean dose administered will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

The *same analysis (adjusted for baseline dose of norepinephrine/noradrenaline) will be performed for the following endpoints:*

- *Catecholamines (defined as the sum of doses of norepinephrine/noradrenaline, epinephrine/adrenaline, dopamine, and phenylephrine)*
- *Catecholamines excluding norepinephrine/noradrenaline*
- *Vasopressin*

For the sum of catecholamine doses we define 100 µg dopamine, 1 µg epinephrine, and 2.2 µg phenylephrine all equivalent to 1 µg norepinephrine.

Also, the number of patients receiving terlipressin will be summarised.

8.2 Reason for Change

To have the analysis of other vasopressors divided into several components and to add imputation rules for norepinephrine/noradrenaline and other vasopressors.

9 Fluid Balance

9.1 Amended Section

9.1.1 Section 9.6.3 Secondary Endpoints, Daily and cumulative fluid balance

Superseded Prior Wording

Daily and cumulative fluid balance (for 7 days or until ICU discharge). Fluid overload is defined as fluid balance as a percentage of baseline weight (e.g. if a patient weighs 90 kg at baseline and has a fluid balance of 9 litres, fluid overload is then 100% * 9 litres / 90 kg = 10%.

Fluid balance and cumulative fluid balance will be presented both unadjusted and adjusted for weight.

Daily and cumulative fluid balance as well as daily and cumulative fluid overload will all be compared between treatment arms using a repeated measures ANCOVA model with baseline (baseline fluid balance or baseline fluid overload) as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

The absolute values and change from baseline will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

New Protocol Wording

Daily and cumulative fluid balance (for 7 days or until ICU discharge). Fluid overload is defined as fluid balance as a percentage of baseline weight (e.g. if a patient weighs 90 kg at baseline and has a fluid balance of 9 litres, fluid overload is then 100% * 9 litres / 90 kg = 10%.

Fluid balance and cumulative fluid balance will be presented both unadjusted and adjusted for weight.

Daily and cumulative fluid balance as well as daily and cumulative fluid overload will all be compared between treatment arms using a repeated measures ANCOVA model with baseline (baseline fluid balance or baseline fluid overload) as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

The absolute values and change from baseline will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

All analyses will be presented for 'all patients' and for 'patients in ICU throughout Day 0-7'.

9.2 Reason for Change

To clarify how the fluid balance will be analysed.

CLARIFICATIONS TO THE PROTOCOL:

10 Blood Sampling for Analysis of Selepressin Concentration in Plasma

10.1 Amended Sections

10.1.1 Synopsis - Additional Endpoints (7th bullet point) and Section 2.2 Endpoints, Additional Endpoints (7th bullet point)

Superseded Prior Wording

- Pharmacokinetic response (in a subset of approximately 200 patients) - to be reported separately according to a pre-specified pharmacokinetic analysis plan ~~(including plasma level of selepressin at the first attempt to wean the IMP infusion)~~

New Protocol Wording

- Pharmacokinetic response (in a subset of approximately 200 patients) - to be reported separately according to a pre-specified pharmacokinetic analysis plan

10.1.2 Section 4.4 Discontinuation and Withdrawal, Premature discontinuation of IMP infusion (1st paragraph)

Superseded Prior Wording

The IMP infusion will continue as long as blood pressure support is deemed necessary (up to a maximum of 30 days). Premature discontinuation of IMP infusion is defined as termination of IMP infusion even though continued intravenous vasopressor treatment for blood pressure support is needed. A premature discontinuation of IMP infusion may occur if the investigator decides that IMP infusion should be discontinued or if the patient, the patient's legal representative, or attending physician requests that IMP infusion be discontinued. If the IMP infusion is prematurely discontinued due to a serious adverse event, an additional blood sample will be collected (see Section 7.2.2).

New Protocol Wording

The IMP infusion will continue as long as blood pressure support is deemed necessary (up to a maximum of 30 days). Premature discontinuation of IMP infusion is defined as termination of IMP infusion even though continued intravenous vasopressor treatment for blood pressure support is needed. A premature discontinuation of IMP infusion may occur if the investigator decides that IMP infusion should be discontinued or if the patient, the patient's legal representative, or attending physician requests that IMP infusion be discontinued. If the IMP infusion is prematurely *paused or* discontinued due to a serious adverse event, an additional blood sample will be collected (see Section 7.2.2).

10.1.3 Section 6.1 Trial Flow Chart

Superseded Prior Wording

2 PK samples during IMP initiation*;	1 PK sample before IMP weaning;	2 PK samples during IMP weaning*;	before and after RRT*; ¥

…

¥ A blood sample will be collected if the IMP infusion is prematurely stopped due to a serious adverse event

New Protocol Wording

2 PK samples during IMP initiation*;	1 PK sample at max plasma conc;	2 PK samples during IMP weaning*;	before and after RRT*; ¥

…

¥ A blood sample will be collected if the IMP infusion is prematurely paused or discontinued due to a serious adverse event

10.1.4 Section 7.2.2 Pharmacokinetics

Superseded Prior Wording

~~A~~ blood sample for measurement of plasma concentration of selepressin will be collected from all patients right before first attempt to wean the IMP infusion.

The IMP infusion rate at the time of the blood sampling will be recorded in the eCRF.

In approximately 200 patients at pre-selected trial sites, additional blood sampling for analysis of pharmacokinetic parameters will be conducted twice during the initiation of the IMP infusion (at approximately 1-3 hours and 6-9 hours after start of infusion) and twice during the weaning of the IMP infusion (at approximately 1-2 hours and 2-3 hours after stop of infusion). If these patients are on RRT, additional blood sampling will also be performed before and after the RRT. The IMP infusion rate at 30 minutes after start of IMP infusion will be recorded in the eCRF.

~~In addition, if the IMP infusion is prematurely discontinued due to a serious adverse event, a blood sample will be collected at the time of IMP discontinuation for measurement of plasma concentration of selepressin~~.

New Protocol Wording

One blood sample for measurement of *maximal* plasma concentration of selepressin will be collected from all patients *at one of the following time points (the one that occurs first): 1)* right before first attempt to wean the IMP infusion*, 2) after at least 7 hours of IMP infusion at maximal infusion rate, 3) at time of first pause of IMP, or 4) at time of IMP discontinuation*.

Moreover, if the IMP infusion is prematurely paused or discontinued due to a serious adverse event, an additional blood sample will be collected at the time of IMP discontinuation or pause for measurement of plasma concentration of selepressin.

The IMP infusion rate at the time of the blood sampling will be recorded in the eCRF.

In approximately 200 patients at pre-selected trial sites, additional blood sampling for analysis of pharmacokinetic parameters will be conducted twice during the initiation of the IMP infusion (at approximately 1-3 hours and 6-9 hours after start of infusion) and twice during the weaning of the IMP infusion (at approximately 1-2 hours and 2-3 hours after stop of infusion). If these patients are on RRT, additional blood sampling will also be performed before and after the RRT. The IMP infusion rate at 30 minutes after start of IMP infusion will be recorded in the eCRF.

10.2 Reason for Change

To allow flexibility in the timing of blood sampling for measurement of maximal plasma concentration of selepressin and to clarify the need for obtaining an additional blood sample if the IMP infusion is prematurely paused or discontinued due to a serious adverse event.

11 Re-start of IMP

11.1 Amended Sections

11.1.1 Synopsis - Methodology (7th paragraph) and Section 3.5.5 Selection and Timing of Dose for Each Patient (4th paragraph)

Superseded Prior Wording

The IMP infusion will continue as long as blood pressure support is deemed necessary with a maximum period of 30 days. After complete weaning, IMP infusion may be re-started during this 30-day~~-~~period~~ if necessary~~. Patients who still need vasopressor treatment after 30 days will be switched to other vasopressors at the discretion of the investigator.

New Protocol Wording

The IMP infusion will continue as long as blood pressure support is deemed necessary with a maximum period of 30 days. After complete weaning, IMP infusion may be re-started during this 30-day period *for treatment of sepsis-induced hypotension if there is no suspicion of mesenteric or cardiac ischaemia*. Patients who still need vasopressor treatment after 30 days will be switched to other vasopressors at the discretion of the investigator.

11.1.2 Synopsis – Duration of treatment

Superseded Prior Wording

All patients will receive IMP (selepressin or placebo) as an intravenous infusion until recovery from the need of vasopressor treatment or for 30 days, whichever comes first. If the need of vasopressor treatment subsides and recurs within the 30-day treatment period, IMP should be used when possible.

New Protocol Wording

All patients will receive IMP (selepressin or placebo) as an intravenous infusion until recovery from the need of vasopressor treatment or for 30 days, whichever comes first. If the need of vasopressor treatment subsides and recurs *due to sepsis-induced hypotension* within the 30-day treatment period, IMP should be used when possible *if there is no suspicion of mesenteric or cardiac ischaemia*.

11.2 Reason for Change

To further clarify when the IMP infusion may be re-started during the 30-day IMP treatment period.

12 Pre-IMP Treatment Period

12.1 Amended Section

12.1.1 Section 6 Trial Procedures (3rd paragraph)

Superseded Prior Wording

The pre-IMP treatment period is defined as the time from ~~onset of vasopressor treatment for septic shock~~ to the start of IMP infusion. To ensure start of IMP treatment without delay, informed consent will be obtained, in compliance with local regulations, as early as possible. During this period, baseline evaluations will be made.

New Protocol Wording

The pre-IMP treatment period is defined as the time from *sepsis-induced hypotension* to the start of IMP infusion. To ensure start of IMP treatment without delay, informed consent will be obtained, in compliance with local regulations, as early as possible. During this period, baseline evaluations will be made.

12.2 Reason for Change

To clarify that the pre-IMP treatment period includes the time from sepsis-induced hypotension to the start of IMP infusion.

13 Sequential Organ Failure Assessment (SOFA)

13.1 Amended Section

13.1.1 Section 7.1.4 Modified Sequential Organ Failure Assessment (SOFA) Score (2nd paragraph)

Superseded Prior Wording

In this trial, a modified version of the SOFA will be used (i.e. SOFA except the Glasgow Coma Scale). As many patients are sedated due to mechanical ventilation a meaningful assessment of the neurological function using the Glasgow Coma Scale cannot be performed.

New Protocol Wording

In this trial, a modified version of the SOFA will be used (i.e. SOFA except the Glasgow Coma Scale). As many patients are sedated due to mechanical ventilation a meaningful assessment of the neurological function using the Glasgow Coma Scale cannot be performed. *In addition, any dose of IMP, vasopressin, terlipressin, or phenylephrine will attribute 3 points on the cardiovascular scale, and any dose of the positive inotropes milrinone and levosimendan will attribute 2 points on the cardiovascular scale.*

13.2 Reason for Change

For the cardiovascular component in the SOFA score, severity is largely based on the need for inotropes and/or vasopressors to stabilise blood pressure. However, the score only defines catecholamines as vasopressors whereby patients treated with non-catecholamine vasopressors will be defined as less ill. The lack of other vasopressors, like vasopressin and phenylephrine, is a methodological weakness of the current score (Evans and Fink. Springer 2002; Mechanisms of Organ Dysfunction in Critical Illness; Vincent and Lopes Ferreira. Multiple Organ Failure: Clinical Syndrome p 394-403). To account for this, any dose of IMP, vasopressin, terlipressin, or phenylephrine will attribute 3 points on the cardiovascular scale, and the positive inotropes milrinone and levosimendan will attribute 2 points on the cardiovascular scale. A similar approach was used for vasopressin in the CHEST trial, Myburgh et al. NEJM 2012;367:1901-1911, and as in the VANISH trial, Gordon et al. to be published.

14 Functional Status, Residence, and Health-related Quality of Life

14.1 Amended Sections

14.1.1 Section 7.1.5 Mortality Rate and Hospitalistion (2nd paragraph)

Superseded Prior Wording

Functional status and residence of each patient before this episode of septic shock (baseline) as well as functional/survival status and residence throughout the trial will be recorded by using one of the following alternatives:

- Home, receiving no support
- Home, receiving paid professional support
- Home, receiving unpaid support
- Rehabilitation site/skilled (or unskilled) nursing facility
- Other acute care hospital (including long-term acute care)
- Still in trial hospital
- Unknown
- Dead

New Protocol Wording

Functional status and residence of each patient before *the infection leading to* this episode of septic shock (baseline) as well as functional/survival status and residence throughout the trial will be recorded by using one of the following alternatives:

- Home, receiving no support
- Home, receiving paid professional support
- Home, receiving unpaid support
- Rehabilitation site/skilled (or unskilled) nursing facility
- Other acute care hospital (including long-term acute care)
- Still in *(or readmitted to)* trial hospital
- Unknown
- Dead

14.1.2 Section 7.1.6 Health-related Quality of Life (3rd paragraph)

Superseded Prior Wording

The baseline value refers to a recall of the time before the septic shock episode and the EQ-5D-5L will be completed as soon as possible after the shock state. A telephone interview will be adequate at Days 30, 60, 90, and 180 if the patient has been discharged. The responses will be entered into the eCRF.

New Protocol Wording

The baseline value refers to a recall of the time before the <u>*infection leading to the*</u> septic shock episode and the EQ-5D-5L will be completed as soon as possible after the shock state. A telephone interview will be adequate at Days 30, 60, 90, and 180 if the patient has been discharged. The responses will be entered into the eCRF.

14.2 Reason for Change

To clarify the baseline time for functional status, residence, and health-related quality of life. Furthermore, to clarify how to document the residence for a patient who have been discharged from trial hospital but readmitted at a given time-point.

15 Adverse Events Recording

15.1 Amended Sections

15.1.1 Section 8.2.2 Recording of Adverse Events, Action Taken to IMP and Other Action Taken

Superseded Prior Wording

Action Taken to IMP

The action taken to the IMP in response to an adverse event must be classified as one of the following:

- No change (medication schedule maintained or no action taken).
- ~~Withdrawn~~ (medication schedule modified through termination of prescribed regimen of medication).
- ~~Interrupted~~ (medication schedule was modified by temporarily terminating a prescribed regimen of medication).

Other Action Taken

Adverse events requiring therapy must be treated with recognised standards of medical care to protect the health and well-being of the patient. Appropriate resuscitation equipment and medicines must be available to ensure the best possible treatment of an emergency situation.

If medication is administered to treat the adverse event, ~~this~~ medication will be entered in the ~~concomitant medication log~~ provided in each patient's eCRF.

New Protocol Wording

Action Taken to IMP

The action taken to the IMP in response to an adverse event must be classified as one of the following:

- No change (medication schedule maintained or no action taken).
- *Discontinued* (medication schedule modified through *permanent* termination of prescribed regimen of medication).
- *Paused* (medication schedule was modified by temporarily terminating a prescribed regimen of medication).

Other Action Taken

Adverse events requiring therapy must be treated with recognised standards of medical care to protect the health and well-being of the patient. Appropriate resuscitation equipment and medicines must be available to ensure the best possible treatment of an emergency situation.

If medication is administered to treat the adverse event, *relevant* medication will be entered in the *medication forms and the serious adverse event form* provided in each patient's eCRF.

15.1.2 Section 8.5.2 Collection, Recording and Reporting of Serious Adverse Events (4th paragraph)

Superseded Prior Wording

Completion of the demographics, adverse event ~~log~~, medical history ~~log~~, and concomitant medication ~~log~~ are mandatory for initial reports and for follow-up reports if any relevant changes have been made since the initial report. Data entries must have been made in the eCRF for Ferring Global Pharmacovigilance to access the information.

New Protocol Wording

Completion of the demographics, adverse event*s*, medical history, and concomitant medication are mandatory for initial reports and for follow-up reports if any relevant changes have been made since the initial report. Data entries must have been made in the eCRF for Ferring Global Pharmacovigilance to access the information.

15.2 Reason for Change

To clarify how adverse events will be recorded.

16 Informed Consent Process

16.1 Amended Sections

16.1.1 Section 9.8.2 Adverse Events (2nd paragraph)

Superseded Prior Wording

A pre-treatment adverse event is any adverse event occurring after ~~signed~~ informed consent and before administration of the IMP.

New Protocol Wording

A pre-treatment adverse event is any adverse event occurring after informed consent and before administration of the IMP.

16.1.2 Section 14.5 Patient Information and Consent (3rd paragraph)

Superseded Prior Wording

The investigator (or the person delegated by the investigator) will obtain a freely given written consent from each patient or his/her legally acceptable representative after an appropriate explanation of the aims, methods, anticipated benefits, potential hazards, and any other aspects of the trial which are relevant to the patient's decision to participate. The patient or the legal representative should be given ample time to consider participation in the trial, before the consent is obtained. The patient (and the legal representative, if applicable) will receive a copy of the patient information and the signed informed consent form.

New Protocol Wording

The investigator (or the person delegated by the investigator) will obtain a freely given written consent *in accordance with national/local regulations* from each patient or his/her legally acceptable representative after an appropriate explanation of the aims, methods, anticipated benefits, potential hazards, and any other aspects of the trial which are relevant to the patient's decision to participate. The patient or the legal representative should be given ample time to consider participation in the trial, before the consent is obtained. The patient (and the legal representative, if applicable) will receive a copy of the patient information and the signed informed consent form.

16.2 Reason for Change

To further clarify that the informed consent process will be conducted in accordance with national/local regulations.

17 Public Disclosure

17.1 Amended Section

17.1.1 Section 13.3.2 Public Disclosure Policy

Superseded Prior Wording

ICMJE member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public, clinical trials registry. Thus, it is the responsibility of Ferring to register the trial in an appropriate public registry, i.e. www.ClinicalTrials.gov which is a website maintained by the National Library of Medicine at the U.S. National Institutes of Health.

New Protocol Wording

ICMJE member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public, clinical trials registry. Thus, it is the responsibility of Ferring to register the trial in an appropriate public registry, i.e. www.ClinicalTrials.gov which is a website maintained by the National Library of Medicine at the U.S. National Institutes of Health. *The trial will also be made publicly available at the EU Clinical Trials Register at www.clinicaltrialsregister.eu. Trial registration may occur in other registries in accordance with local regulatory requirements. A summary of the trial results is made publicly available in accordance with applicable regulatory requirements.*

17.2 Reason for Change

To clarify that the trial will be publicly available at the EU Clinical Trials Register and other registries in accordance with local regulatory requirements. The master informed consent has been updated accordingly.

18 Various Editorial Changes

18.1 Amended Sections

18.1.1 Front page

Superseded Prior Wording

~~SEPSIS-ACT~~

~~Selepressin Evaluation Programme for Sepsis-Induced Shock – Adaptive Clinical Trial~~

New Protocol Wording

SEPSIS-ACT

Selepressin Evaluation Programme for Sepsis-Induced Shock–Adaptive Clinical Trial

18.1.2 Section 4.1.3 – Eligibility Criteria – Post-randomisation / Before Start of IMP Infusion

Superseded Prior Wording

In addition, the following criteria must be met at start of IMP infusion:

1. Received a minimum of 30 mL/kg fluid in total from the onset of hypotension (or less if evidence of fluid replete/overload).
2. Received a continuous infusion of norepinephrine/noradrenaline base greater than 5 µg/min for at least one hour and is still receiving at least 5 µg/min norepinephrine/noradrenaline base.
3. Less than 12 hours since onset of vasopressor treatment for septic shock.

   The requirement of at least one hour duration of vasopressor support is intended to ensure a certain severity of the septic shock while balancing the need to recruit patients early during the initial hours of resuscitation.

New Protocol Wording

In addition, the following criteria must be met at start of IMP infusion:

1. Received a minimum of 30 mL/kg fluid in total from the onset of hypotension (or less if evidence of fluid replete/overload).

2. Received a continuous infusion of norepinephrine/noradrenaline base greater than 5 µg/min for at least one hour and is still receiving at least 5 µg/min norepinephrine/noradrenaline base.

   The requirement of at least one hour duration of vasopressor support is intended to ensure a certain severity of the septic shock while balancing the need to recruit patients early during the initial hours of resuscitation.

3. Less than 12 hours since onset of vasopressor treatment for septic shock.

18.1.3 Section 7.1.1 Vasopressors (1st paragraph, 1st sentence)

Superseded Prior Wording

The integrity of the primary endpoint depends on accurate start and stop times of vasopressor treatment and therefore start -and stop time of all vasopressor periods must be recorded in the eCRF.

New Protocol Wording

The integrity of the primary endpoint depends on accurate start and stop times of vasopressor treatment and therefore start and stop time of all vasopressor periods must be recorded in the eCRF.

18.1.4 Section 7.1.2 Mechanical Ventilation (2nd paragraph)

Superseded Prior Wording

The use of mechanical ventilation (as defined in Section 2.2) must be thoroughly documented up to Day 30. Start and stop time of all mechanical ventilation periodsmust be recorded in the eCRF.

New Protocol Wording

The use of mechanical ventilation (as defined in Section 2.2) must be thoroughly documented up to Day 30. Start and stop time of all mechanical ventilation periods_must be recorded in the eCRF.

18.1.5 Section 9.6.3 Secondary Endpoints, Incidence of RRT up to Day 30 (2nd paragraph)

Superseded Prior Wording

Incidence of RRT will be analysed by a logistic regression model with time from onset of shock to start of treatment, baseline creatinine and norepinephrine/noradrenaline requirement at baseline, as covariates, and treatment, and need for ventilation as factors. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method. Patients already on RRT at time of inclusion will be excluded from the analysis of incidence of RRT.

New Protocol Wording

Incidence of RRT will be analysed by a logistic regression model with time from onset of shock to start of treatment, baseline creatinine and norepinephrine/noradrenaline requirement at baseline *(µg/kg/min)*, as covariates, and treatment, and need for ventilation as factors. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method. Patients already on RRT at time of inclusion will be excluded from the analysis of incidence of RRT.

18.1.6 Section 9.6.3 Secondary Endpoints, Duration of septic shock up to Day 30 (2nd paragraph)

Superseded Prior Wording

Duration of septic shock will be analysed separately for survivors, non-survivors (within the first 30 days) and overall, comparing treatment arms by an analysis of covariance (ANCOVA) model with time from onset of shock to start of treatment and norepinephrine/noradrenaline requirement at baseline as covariates, and treatment and need for ventilation (Yes/No) as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented.

New Protocol Wording

Duration of septic shock will be analysed separately for survivors, non-survivors (within the first 30 days) and overall, comparing treatment arms by an analysis of covariance (ANCOVA) model with time from onset of shock to start of treatment and norepinephrine/noradrenaline requirement at baseline *(µg/kg/min)* as covariates, and treatment and need for ventilation (Yes/No) as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented.

18.1.7 Section 9.6.3 Secondary Endpoints, Incidence of new organ dysfunction and new organ failure (3rd and 5th paragraph)

Superseded Prior Wording

Incidence of at least one new organ failure will be analysed for any new organ failure (across all organ systems) and by individual organ systems, and compared between treatment arms using a logistic regression model with age, modified SOFA score, and norepinephrine/noradrenaline requirement at baseline as covariates and gender and treatment arm as factors, presenting odds ratios with 95% confidence intervals.

…

The number of new organ dysfunctions and new organ failures will be compared between treatment arms using a negative binominal model with age, modified SOFA score, and norepinephrine/ noradrenaline requirement at baseline as covariates, and gender and treatment as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method.

New Protocol Wording

Incidence of at least one new organ failure will be analysed for any new organ failure (across all organ systems) and by individual organ systems, and compared between treatment arms using a logistic regression model with age, modified SOFA score, and norepinephrine/noradrenaline requirement at baseline *(µg/kg/min)* as covariates and gender and treatment arm as factors, presenting odds ratios with 95% confidence intervals.

…

The number of new organ dysfunctions and new organ failures will be compared between treatment arms using a negative binominal model with age, modified SOFA score, and norepinephrine/noradrenaline requirement at baseline *(µg/kg/min)* as covariates, and gender and treatment as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method.

18.1.8 Section 9.6.4 Other Efficacy Endpoints (12th bullet point)

Superseded Prior Wording

- Creatinine clearance

Creatinine clearance will be analysed as for fluid balance with baseline creatinine as a covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit.

New Protocol Wording

- Creatinine clearance

Creatinine clearance will be analysed as for fluid balance with baseline creatinine *clearance* as a covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit.

18.2 Reason for Change

Various editorial changes.

CLINICAL TRIAL PROTOCOL

A Double-blind, Randomised, Placebo-controlled, Phase 2b/3 Adaptive Clinical Trial Investigating the Efficacy and Safety of Selepressin as Treatment for Patients with Vasopressor-dependent Septic Shock

Trial Code: 000133

SEPSIS-ACT
Selepressin Evaluation Programme for Sepsis-Induced Shock–Adaptive Clinical Trial

Note: This is a consolidated protocol including all changes stated in:

- ***Protocol Amendment 01 (implemented prior to trial start)***
- ***Protocol Amendment 02***

EudraCT Number:	2014-003973-41
IND Number:	77246
Investigational Medicinal Products:	Selepressin; concentrate for solution for infusion Placebo; sterile 0.9% sodium chloride solution
Indication:	Vasopressor-dependent septic shock
Phase:	2b/3
Name and Address of Sponsor:	Ferring Pharmaceuticals A/S Clinical Research and Development Kay Fiskers Plads 11 2300 Copenhagen S, Denmark Telephone number: +45 88 33 88 34
GCP Statement:	This trial will be performed in compliance with GCP

SYNOPSIS

TITLE OF TRIAL A Double-blind, Randomised, Placebo-controlled, Phase 2b/3 Adaptive Clinical Trial Investigating the Efficacy and Safety of Selepressin as Treatment for Patients with Vasopressor-dependent Septic Shock SEPSIS-ACT / Selepressin Evaluation Programme for Sepsis-Induced Shock - Adaptive Clinical Trial	
SIGNATORY INVESTIGATORS [redacted] Department of Critical Care Medicine, University of Pittsburgh, United States of America. [redacted] Medical-surgical Intensive Care Unit, Saint Luc University Hospital at the Université Catholique de Louvain, Brussels, Belgium.	
TRIAL SITES The trial will be conducted predominately across Europe and North America at approximately 60-100 trial sites in total.	
PLANNED TRIAL PERIOD First visit for the first patient is planned for Q3 2015. Last visit for the last patient is expected in Q4 2018.	**CLINICAL PHASE** 2b/3

OBJECTIVES

Primary objective

- To demonstrate superiority of selepressin plus standard care versus placebo plus standard care in the number of vasopressor- and mechanical ventilator-free days (with penalty for mortality) in patients with vasopressor-dependent septic shock

Secondary objectives

- To determine the efficacy of selepressin on:
  - Organ dysfunction
  - Morbidity and mortality
  - Fluid balance
  - Health-related quality of life
- To determine the safety profile of selepressin
- To determine the pharmacokinetics of selepressin
- To determine the health economics of selepressin
- To further evaluate a range of biomarkers in relation to the mode of action of selepressin

ENDPOINTS

Primary endpoint

- Vasopressor- and mechanical ventilator-free days (P&VFDs) up to Day 30

  This composite endpoint is defined as the number of days (reported to one decimal place [0.0 to 30.0 days]) from start of treatment with the investigational medicinal product (IMP) [selepressin or placebo] to 30.0 days thereafter during which the patient is: 1) alive; 2) free of treatment with intravenous vasopressors; and 3) free of any invasive mechanical ventilation (see definition below).

  Any patient that dies within this 30-day period is assigned zero P&VFDs, even if there is a period during which the patient is free of both vasopressor treatment and mechanical ventilation. If vasopressors need to be restarted or mechanical ventilation needs to be initiated or restarted, and the use of either is greater than 60 minutes within a 24-hour period, then the clock is reset and the patient is not considered free of vasopressors and/or mechanical ventilation until after those therapies are again finally discontinued. Vasopressor use or mechanical ventilation during - and up to three hours after - surgery / procedure (including bedside) is exempt from this rule (i.e. does not reset the calculation of P&VFDs). The intent is for the endpoint to reflect the speed of recovery from septic shock and respiratory failure, with appropriate penalties for recurrent shock, new or recurrent respiratory failure, and death.

  Vasopressor use is defined as any intravenous dose of norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, terlipressin, and IMP (i.e. selepressin and placebo).

  Mechanical ventilation is defined as use of endotracheal or tracheostomy tube assisted ventilation (>5 cm H_2O continuous positive airway pressure and >5 cm H_2O of pressure support from the ventilator in tracheostomy patients). End of mechanical ventilation is defined as: 1) extubation of intubated patients or 2) ≤5 cm H_2O continuous positive airway pressure and ≤5 cm H_2O of pressure support from the ventilator in tracheostomy patients. If non-invasive ventilation by mask or bag (>5 cm H_2O of pressure support) is deployed to avoid (re)intubation, it also counts as mechanical ventilation. However, all other uses of non-invasive ventilation such as chronic night-time use of positive airway pressure for chronic obstructive pulmonary disease (COPD) or sleep apnea does not count as mechanical ventilation (regardless of pressure).

Key secondary endpoints

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Day 90
- Renal replacement therapy (RRT)-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)
- Intensive care unit (ICU)-free days up to Day 30

Secondary efficacy endpoints

Organ dysfunction

- Vasopressor-free days up to Day 30
- Mechanical ventilator-free days up to Day 30
- Duration of septic shock (i.e. vasopressor use) up to Day 30
- Duration of mechanical ventilation up to Day 30
- Incidence of RRT up to Day 30 (counting patients who die as on RRT and excluding patients on RRT for chronic renal failure at time of randomisation)
- Duration of RRT up to Day 90 (excluding patients on RRT for chronic renal failure at time of randomisation)
- Daily overall and individual organ (cardiovascular, respiratory, renal, hepatic, coagulation) scores using a modified version of the Sequential Organ Failure Assessment (SOFA) until ICU discharge
- Incidence of new organ dysfunction and new organ failure (based on the SOFA score) up to Days 7 and 30

Morbidity and mortality

- ICU length of stay up to Day 30
- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Days 30 and 180

Fluid balance

- Daily and cumulative fluid balance until ICU discharge (for a maximum of 7 days)
- Daily and cumulative urine output until ICU discharge (for a maximum of 7 days)

Health-related quality of life

- Change in utility, based on the EuroQol group's 5-dimension 5-level (EQ-5D-5L) questionnaire, up to Day 180

Safety endpoints

- Incidence of adverse events (type, frequency, and intensity) with specific emphasis on:
    - Ischaemic events (e.g. myocardial, skin, cerebral, mesenteric, and limb ischaemia)
- Changes in vital signs and safety laboratory variables, including:
    - Number of clinically significant results assessed as unanticipated in the setting of septic shock
- Episodes of hypotension (mean arterial pressure <60 mmHg for longer than one hour)

Additional endpoints

- Hospital-free days up to Day 90
- Hospital length of stay up to Day 90

- Patient residence at Day 30, Day 60, Day 90, and Day 180
- Health economic evaluation - to be reported separately according to a pre-specified health economic analytical plan
- Mean arterial pressure (MAP), until ICU discharge (for a maximum of 7 days)
- Norepinephrine/noradrenaline and other vasopressor doses
- Pharmacokinetic response (in a subset of approximately 200 patients) - to be reported separately according to a pre-specified pharmacokinetic analysis plan
- Creatinine clearance
- Ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2 ratio) (in a subset of 100-350 patients)
- Extravascular lung water and pulmonary permeability index (in a subset of 100-350 patients)
- Cardiac output (in a subset of 100-350 patients)
- Cytokines (in a subset of 100-350 patients)
- Angiopoietin-1 and -2 (in a subset of 100-350 patients)

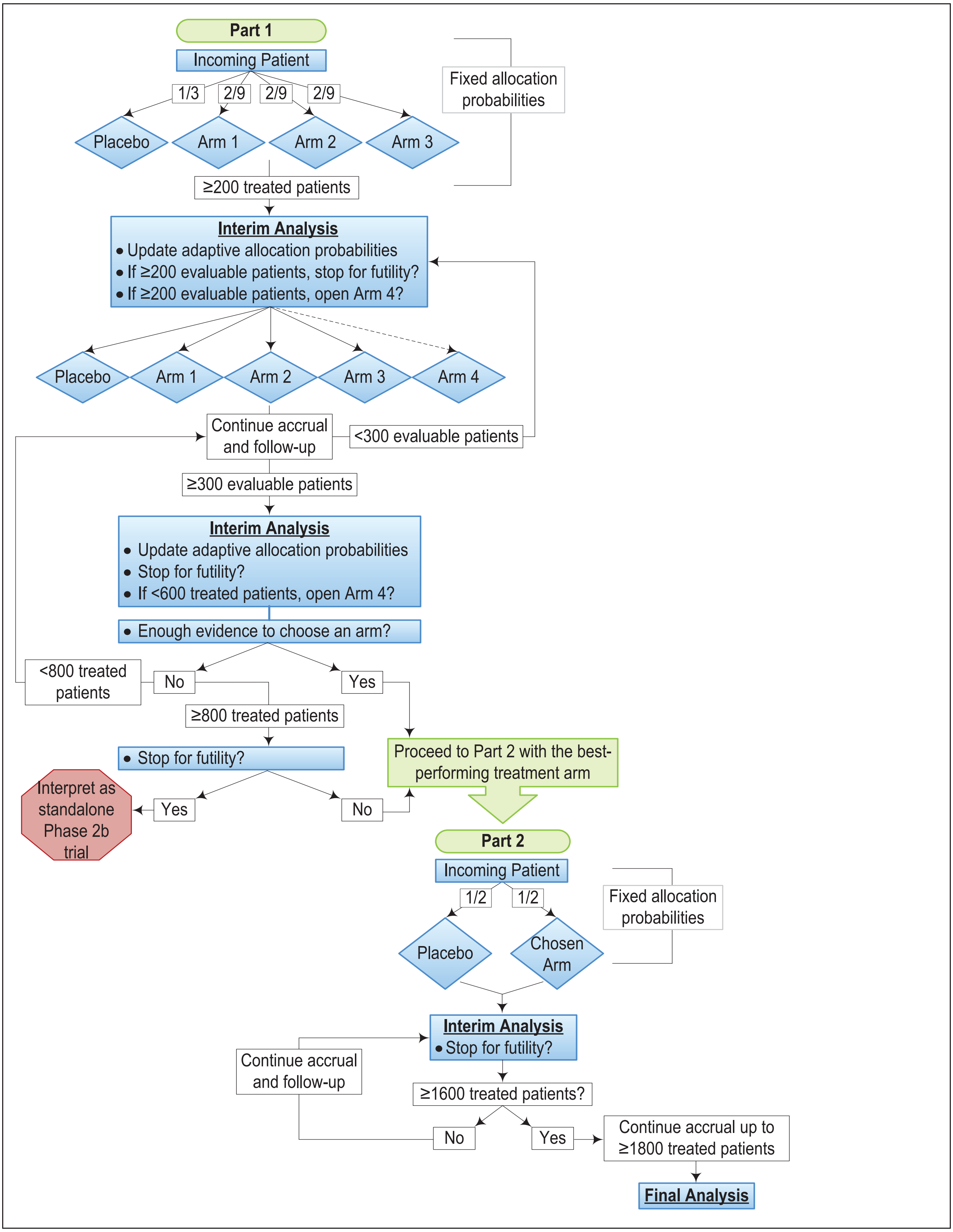
METHODOLOGY

This is a double-blind, randomised, placebo-controlled, two-part adaptive clinical trial. The trial is designed to investigate the efficacy and safety of multiple dosing regimens of selepressin and to confirm the efficacy and safety of one dosing regimen in treatment of adult patients with septic shock requiring vasopressor.

Up to four dosing regimens of selepressin will be investigated:

Treatment Arm	Starting Dose (ng/kg/min)	Maximum Dose (ng/kg/min)	Range (ng/kg/min)
Arm 1	1.7	2.5	0-2.5
Arm 2	2.5	3.75	0-3.75
Arm 3	3.5	5.25	0-5.25
Arm 4	5.0	7.5	0-7.5

The overall trial design includes two parts (Part 1 – Phase 2b and Part 2 – Phase 3) as illustrated on the next page.


Part 1
Incoming Patient
1/3
2/9
2/9
2/9
Fixed allocation probabilities
Placebo
Arm 1
Arm 2
Arm 3
≥200 treated patients
Interim Analysis
• Update adaptive allocation probabilities
• If ≥200 evaluable patients, stop for futility?
• If ≥200 evaluable patients, open Arm 4?
Placebo
Arm 1
Arm 2
Arm 3
Arm 4
Continue accrual and follow-up
<300 evaluable patients
≥300 evaluable patients
Interim Analysis
• Update adaptive allocation probabilities
• Stop for futility?
• If <600 treated patients, open Arm 4?
• Enough evidence to choose an arm?
<800 treated patients
No
Yes
≥800 treated patients
• Stop for futility?
Proceed to Part 2 with the best-performing treatment arm
Interpret as standalone Phase 2b trial
Yes
No
Part 2
Incoming Patient
1/2
1/2
Fixed allocation probabilities
Placebo
Chosen Arm
Interim Analysis
• Stop for futility?
Continue accrual and follow-up
≥1600 treated patients?
No
Yes
Continue accrual up to ≥1800 treated patients
Final Analysis

Part 1 is a dose-finding stage with response-adaptive randomisation to preferentially allocate patients to the dosing regimens that appear to have the maximum benefit with respect to the primary endpoint. Interim analyses will be conducted regularly in Part 1 to optimise the efficiency of selecting the optimal dosing regimen and to allow early termination of the trial for futility. If Part 1 results in a decision to run the second part of the trial, Part 2 will be a 1:1 randomised comparison of the best-performing dosing regimen of selepressin selected in Part 1 versus placebo on top of standard care. All patients will be on norepinephrine/noradrenaline treatment as part of standard of care at the time when the IMP (selepressin or placebo) infusion is initiated.

Before administration, selepressin will be diluted to one of four specific concentrations to allow for blinded administration using similar infusion rates. The infusion of IMP (selepressin or placebo) will start as early as possible and no later than 12 hours after initiation of continuous infusion of vasopressor treatment for septic shock. To ensure start of IMP treatment without delay, informed consent will be obtained, in compliance with local regulations, as early as possible.

During the course of treatment, the IMP infusion rate will be adjusted within pre-defined infusion rates to keep the MAP at the target of 65 mmHg. A different MAP target is acceptable, if it is pre-specified and if it is appropriate, as judged by the investigator, for the clinical management e.g. previous history of hypotension or hypertension (if deemed necessary to maintain adequate organ perfusion). A detailed IMP and vasopressor administration guide will be provided to trial sites. If the administration of IMP in addition to norepinephrine/noradrenaline increases MAP to above the target, norepinephrine/noradrenaline will be weaned first while aiming to keep MAP at the target. Norepinephrine/noradrenaline must be completely weaned prior to weaning of IMP. If norepinephrine/noradrenaline cannot be completely weaned, the infusion rate of IMP is to be increased up to the maximum allowed infusion rate in an attempt to use IMP as the sole vasopressor. If infusion of IMP alone increases the MAP to above the target, the IMP will be weaned step-wise while aiming to keep MAP at the target. If the maximum allowed infusion rate of the IMP is not sufficient to maintain MAP at the target, norepinephrine/noradrenaline will be added to achieve the targeted MAP. If target MAP cannot be maintained despite maximum allowed infusion rate of IMP and ≥1 µg/kg/min norepinephrine/noradrenaline base (or a total catecholamine equivalent of 1 µg/kg/min norepinephrine/noradrenaline base), vasopressin may be added.

The IMP infusion will continue as long as blood pressure support is deemed necessary with a maximum period of 30 days. After complete weaning, IMP infusion may be re-started during this 30-day period for treatment of sepsis-induced hypotension if there is no suspicion of mesenteric or cardiac ischaemia. Patients who still need vasopressor treatment after 30 days will be switched to other vasopressors at the discretion of the investigator.

NUMBER OF PATIENTS

Enough patients will be enrolled in the trial to obtain 1800 evaluable patients in the entire programme (including both parts of the trial).

CRITERIA FOR INCLUSION / EXCLUSION

The intention is to enrol a typical sample of patients presenting with septic shock and commence treatment with the IMP during the initial hours of resuscitation, within 12 hours from onset of vasopressor treatment, targeting those who do not respond rapidly to fluids and whose vasopressor-need persists for at least one hour.

Inclusion criteria

1. 18 years of age or older.
2. Proven or suspected infection.
3. Septic shock defined as hypotension (systolic blood pressure less than 90 mmHg OR MAP less than 65 mmHg) requiring vasopressor treatment (i.e. any dose of norepinephrine / noradrenaline base greater than 5 µg/min) despite adequate fluid resuscitation (at least one litre for hypotension).
4. Informed consent obtained in accordance with local regulations.

Exclusion criteria

1. Not possible to initiate IMP treatment within 12 hours from onset of vasopressor treatment for septic shock.
2. Primary cause of hypotension not due to sepsis (e.g. major trauma including traumatic brain injury, haemorrhage, burns, or congestive heart failure/cardiogenic shock).
3. Previous severe sepsis with ICU admission within this hospital stay.
4. Known/suspected acute mesenteric ischaemia.
5. Suspicion of concomitant acute coronary syndrome based on clinical symptoms and/or ECG during this episode of septic shock.
6. Chronic mechanical ventilation for any reason OR severe COPD requiring either continuous daily oxygen use during the preceding 30 days or mechanical ventilation (for acute exacerbation of COPD) during the preceding 30 days.
7. Received bone marrow transplant during the preceding 6 months or chemotherapy during the preceding 30 days for lymphoma or leukemia.
8. Known to be pregnant.
9. Decision to limit full care taken before obtaining informed consent.
10. Use of vasopressin in the past 12 hours prior to start of the IMP infusion or use of terlipressin within 7 days prior to start of the IMP infusion.
11. Prior enrolment in the trial.
12. Prior use of an investigational medicinal product within the last month OR planned or concurrent participation in a clinical trial for any investigational drug or investigational device.

Eligibility criteria – post-randomisation / before start of IMP infusion
In addition, the following criteria must be met at start of IMP infusion:

1. Received a minimum of 30 mL/kg fluid in total from the onset of hypotension (or less if evidence of fluid replete/overload).
2. Received a continuous infusion of norepinephrine/noradrenaline base greater than 5 µg/min for at least one hour and is still receiving at least 5 µg/min norepinephrine/noradrenaline base.
3. Less than 12 hours since onset of vasopressor treatment for septic shock.

INVESTIGATIONAL MEDICINAL PRODUCTS

- Selepressin 0.3 mg/mL (10 mM acetate buffer, pH 4). Selepressin is provided as a stock solution which will be diluted with sterile 0.9% sodium chloride solution prior to infusion according to a specific dilution protocol.
- Placebo: sterile 0.9% sodium chloride solution.

DURATION OF TREATMENT

All patients will receive IMP (selepressin or placebo) as an intravenous infusion until recovery from the need of vasopressor treatment or for 30 days, whichever comes first. If the need of vasopressor treatment subsides and recurs due to sepsis-induced hypotension within the 30-day treatment period, IMP should be used when possible if there is no suspicion of mesenteric or cardiac ischaemia.

STATISTICAL METHODS

The trial is powered to demonstrate superiority of selepressin plus standard care versus placebo plus standard care in the number of P&VFDs in patients with vasopressor-dependent septic shock.

Primary analysis
If Part 2 of the trial is run, the primary endpoint, P&VFDs, will be analysed using a van Elteren test. The primary analysis will compare all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) to all patients on the placebo arm from both parts of the trial.

The primary analysis will be a test of superiority using a two-sided 5% significance level test. This test, within the trial, controls the type 1 error at a two-sided 5% level.

The analysis will be based on both the full analysis set (FAS) and the per protocol (PP) analysis set, with the FAS being considered the primary analysis to judge statistical significance and the PP analysis considered as supportive. The FAS comprises all randomised (as planned) patients who have received IMP treatment.

Power
The overall power for obtaining statistical significance based on combined evidence from Part 1 and Part 2 is 91% in situations where all 4 arms have a true underlying 1.5% lower mortality rate

and a 1.5-day higher expected number of P&VFDs for survivors (corresponding to an overall treatment effect of 1.5 P&VFDs) as compared to placebo. If the effect sizes are 2% on mortality and 2 days for P&VFDs in survivors for all 4 arms (corresponding to an overall treatment effect of 2 P&VFDs) then the overall power is 98%. In this latter case the probability of engaging into Part 2 is ~99%.

Secondary analyses

The secondary endpoints are aimed at supporting primary efficacy by further demonstrating treatment effect accompanied by an acceptable safety profile.

Endpoints defined as '-free days' will be defined and analysed in a similar manner as the primary endpoint. Endpoints addressing SOFA score, fluid balance, and health-related quality of life as well as all endpoints measuring duration and split in survivors/non-survivors will be analysed by analysis of covariance (ANCOVA) methods and presented graphically. Mortality, incidence of new organ dysfunction, and new organ failure will be analysed by logistic regression. Mortality will be presented graphically by a Kaplan-Meier plot. All secondary endpoints will be analysed using both the FAS and the PP analysis set.

For the purpose of a possible label inclusion, the Hochberg procedure for adjustment on multiplicity will be implemented to selected secondary endpoints. Only if the primary efficacy analysis leads to a statistically significant result at the (one-sided) 2.5% level, then the Hochberg procedure, which is described below, will be applied to selected secondary analyses. If the primary efficacy analysis does not result in statistical significance at the (one-sided) 2.5% level, then statistical significance (for the purpose of a possible label inclusion only) will not be declared for any of the secondary analyses, regardless of their p-values.

The selected key secondary endpoints aimed at further demonstrating treatment effect are:

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Day 90
- RRT-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)
- ICU-free days up to Day 30

In this application of the Hochberg procedure there are three hypothesis tests of superiority for each of the selected secondary endpoints. The target alpha level is (one-sided) 2.5%. The Hochberg procedure is as follows:

- Order the p-values from the smallest to the largest value, $p(1) < p(2) < p(3)$, with corresponding null hypothesis $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$.
- Start with the highest p-value. If $p(3) < 2.5\%$ (one-sided), then stop and declare all three comparisons significant at the 2.5% (one-sided) level (i.e. reject $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$). Otherwise, accept $H_{(3)}$ for the endpoint related to p(3), and go to p(2) the second highest p-value.
- If $p(2) < 2.5/2 = 1.25\%$ (one-sided), then stop and declare significance for $H_{(1)}$ and $H_{(2)}$.

Otherwise, accept $H_{(2)}$, for the endpoint related to p(2), and go to p(1) the lowest p-value.

- If $p(1) < 2.5/3 = 0.833\%$ (one-sided), then stop and declare significance for $H_{(1)}$. Otherwise, accept $H_{(1)}$, for the endpoint related to p(1).

Regardless of the statistical significance declared according to the Hochberg procedure, all analysis will be included and presented in the statistical report.

The safety profile, including adverse events, vital signs, and safety laboratory variables, will be summarised descriptively. The safety analyses will be performed using the safety analysis set. The safety analysis set comprises all IMP-treated patients and are analysed according to the actual treatment received.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

List of Abbreviations

ANCOVA	Analysis of Covariance
ATC	Anatomical Therapeutic Chemical (Classification System)
CCC	Clinical Coordinating Centre
CCL	CC Chemokine Ligand
COPD	Chronic Obstructive Pulmonary Disease
CSF	Colony-stimulating Factor
CVP	Central Venous Pressure
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ED	Emergency Department
EQ-5D-5L	EuroQoL group's 5-dimension 5-level Questionnaire
EudraCT	European Union Clinical Trial Database
EVLW	Extravascular Lung Water
FAS	Full Analysis Set
FiO_2	Fraction of Inspired Oxygen
FPFV	First Patient First Visit
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HCO_3	Bicarbonate
ICMJE	the International Committee of Medicinal Journal Editors
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IL	Interleukin
IMP	Investigational Medicinal Product
IND	Investigational New Drug

IRB	Institutional Review Board
ITT	Intention-to-Treat
KM	Kaplan Meier
LOCF	Last Observation Carried Forward
LPLV	Last Patient Last Visit
LTA	Lymphotoxin Alpha
MAP	Mean Arterial Pressure
MedDRA	Medical Dictionary for Regulatory Activities
P&VFD	Vasopressor- and Mechanical Ventilator-free Day
$PaCO_2$	Arterial Carbon Dioxide Partial Pressure
PaO_2	Arterial Oxygen Partial Pressure
PK	Pharmacokinetic
PP	Per Protocol
PPI	Pulmonary Permeability Index
PT	Preferred Term
QALY	Quality-adjusted Life Years
RRT	Renal Replacement Therapy
SaO_2	Arterial Oxygen Saturation
$ScvO_2$	Central Venous Oxygen Saturation
SOC	System Organ Class
SOFA	Sequential Organ Failure Assessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
TSC	Trial Steering Committee
VEGFA	Vascular Endothelial Growth Factor

Definition of Terms

Audit	A systematic and independent examination of trial-related activities and documents to determine whether the evaluated trial-related activities were conducted, and the data were recorded, analysed, and accurately reported according to the protocol, sponsor's standard operating procedures, good clinical practice, and the applicable regulatory requirement(s).
Clinical Coordinating Center	A third party contracted by Ferring to provide support to the trial sites for certain aspects of conduct of the trial, such as assessment of eligibility and medical support.
Compliance	Adherence to all the trial-related requirements, good clinical practice requirements, and the applicable regulatory requirements.
Evaluable Patient	A patient who has been treated with the investigational medicinal product (IMP) and 30 days have passed since initiation of IMP infusion.
Good Clinical Practice	A standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of trial participants are protected.
Investigator	The person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator.
Legal Representative	An individual or juridical or other body authorised under applicable law to consent, on behalf of a prospective patient, to the patient's participation in the clinical trial.
Treated Patient	A patient who has been treated with the IMP.
Trial Entry Terms	*Screening* The act of determining if an individual meets requirements for participation in the clinical trial. *Enter (=Consent)* The act of obtaining informed consent for participation in the clinical trial from patients deemed eligible or potentially eligible to participate in the clinical trial. Patients who have entered into a trial are those who sign the informed consent document directly or through their legally acceptable representatives. *Enrolment (=Randomisation)* The act of assigning a patient to a treatment. Patients who are enrolled in the trial are those who have been assigned to a treatment.

1 INTRODUCTION

1.1 Background

Ferring Pharmaceuticals A/S (hereinafter called Ferring) is currently developing selepressin, a novel vasoconstrictor agent for treatment of patients with vasopressor-dependent septic shock.

Patients suffering from septic shock, defined by the need for vasopressor treatment despite adequate fluid resuscitation, represent an extremely ill patient population with a common need for prolonged intensive care, frequent multisystem organ failure, and a high mortality. Septic shock is one of the most common causes of death in intensive care units (ICUs) and its incidence is rising. The growing incidence is most likely due to increased use of invasive devices and immunosuppressive therapies, higher numbers of immunocompromised patients, more antibiotic resistance, and an aging population.

Selepressin is a cyclic nonapeptide vasopressin analogue with high affinity and selectivity for the human vasopressin V_{1a}-receptor versus the V_{1b}-, V_2-, and oxytocin receptors and no known affinity to other receptors, ion-channels, and transporters (Laporte R et al, 2011). The lack of V_{1b} and V_2 activity is the main differentiator of selepressin from existing vasopressin analogues such as arginine vasopressin.

In addition to its vasopressor activity, one of the key characteristic of selepressin is its apparent anti-leakage properties (Maybauer et al., 2014; Rehberg et al., 2012(a); Rehberg et al., 2011; Su et al., 2012). The ability to limit vascular leakage is a clinically relevant and very important feature of selepressin. While fluid therapy is fundamental to the acute resuscitation of critically ill patients, in those with sepsis accompanied by increased capillary permeability from microvascular injury, it can contribute to tissue oedema and eventually organ dysfunction.

Thus, selepressin may serve a dual role of providing haemodynamic benefit while reducing the leakage of intravascular fluid into the extracellular space. It is believed that these unique characteristics could help address the unmet need in the treatment of vasopressor-dependent septic shock and provide significant benefit for the patients.

Moreover, due to the lack of V_2 activity, selepressin does not cause V_2-mediated antidiuresis and release of coagulation factors (Rehberg et al, 2012(b)), important safety consideration in septic shock patients that often have a positive fluid balance and coagulation disturbances.

1.2 Scientific Justification for Conducting the Trial

Septic shock is characterised by hypotension and decreased tissue perfusion due to vasodilation and capillary leakage. In this setting, vasopressors such as norepinephrine / noradrenaline, epinephrine / adrenaline, and dopamine are considered to be critical life-support necessary to quickly restore perfusion pressure. However, at the same time these drugs may cause serious adverse effects such as arrhythmias, myocardial, mesenteric, cerebral, or digital ischaemia/infarction, and acute kidney injury. Such complications may prevent or prolong the recovery from septic shock and may cause long-term sequelae. Therefore, interventions that decrease the duration of the need for vasopressors are expected to result in reduced risk of complications and possibly improved patient outcome.

In the first clinical trial with selepressin in septic shock patients (FE 202158 CS02), selepressin reduced the need for norepinephrine/noradrenaline in a dose-dependent manner (Ferring report, CS02). Furthermore, selepressin significantly reduced the duration of mechanical ventilation compared to placebo during the first 7 days. Also, cumulative fluid balance and fluid overload tended to be reduced compared to placebo. However, the FE 202158 CS02 trial was not powered to assess clinical outcomes and therefore, a large pivotal trial is needed to fully assess the effects and clinical outcomes associated with the use of selepressin.

The present trial (trial code: 000133) is an adequately powered Phase 2b/3 clinical trial designed to assess and confirm the efficacy and safety of selepressin as a treatment for patients with vasopressor-dependent septic shock. The trial design has been reviewed by both the United States Food and Drug Administration and the European Medicines Agency.

1.3 Benefit / Risk Aspects

Selepressin is a potent vasoconstrictor and expected pharmacological effects include correction of hypotension which makes treatment potentially beneficial to the septic shock patients participating in the trial. The vasoconstrictor effect of selepressin has been investigated in a number of studies in vitro and in vivo in healthy animals, in sheep models of severe sepsis and septic shock, in isolated human resistance arteries, and in septic shock patients. In animal models of severe sepsis and septic shock, selepressin was shown to limit fluid accumulation and pulmonary oedema formation (Maybauer et al, 2014; Su et al, 2012). In patients with septic shock, this may provide clinical benefit by reducing mortality and/or the total duration of time that the patient requires life support with vasopressors and mechanical ventilation, speeding up the recovery, and reducing the risk of short- and long-term sequelae from organ dysfunction.

In healthy adult men and women, the safety profile of selepressin was consistent with the pharmacological effects of a V_{1a} agonist, mainly related to its vasoconstrictor properties, and did not cause any safety concerns (Ferring report, FE 202158 CS01). The dose-limiting effect was reduction in cardiac output. The infusion of selepressin was interrupted in 7 out of 10 subjects in the highest dose group (13.1 µg/h [i.e. 3.1 ng/kg/min assuming 70 kg body weight]) due to a decrease in cardiac output by more than 25%, which was a pre-defined criterion for termination of infusion. No negative renal or hepatic effects were observed. Some subjects reported signs of gastrointestinal discomfort, pallor, and feeling of body temperature change (i.e. signs of effects on the peripheral blood flow). The ECG, blood gases, lactate, and the other safety laboratory parameters were not affected to any measurable extent by the infusion of selepressin. QTcF values of >450 msec, or an increase of >30 msec, were sporadically observed in all dose groups at all time-points, including 7 subjects that received placebo. V_{1a}-induced mesenteric vasoconstriction is a general safety concern. However, no signs of mesenteric or myocardial ischaemia were observed in the healthy subjects. The lack of signs of myocardial ischaemia is consistent with previous findings that selepressin did not demonstrate specific coronary vasoconstrictive properties in anaesthetised dogs.

Two clinical trials in septic shock patients have been conducted. In the first trial (trial code: FE 202158 CS02), selepressin was safe and well tolerated up to an infusion rate of 2.5 ng/kg/min. Too few patients were enrolled in the highest dose group (i.e. 3.75 ng/kg/min), and hence it was not possible to conclude on the safety of this dose level. In approximately 50% of the patients receiving 2.5 ng/kg/min of selepressin, norepinephrine/noradrenaline could be completely weaned within 12 hours whereas all patients receiving 1.25 ng/kg/min required norepinephrine/noradrenaline at 12 hours (Ferring report, FE 202158 CS02). There were neither apparent differences between treatment with selepressin and placebo with respect to adverse event reporting nor apparent shifts or trends in the means of the ECG parameters during the treatment period that could be attributed to the treatment. A number of patients in all treatment groups, including >75% of the placebo patients, had occasional QTcF values >450 ms, several of whom already had this finding at baseline, or an increase of >30 ms. These observations were regarded as caused by the underlying disease.

The second trial in septic shock patients (trial code: 000025) investigated the dose-range from 2.5-7.5 ng/kg/min and allowed free up- as well as down-titration of selepressin within a certain range. Data from this trial showed that: 1) it was possible to wean norepinephrine/noradrenaline completely within few hours in most of the patients when the selepressin dose was increased to 5-7.5 ng/kg/min, 2) it was possible to maintain mean arterial pressure (MAP) within a target range by adjusting the dose, and 3) in the majority of patients, doses above 3.75 ng/kg/min were only needed during the first 6 hours. Five patients reported a total of eight treatment-emergent serious adverse events which were regarded as related to the treatment. Four of these events occurred in the same patient including one with fatal outcome. Although it cannot be ruled out that the infusion of selepressin, which was stopped after 9.5 hours, approximately 17 hours before the fatal outcome, contributed to the development of the adverse event, it should be recognised that the condition of the patient at enrolment was very serious with a very high baseline infusion rate of noradrenaline (1 µg/kg/min) and a high lactate level (5.4 mmol/L).

The pharmacokinetic profile of selepressin in septic shock patients is based on data from the FE 202158 CS02 and the 000025 trials. As the infusion rate of selepressin was adjusted according to the need of the patient, the pharmacokinetic parameters were calculated by means of population pharmacokinetic modelling. Clearance was on average estimated to 11.28 L/h in a typical patient with a body weight of 70 kg, with inter-individual variability of 22 (CV%). The time to steady state concentration was approximately 7 hours and the steady-state concentrations were proportional to the initial infusion rate. The terminal half-life was approximately 1.4 hours irrespective of dose. However, the initial distribution phase half-life was short, approximately 10 minutes.

The evaluation of benefits and risks indicate that participation in this trial is associated with a favourable benefit-risk ratio. The trial is justified by the potential clinical benefit of a selective V_{1a} receptor agonist treatment in patients with vasopressor-dependent septic shock and the risk posed to the patients participating in the trial is deemed low as the standard septic shock treatment will be in line with the Surviving Sepsis Campaign guidelines (Dellinger et al, 2013). Patients participating in this trial will be closely monitored and they will have either the same or more frequent contacts with treating clinicians compared to routine treatment, depending on local clinical practice.

For further information regarding selepressin, please refer to the current investigator's brochure.

2 TRIAL OBJECTIVES AND ENDPOINTS

2.1 Objectives

Primary Objective

- To demonstrate superiority of selepressin plus standard care versus placebo plus standard care in the number of vasopressor- and mechanical ventilator-free days (with penalty for mortality) in patients with vasopressor-dependent septic shock

Secondary Objectives

- To determine the efficacy of selepressin on:
  - Organ dysfunction
  - Morbidity and mortality
  - Fluid balance
  - Health-related quality of life
- To determine the safety profile of selepressin
- To determine the pharmacokinetics of selepressin
- To determine the health economics of selepressin
- To further evaluate a range of biomarkers in relation to the mode of action of selepressin

2.2 Endpoints

Primary Endpoint

- Vasopressor- and mechanical ventilator-free days (P&VFDs) up to Day 30

This composite endpoint is defined as the number of days (reported to one decimal place [0.0 to 30.0 days]) from start of treatment with the investigational medicinal product (IMP) [selepressin or placebo] to 30.0 days thereafter during which the patient is: 1) alive; 2) free of treatment with intravenous vasopressors; and 3) free of any invasive mechanical ventilation (see definition below).

Any patient that dies within this 30-day period is assigned zero P&VFDs, even if there is a period during which the patient is free of both vasopressor treatment and mechanical ventilation. If vasopressors need to be restarted or mechanical ventilation needs to be initiated or restarted, and the use of either is greater than 60 minutes within a 24-hour period, then the clock is reset and the patient is not considered free of vasopressors and/or mechanical ventilation until after those therapies are again finally discontinued. Vasopressor use or mechanical ventilation during - and up to three hours after - surgery / procedure (including bedside) is exempt from this rule (i.e. does not reset the calculation of P&VFDs). The intent is for the endpoint to reflect the speed of recovery from septic shock and respiratory failure, with appropriate penalties for recurrent shock, new or recurrent respiratory failure, and death.

Vasopressor use is defined as any intravenous dose of norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, terlipressin, and IMP (i.e. selepressin and placebo).

Mechanical ventilation is defined as use of endotracheal or tracheostomy tube assisted ventilation (>5 cm H_2O continuous positive airway pressure and >5 cm H_2O of pressure support from the ventilator in tracheostomy patients). End of mechanical ventilation is defined as: 1) extubation of intubated patients or 2) ≤5 cm H_2O continuous positive airway pressure and ≤5 cm H_2O of pressure support from the ventilator in tracheostomy patients. If non-invasive ventilation by mask or bag (>5 cm H_2O of pressure support) is deployed to avoid (re)intubation, it also counts as mechanical ventilation. However, all other uses of non-invasive ventilation such as chronic night-time use of positive airway pressure for chronic obstructive pulmonary disease (COPD) or sleep apnea does not count as mechanical ventilation (regardless of pressure).

Key Secondary Endpoints

The following key secondary endpoints have been selected for the purpose of a possible label inclusion (see Section 9.6.3).

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Day 90
- Renal replacement therapy (RRT)-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)

- Intensive care unit (ICU)-free days up to Day 30

Secondary Efficacy Endpoints

Other secondary endpoints are listed below. All ssessments related to the endpoints are described in Section 7.1.

Organ dysfunction

- Vasopressor-free days up to Day 30
- Mechanical ventilator-free days up to Day 30
- Duration of septic shock (i.e. vasopressor use) up to Day 30
- Duration of mechanical ventilation up to Day 30
- Incidence of RRT up to Day 30 (counting patients who die as on RRT and excluding patients on RRT for chronic renal failure at time of randomisation)
- Duration of RRT up to Day 90 (excluding patients on RRT for chronic failure at time of randomisation)
- Daily overall and individual organ (cardiovascular, respiratory, renal, hepatic, coagulation) scores using a modified version of the Sequential Organ Failure Assessment (SOFA) until ICU discharge
- Incidence of new organ dysfunction and new organ failure (based on the SOFA score) up to Days 7 and 30

Morbidity and mortality

- ICU length of stay up to Day 30
- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Days 30 and 180

Fluid balance

- Daily and cumulative fluid balance until ICU discharge (for a maximum of 7 days)
- Daily and cumulative urine output until ICU discharge (for a maximum of 7 days)

Health-related quality of life

- Change in utility, based on the EuroQol group's 5-dimension 5-level (EQ-5D-5L) questionnaire, up to Day 180

Safety Endpoints

- Incidence of adverse events (type, frequency, and intensity) with specific emphasis on:
  - Ischaemic events (e.g. myocardial, skin, cerebral, mesenteric, and limb ischaemia)
- Changes in vital signs and safety laboratory variables, including:
  - Number of clinically significant results assessed as unanticipated in the setting of septic shock
- Episodes of hypotension (MAP <60 mmHg for longer than one hour)

Additional Endpoints

- Hospital-free days up to Day 90
- Hospital length of stay up to Day 90
- Patient residence at Day 30, Day 60, Day 90, and Day 180
- Health economic evaluation - to be reported separately according to a pre-specified health economic analytical plan
- Mean arterial pressure (MAP), until ICU discharge (for a maximum of 7 days)
- Norepinephrine/noradrenaline and other vasopressor doses
- Pharmacokinetic response (in a subset of approximately 200 patients) - to be reported separately according to a pre-specified pharmacokinetic analysis plan
- Creatinine clearance
- Ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2 ratio) (in a subset of 100-350 patients)
- Extravascular lung water and pulmonary permeability index (in a subset of 100-350 patients)
- Cardiac output (in a subset of 100-350 patients)
- Cytokines (in a subset of 100-350 patients)
- Angiopoietin-1 and -2 (in a subset of 100-350 patients)

3 INVESTIGATIONAL PLAN

3.1 Overall Trial Design

The overall trial design is a multi-centre, double-blind, randomised, placebo-controlled, two-part adaptive clinical trial. The trial is designed to investigate the efficacy and safety of multiple dosing regimens of selepressin and to confirm the efficacy and safety of one dosing regimen in treatment of adult patients with septic shock requiring vasopressor treatment.

Up to four dosing regimens of selepressin, as described in Table 1, will be investigated in the first part of the trial and the best-performing dosing regimen will be selected for the second part of the trial.

Table 1 Dosing Regimens

	Starting Dose (ng/kg/min)	Maximum Dose (ng/kg/min)	Range (ng/kg/min)
Arm 1	1.7	2.5	0-2.5
Arm 2	2.5	3.75	0-3.75
Arm 3	3.5	5.25	0-5.25
Arm 4	5.0	7.5	0-7.5

3.1.1 Trial Design Diagram

The overall trial design is illustrated in Figure 1. The entire trial duration for an individual patient is up to 6 months (see Section 6).

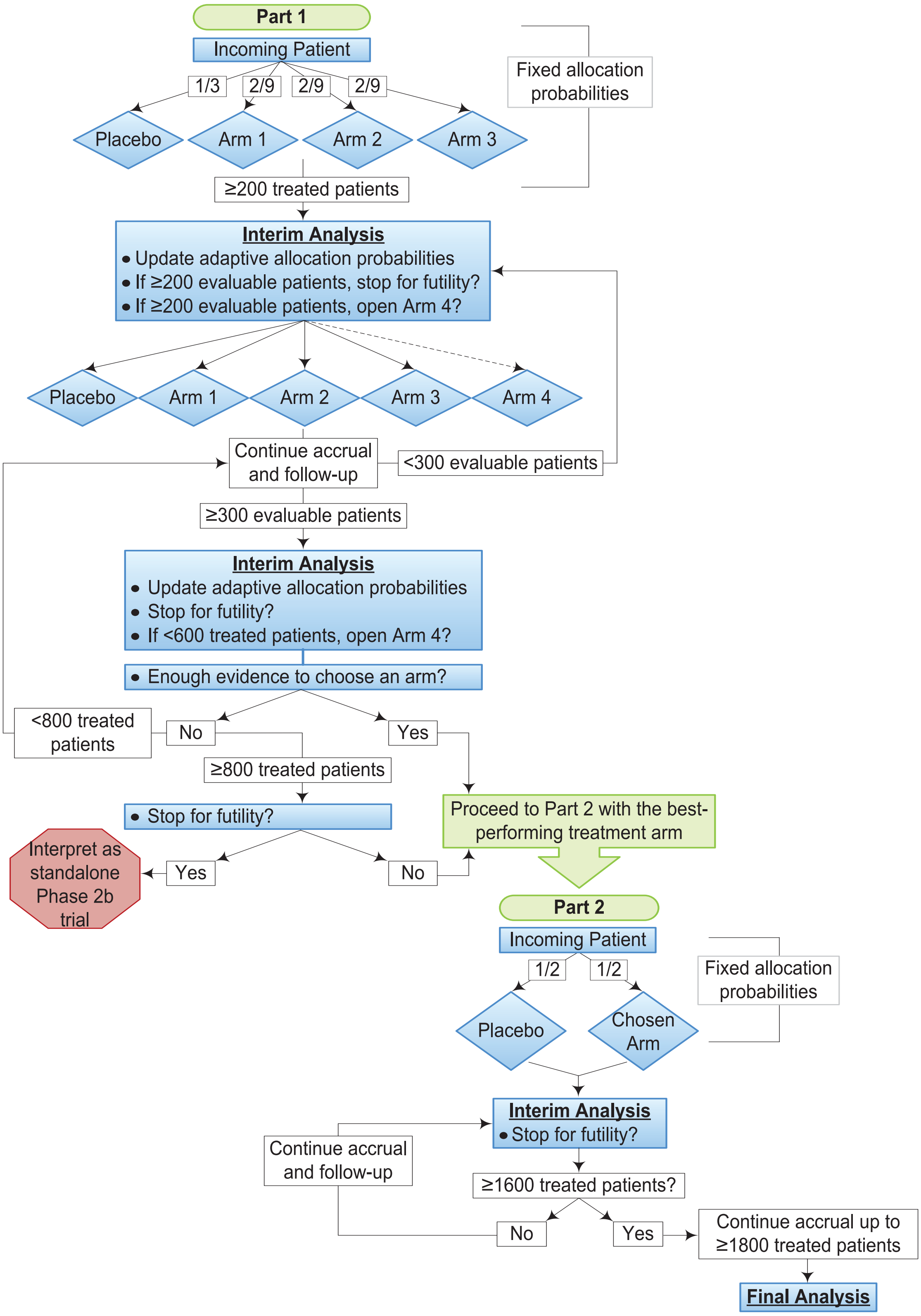


Figure 1 Trial Design

3.1.2 Overall Design and Control Methods

This is a Phase 2b/3 adaptive clinical trial with two parts: Part 1 - Phase 2b and Part 2 - Phase 3 (Figure 1). The entire trial, combining both parts, represents an adequate and well-controlled comparison of selepressin versus placebo. Part 1 comprises 300 evaluable – 800 treated patients. The size of Part 2 will include enough patients to bring the total number of patients in Part 1 and Part 2 to 1800 evaluable patients, ensuring a minimum sample size of 1000 patients in Part 2. The final analysis uses all patients from both parts of the trial.

Part 1 will begin with a 200-patient ‘burn-in’ period during which fixed randomisation across Arms 1, 2, and 3 will be used (the arms are described in Table 1) with:

- One-third of the patients randomised to placebo
- Two-third of the patients randomised to selepressin (two-ninths of the patients to each of the Arms 1, 2, and 3).

After completion of the ‘burn-in’ period, Part 1 will utilise response-adaptive randomisation to preferentially allocate patients to the treatment arms that appear to have the maximum benefit with respect to the primary endpoint (i.e. vasopressor- and mechanical ventilator-free days [P&VFD]). A fixed fraction (one-third) of patients will be randomised to placebo throughout Part 1 to ensure contemporaneous control patients are enrolled throughout the trial. Arm 4 will only be opened between 200 evaluable and 600 treated patients and if there is at least a 50% posterior probability that Arm 3 has a higher expected P&VFD than Arm 2 and data from the lower dosing regimens do not suggest any significant safety concerns.

If Part 1 results in the decision to run Part 2, Part 2 will utilise a fixed 1:1 randomisation proportion between placebo and the best-performing dosing regimen of selepressin. The best-performing dosing regimen will be identified at the end of Part 1.

Pre-defined interim analyses will be conducted as described in Section 3.3 and Section 9.9. Patient enrolment will continue without any stop at the interim analyses and a seamless transition to Part 2 can occur after any interim analysis after 300 evaluable - 800 treated patients in Part 1.

3.1.3 Trial Schedule

The estimated timelines are:

- First patient first visit (FPFV): Q3 2015
- Last patient at Day 30 (primary database lock): Q2 2018
- Last patient last visit (LPLV) / end-of-trial: Q4 2018

It is expected that all patients will be recruited within a period of up to 3 years. Hence, the duration of the entire trial is expected to be no longer than 3.5 years.

The primary database will be locked when all ongoing patients have passed Day 30.

3.2 Planned Number of Trial Sites and Patients

Enough patients will be randomised to bring the total number of evaluable patients up to 1800 in the entire trial programme (including both parts of the trial). Patient recruitment will be competitive between trial sites. To achieve the requested number of patients within the given timelines in Section 3.1.3, approximately 60 - 100 trial sites will actively participate in the trial, with additional sites identified as 'back-up' sites to replace non-performing sites.

3.3 Interim Analysis

There will be no interim analyses with the potential to stop the trial early for success. The trial will either stop for futility or run to 1800 evaluable patients.

Once the 'burn-in' period in Part 1 is completed, interim analyses will be conducted regularly during Part 1 to improve the efficiency of dosing regimen selection and to allow early termination for futility or for successful dosing regimen selection.

During Part 2, regular interim analyses for futility will be performed until 1600 patients have been treated.

The interim analyses reports will be prepared by an external company who is not otherwise involved in the conduct of the trial, and who will not divulge these data to Ferring personnel. The measures taken to protect the overall blinding during the interim analyses are illustrated in Figure 2 in Section 5.5.1.

The statistical considerations of the interim analyses are further described in Section 9.9.

3.4 Data and Safety Monitoring Board

A data and safety monitoring board (DSMB) will be established for this trial. The DSMB will be an independent group of critical care and emergency medicine experts not otherwise involved in the trial and a statistician with expertise in adaptive designs. The DSMB will oversee safety and ensure appropriate trial conduct, which includes overseeing that the adaptive design is implemented and performing as intended.

The DSMB will have access to unblinded interim efficacy and safety data as well as recruitment data so that they are able to monitor the enrolment of the intended patient population. The DSMB will keep the interim results confidential from any individuals involved in the trial conduct. A trial-specific DSMB charter specifies the composition of the DSMB and its responsibilities and working procedures.

3.5 Discussion of Overall Trial Design and Choice of Control Groups

3.5.1 Trial Design

This is a multi-centre, randomised, placebo-controlled, double-blind Phase 2b/3 adaptive clinical trial, which will be conducted in accordance with the protocol, good clinical practice, and applicable regulatory requirements.

The trial will be conducted at multiple trial sites predominantly across Europe and North America. High-quality trial sites with shared standards of practice and values will be selected; all trial sites follow the globally accepted Surviving Sepsis Campaign guidelines (Dellinger et al, 2013) which provides recommendations for the best care of patients with severe sepsis and septic shock.

Patients will be enrolled in the trial and the IMP infusion will be initiated as early as possible. To ensure, as far as possible, that only eligible patients enter the trial (see Section 4.1), all potential patients will be discussed with one of the assigned clinical coordinating centres (CCCs) prior to randomisation. The CCCs will be available 24 hours a day throughout the trial to answer investigators' medical questions (such as assessment of eligibility and medical support).

As described in Section 3.4, the trial will be overseen by an independent DSMB. In addition, a trial steering committee (TSC) will oversee the overall conduct of the trial in a blinded manner. The TSC includes representatives of Ferring and the CCCs, trial investigators, and experts in the fields of critical care, emergency medicine and clinical trial methodology. The TSC will make recommendations to Ferring regarding all trial-related decisions including those based on recommendations from the DSMB. A TSC charter specifies the composition of the TSC and its responsibilities and working procedures.

The trial design is robust with clear, prospectively determined clinical and statistical analytic criteria. The design achieves control of type 1 error through analytical means. While the trial can stop early for futility, a successful Phase 3 trial can only be achieved with at least 1800 evaluable patients. At the end of the trial, a single test statistic will be calculated to compare two populations that are defined before the trial begins, namely patients allocated to placebo compared to patients allocated to any dosing regimen of selepressin.

3.5.2 Selection of Endpoints

Mortality is indisputably a critical endpoint to consider in septic shock patients; however, as more and more patients survive septic shock it becomes increasingly important to focus on patient-centred outcomes in survivors such as improving the speed of recovery, the ability to limit the need for life support, and long-term sequelae of septic shock. Therefore in this trial, we will not only assess mortality, but also compare survival free of both vasopressor and mechanical ventilation – the two primary forms of life-support provided to septic shock patients in the ICUs. The primary endpoint is constructed to capture selepressin's ability to hasten the resolution of septic shock and to reduce the time the patient is dependent on life-support. A faster resolution of septic shock is expected to lead to a reduction in irreversible morbidities and serious outcomes resulting from fluid overload and ischaemic damage to organs.

The primary endpoint is a composite endpoint capturing the need for vasopressor, the need for mechanical ventilation, and mortality. All three components on their own represent important beneficial effects for septic shock patients and will also be captured separately as secondary endpoints. This combined endpoint (vasopressor- and mechanical ventilator-free days [P&VFDs])

is well aligned with the currently understood beneficial actions of selepressin and supported by the effects of selepressin found in previous non-clinical and clinical trials as discussed in Section 1.

Septic shock is associated with a wide array of serious and troublesome sequelae that may impact long-term outcomes. The secondary efficacy and safety endpoints (as listed in Section 2.2) were selected to focus on key effects related to the primary mode of action of selepressin (i.e. vasopressor and anti-leakage effects) and at the same time to capture other clinically meaningful outcomes such as incidence of organ dysfunction, mortality, quality of life, and general safety of selepressin. Hence, the secondary endpoints will capture both efficacy and safety aspects of the treatment. Furthermore, the length of stay at the ICU as well as health-related quality of life represent society and life impact focused endpoints.

Patients with septic shock represent a notoriously heterogeneous patient population. To account for potential differences in outcome based on baseline characteristics, patients will be randomised in a stratified manner as described in Section 4.2.2.

3.5.3 Choice of Control Group

In this trial, selepressin plus standard of care will be compared to placebo plus standard of care with norepinephrine/noradrenaline as primary vasopressor. Use of placebo allows provision of usual standard of care in both the selepressin-treated group and the placebo-treated group. The investigators can continue the use of norepinephrine/noradrenaline, continue to add (or not) antibiotics, fluids, inotropic agents (such as dobutamine), oxygen, mechanical ventilation, corticosteroids, feeding, and other supportive care in accordance with standard clinical practice following the Surviving Sepsis Campaign guidelines (Dellinger et al, 2013). Thus, this background of ethical usual clinical care would occur in both the selepressin-treated group and the placebo-treated group.

3.5.4 Selection of Doses in the Trial

Four dosing regimens of selepressin ranging from 1.7 ng/kg/min to 7.5 ng/kg/min (as detailed in Table 1) have been selected for this trial. These dosing regimens are chosen to cover the relevant dose range based on dosing experience from the previous clinical trials (i.e. FE 202158 CS02 and 000025).

In FE 202158 CS02, which was the first clinical trial in septic shock patients, selepressin was safe and well tolerated up to 2.5 ng/kg/min. In approximately 50% of the patients receiving 2.5 ng/kg/min of selepressin, norepinephrine/noradrenaline could be completely weaned within 12 hours whereas all patients receiving 1.25 ng/kg/min of selepressin required norepinephrine/noradrenaline at 12 hours (Ferring report FE 202158 CS02). This suggests that some patients may require more than 2.5 ng/kg/min of selepressin in order to wean norepinephrine/noradrenaline completely.

The second clinical trial (000025) was initiated to assess whether use of higher doses of selepressin would allow for a faster and more complete weaning of norepinephrine/noradrenaline. In that trial, the dose-range from 2.5 to 7.5 ng/kg/min with free up- and down-titration of selepressin within a certain range was investigated. The flexible dose-adjusting was designed to mimic the current clinical practice for the use of vasopressors. The 000025 trial showed that it was possible to wean norepinephrine/noradrenaline completely within a few hours in most of the patients when the selepressin dose was increased to 5 to 7.5 ng/kg/min. Despite the pharmacokinetic characteristics of selepressin (i.e. approximately 7 hours to reach steady state plasma levels and a half-life of approximately 1.4 hours), it was possible to maintain MAP within the target range by adjusting the dose. In the majority of patients, doses above 3.75 ng/kg/min were only needed in the first 24 hours. However, as the 000025 trial was not designed (or powered) to provide data on efficacy and safety, it is not known whether allowing the use of higher doses of selepressin is associated with an increased benefit or safety risk; hence, four dosing regimens (see Table 1) have been selected for investigation in the 000133 trial.

The lowest dosing regimen selected for the 000133 trial is expected to allow partial weaning of norepinephrine/noradrenaline and will address the question whether low doses of selepressin or co-treatment of selepressin and norepinephrine/noradrenaline is superior to full substitution of norepinephrine/noradrenaline with selepressin. The higher dosing regimens will allow progressively faster and more complete weaning of norepinephrine/noradrenaline and will answer the question whether higher doses of selepressin are associated with increased clinical benefit. The high granularity in the dose-selection is chosen to increase the ability to detect the dosing regimen that provides the optimal risk-benefit ratio.

3.5.5 Selection and Timing of Dose for Each Patient

Treatment with the IMP (selepressin or placebo) has to start as early as possible following fulfilment of the eligibility criteria and no later than 12 hours after initiation of the required continuous infusion of vasopressor treatment for septic shock. All patients will be on norepinephrine/noradrenaline treatment as part of standard of care at the time when the IMP infusion is initiated.

During the course of treatment, the IMP will be continuously administered and adjusted within pre-defined infusion rates. An administration guide will be provided with recommendations on how to adjust the IMP infusion rate.

As the vasopressor-need varies significantly from patient to patient and over time in any given patient, it is necessary to allow dose-adjustments. A lower starting infusion rate of the IMP has been included to minimise the risk of overshooting when IMP is added on top of the norepinephrine/noradrenaline infusion the patient is receiving to maintain the target MAP. The infusion rate of the IMP will be increased if the starting rate is insufficient to wean norepinephrine/ noradrenaline completely. If the MAP is increased above the target, norepinephrine/noradrenaline will be weaned first while aiming to keep MAP at the target. Norepinephrine/noradrenaline must be completely weaned prior to weaning of the IMP. If the IMP alone increases the MAP above the target, the IMP will be weaned step-wise while aiming to keep MAP at the target. If the maximum allowed infusion rate of the IMP is not sufficient to maintain the MAP at the target, norepinephrine/

noradrenaline will be added to achieve the targeted MAP. If target MAP cannot be maintained despite maximum allowed infusion rate of IMP and ≥1 µg/kg/min norepinephrine/noradrenaline base (or a total catecholamine equivalent of 1 µg/kg/min norepinephrine/noradrenaline base), vasopressin may be added.

The IMP infusion will continue as long as blood pressure support is deemed necessary with a maximum of 30 days. After complete weaning, IMP infusion may be re-started during this 30-day period for treatment of sepsis-induced hypotension if there is no suspicion of mesenteric or cardiac ischaemia. Patients who still need vasopressor treatment after 30 days will be switched to other vasopressors at the discretion of the investigator.

4 SELECTION OF TRIAL POPULATION

4.1 Trial Population

The intention is to enrol a typical sample of patients presenting with septic shock and commence treatment with the IMP during the initial hours of resuscitation, within 12 hours from the onset of vasopressor treatment, targeting those who do not respond rapidly to fluids and whose vasopressor-need persists for at least one hour.

4.1.1 Inclusion Criteria

1. 18 years of age or older.

2. Proven or suspected infection.

3. Septic shock defined as hypotension (systolic blood pressure less than 90 mmHg OR MAP less than 65 mmHg) requiring vasopressor treatment (i.e. any dose of norepinephrine / noradrenaline base greater than 5 µg/min) despite adequate fluid resuscitation (at least one litre for hypotension).

   The MAP threshold for inclusion in the trial is a MAP below 65 mmHg before vasopressor support is started. However, it is not a requirement that patients are under the target MAP during vasopressor treatment with norepinephrine/noradrenaline. The patients must require vasopressor treatment to stay on the target MAP i.e. a patient can be at the target of 65 mmHg or higher while on at least 5 µg/min of norepinephrine/noradrenaline base at least for one hour before inclusion and when IMP is started.

   A patient can also be included based on systolic blood pressure less than 90 mmHg even if the MAP is above 65 mmHg if the patient is judged in need of vasopressor treatment based on evidence of poor organ perfusion.

   The requirement of at least one litre of fluid for hypotension to start the randomisation process balances the need to ensure that patients have been properly fluid resuscitated while still allowing for early enrolment before there is marked endothelial injury and increased permeability so that the proposed permeability-protection of selepressin can be assessed. Fluid resuscitation should continue according to the recommendations in the Surviving Sepsis Campaign guidelines (Dellinger et al, 2013) and therefore the patients should have received the recommended 30 mL/kg fluid from the onset of hypotension and to the time IMP infusion is started (unless evidence of fluid replete/overload) (see Section 4.1.3).

4. Informed consent obtained in accordance with local regulations.

4.1.2 Exclusion Criteria

1. Not possible to initiate IMP treatment within 12 hours from onset of vasopressor treatment for septic shock.

   Patients will be excluded if IMP infusion cannot be started within 12 hours from onset of vasopressor treatment. This time limit exclusion is added to ensure that patients are included early in the septic shock state. If the inclusion is left too late, then there is often irreparable organ dysfunction and endothelial injury with increased permeability in septic shock. Thus, even an effective intervention could fail if applied later when there is irreversible injury.

2. Primary cause of hypotension not due to sepsis (e.g. major trauma including traumatic brain injury, haemorrhage, burns, or congestive heart failure/cardiogenic shock).

3. Previous severe sepsis with ICU admission within this hospital stay.

   Patients who have had a prior episode of severe sepsis have a poorer prognosis and may still be recovering from the associated organ dysfunction so patients with previous severe sepsis within this hospital stay are not eligible.

4. Known/suspected acute mesenteric ischaemia.

   Selepressin is a potent V_{1a} agonist and V_{1a}-induced mesenteric vasoconstriction is a safety concern so patients with known or suspected acute mesenteric ischaemia are not allowed for safety reasons.

5. Suspicion of concomitant acute coronary syndrome based on clinical symptoms and/or ECG during this episode of septic shock.

   V_{1a} agonism could also induce coronary vasoconstriction and so, for safety reasons, patients are not allowed in the trial if the investigator believes the ECG and clinical symptoms suggest a concomitant acute coronary syndrome.

6. Chronic mechanical ventilation for any reason OR severe COPD requiring either continuous daily oxygen use during the preceding 30 days or mechanical ventilation (for acute exacerbation of COPD) during the preceding 30 days.

   A potential confounder to interpretation of the efficacy of selepressin on ventilator-free days would be inclusion of patients who have severe COPD requiring chronic oxygen use or mechanical ventilation. Such patients may recover from the acute pulmonary effects of septic shock (such as acute respiratory distress syndrome) because of the proposed beneficial effects of selepressin but then prove difficult and slow to wean from mechanical ventilation because of their significant underlying disease. Accordingly such patients are not allowed in the trial. However, patients with less severe COPD are allowed.

7. Received bone marrow transplant during the preceding 6 months or chemotherapy during the preceding 30 days for lymphoma or leukemia.

   Patients who have had bone marrow transplant during the preceding 6 months or chemotherapy during the preceding 30 days for lymphoma or leukemia are excluded because these patients can have a significantly worse prognosis compared to the average septic shock patient due to their impaired immunity and other effects of their underlying disease and its treatment. Depending on the state of immune dysfunction, the mortality rate of these patients when they get septic shock - even with aggressive intensive care, resuscitation, and appropriate intravenous broad spectrum antibiotics - can exceed 90%. Furthermore, many of these patients have thrombocytopenia secondary to their disease or their therapies and this underlying thrombocytopenia increases the risks of worsening to profound thrombocytopenia during septic shock because septic shock induces thrombocytopenia directly and independent of prior chemotherapy. Finally, many of these patients have other mortality risk factors such as anemia, hepatic and renal dysfunction, all of which would be worsened during septic shock.

8. Known to be pregnant.

9. Decision to limit full care taken before obtaining informed consent.

10. Use of vasopressin in the past 12 hours prior to start of the IMP infusion or use of terlipressin within 7 days prior to start of the IMP infusion.

11. Prior enrolment in the trial.

12. Prior use of an investigational medicinal product within the last month OR planned or concurrent participation in a clinical trial for any investigational drug or investigational device.

    In order to be able to assess the safety and efficacy of selepressin without confounding factors from the use of other investigational drugs or devices, co-enrolment in trials involving investigational products are not allowed. Co-enrolment in a non-investigational trial requires preapproval of the TSC and will be assessed on a case by case basis. In principle, co-enrolment is allowed unless it is expected to impact the outcome of this clinical trial.

4.1.3 Eligibility Criteria – Post-randomisation / Before Start of IMP Infusion

In addition, the following criteria must be met at start of IMP infusion:

1. Received a minimum of 30 mL/kg fluid in total from the onset of hypotension (or less if evidence of fluid replete/overload).
2. Received a continuous infusion of norepinephrine/noradrenaline base greater than 5 µg/min for at least one hour and is still receiving at least 5 µg/min norepinephrine/noradrenaline base.

The requirement of at least one hour duration of vasopressor support is intended to ensure a certain severity of the septic shock while balancing the need to recruit patients early during the initial hours of resuscitation.

3. Less than 12 hours since onset of vasopressor treatment for septic shock.

4.2 Method of Assigning Patients to Treatment Groups

4.2.1 Recruitment

Both emergency departments (EDs) and ICUs will recruit patients. Each patient considered for the trial will receive a unique screening number. The screening number will be allocated sequentially within each trial site in the order in which the patients are screened. The screening number and the result of each screening will be recorded.

A screening log with patient identification details for consecutive patients with septic shock must be maintained at each trial site to capture how many patients that were screened to include the 1800 evaluable patients in the trial. The main reason for exclusion will be described.

4.2.2 Randomisation

A computer-generated randomisation list will be prepared prior to enrolment of the first patient into the trial and updated during the trial via the response-adaptive randomisation procedure.

To minimise the risk of imbalance between the treatment arms, the randomisation will be stratified by: 1) trial site, 2) need for mechanical ventilation (‘Yes’ or ‘No’), 3) norepinephrine/noradrenaline requirement (< or ≥30 µg/min), and 4) plasma/serum creatinine (< or ≥150 µmol/L).

The patient-specific randomisation number will be allocated in the order at which the patients are being randomised into the trial. The actual patient randomisation number will be added on the screening log. No patient can be enrolled twice.

The first 200 patients will be randomised to treatment in a fixed manner. The randomisation is 3:2:2:2:0 for placebo and active treatment arms 1, 2, 3, and 4, respectively. After the first 200 patients and throughout the rest of Part 1, the patients will be randomised with one-third probability to placebo and the remaining probability allocated to active treatment arms in a response-adaptive randomisation manner. A pre-defined algorithm will be used to determine the relative randomisation to each of the treatment arms. Interim analyses will be conducted regularly in Part 1 to adjust the adaptive randomisation probabilities for the active treatment arms. The enrolment of patients will continue without any stop at the interim analyses.

During Part 2, a minimum of 1000 patients will be randomised in a 1:1 fashion to placebo and the active treatment arm selected from Part 1 in order to get the 1800 evaluable patients needed for the final analysis.

4.3 Restrictions

4.3.1 Prior and Concomitant Medications/Procedures

Medicinal products may be administered and concomitant procedures may be conducted for the well-being of the patients at the discretion of the investigators.

Use of vasopressors, mechanical ventilation, RRTs, and fluids must be documented thoroughly as these are related to the endpoints of the trial (see Section 2.2). Concomitant medications/procedures that are likely to influence the outcome from septic shock have to be detailed.

4.3.2 Prohibited Medications/Procedures

The following medications/procedures are prohibited:

- Vasopressin in the past 12 hours prior to start of IMP infusion and during IMP infusion unless the patient remains hypotensive despite the maxium allowed infusion rate of IMP and ≥1 µg/kg/min norepinephrine/noradrenaline base (or a total catecholamine equivalent of 1 µg/kg/min norepinephrine/noradrenaline base).
- Terlipressin (from 7 days prior to start of IMP infusion to end of ICU stay).
- Another investigational medicinal product (from 1 month prior to trial enrolment to 30 days after initiation of IMP infusion).
- Participation in another clinical trial for an investigational drug or investigational device or co-enrolment in a non-investigational trial that is not pre-approved by the TSC (from 1 month prior to trial enrolment to 30 days after initiation of IMP infusion).

4.4 Discontinuation and Withdrawal

For all discontinuations and withdrawals, the investigator will document the date of the termination and the main reason.

Premature discontinuation of IMP infusion

The IMP infusion will continue as long as blood pressure support is deemed necessary (up to a maximum of 30 days). Premature discontinuation of IMP infusion is defined as termination of IMP infusion even though continued intravenous vasopressor treatment for blood pressure support is needed. A premature discontinuation of IMP infusion may occur if the investigator decides that IMP infusion should be discontinued or if the patient, the patient's legal representative, or attending physician requests that IMP infusion be discontinued. If the IMP infusion is prematurely paused or discontinued due to a serious adverse event, an additional blood sample will be collected (see Section 7.2.2).

Patients whose IMP infusion is prematurely discontinued, regardless of reason, are not discontinued from the trial. These patients will continue in the trial and undergo the trial assessments following the trial protocol in order to provide the data needed for the analyses and to determine their survival status.

If a patient who does not meet the eligibility criteria is inadvertently enrolled and the IMP infusion has been started, the investigator will consult with the CCC regarding termination or continuation of the IMP infusion.

Withdrawal from the trial

A patient has the right to withdraw from the trial at any time for any reason, without the need to justify the decision.

In the event that the patient (or the patient's legal representative) withdraws consent or the investigator or Ferring, for any reason, prematurely stops the patient's participation in the trial, the IMP infusion and all scheduled trial-related assessments and laboratory testings will be stopped. Data collected up to withdrawal will remain in the database but data obtained after the patient has withdrawn his/her consent will not be entered into the database. However, results from assessments and blood samples collected prior to the withdrawal of the consent but not analysed at the time of the withdrawal will be entered into the database unless the patient refuses. The patient can request destruction of samples which would otherwise have been kept in storage.

Refer to Section 12.3 for information regarding premature trial termination.

5 TREATMENTS

5.1 Treatments Administered

5.1.1 Investigational Medicinal Product (IMP)

The IMP (selepressin and placebo) will be administered through a central venous catheter as a continuous intravenous infusion at a controlled flow rate using a syringe pump or an infusion pump suited for vasopressor administration. The administration is discussed in Section 3.5.5. A detailed IMP and vasopressor administration guide will be provided to the trial sites.

5.1.2 Norepinephrine/Noradrenaline

In order to be eligible for the trial, all patients will be on norepinephrine/noradrenaline treatment as part of standard of care (see Section 4.1.1). During the trial, the norepinephrine/noradrenaline treatment will continue as deemed necessary. Norepinephrine/noradrenaline will be from the commercial batches that the trial sites are using as part of usual clinical care and no modification to their commercial state will be made.

5.2 Characteristics and Source of the IMPs

Selepressin

Selepressin 0.3 mg/mL is a concentrate for solution for infusion. It has been manufactured in accordance with the principles of Good Manufacturing Practice (GMP) and will be provided by Ferring in vials in which the drug substance has been dissolved in an isotonic 10 mM acetate buffer of pH 4.0.

The concentrate will be diluted with sterile 0.9% sodium chloride solution to one of four different concentrations as detailed in a dilution protocol. Sterile 0.9% sodium chloride solution will be provided together with the vial with selepressin concentrate. The dilution will be prepared by dedicated and trained personnel at the hospital pharmacy or at another approved facility at the hospital. A dilution log will be provided in which the dilution will be documented.

Placebo

Sterile 0.9% sodium chloride solution will be used as the placebo. It will be provided by Ferring from commercial batches and no modification to their commercial state will be made.

5.3 Packaging and Labelling

Packaging and labelling of the IMPs (selepressin and placebo) will be performed under the responsibility of the IMP department at Ferring in accordance with GMP and national regulatory requirements.

The IMPs (selepressin and placebo) will be labelled with trial-specific labels and the content on the labels will be in accordance with Annex 13, EudraLex, volume 4 and national requirements. The labels will contain a self-adhesive tear-off portion to be affixed to the dispensing log maintained at the trial site.

5.4 Conditions for Storage and Use

The IMPs will be stored in accordance with the label.

The investigator will ensure that the IMPs will be stored in appropriate conditions in a secure location with controlled access. The storage compartment shall be monitored regularly and the temperature shall be documented. Deviations in storage temperature must be reported to Ferring without delay and the IMPs must not be used until further instructions are received.

Diluted IMP dosing solutions must be used within 26 hours after the preparation if prepared in controlled and validated aseptic conditions, otherwise within 24 hours after the preparation.

5.5 Blinding / Unblinding

5.5.1 Blinding

This is a double-blind, placebo-controlled trial in which patients, investigators and other trial site staff, the CCCs, the TSC, the clinical trial team at Ferring and its representatives will be blinded to the treatment assignment. As discussed in Section 3.4, the DSMB will have access to unblinded data during the trial. The personnel preparing the IMPs and the drug accountability monitors will also be unblinded. Figure 2 illustrates the applicable safeguards to maintain the overall blinding during conduct of trial. Three independent parties will be involved in forming the safeguard during the adaptive portion of the trial. One part (the randomisation company) will provide the expertise to run the adaptive algorithm. The second part (the statistical consulting company) will provide expertise in producing the DSMB reports and provide statistical and project management support for the DSMB. The third part (the adaptive design company) will provide expertise in adaptive clinical trial design to oversee the performance of the adaptive algorithm and provide statistical support for the DSMB in relation to the adaptive trial design.

Adequate blinding of the treating investigators and nurses is important to ensure the integrity of the results. Use of placebo will ensure effective blinding due to the significant individual variability in the vasopressor need from patient to patient. Thus, in some patients the vasopressor need declines quickly and it will not be possible to tell whether fast weaning of norepinephrine/noradrenaline is due to the fact that the patient receives active treatment or whether it is just because the patient is improving. Also, the potential side effects of selepressin are similar to side effects that can be seen with norepinephrine/noradrenaline or that may be caused by worsening of septic shock so there are no obvious effects of selepressin that will lead to unblinding.

The IMPs will be prepared in accordance with the trial-specific computer-generated randomisation numbers. The randomisation will be securely kept without access for personnel involved in the conduct of the trial with exception of the personnel preparing the IMPs until the trial database is declared clean and released to the trial team statistician. The prepared IMPs (i.e. the four diluted selepressin dosing solutions and the placebo) will be indistinguishable i.e. identical appearance and smell and will be administered using similar infusion rates.

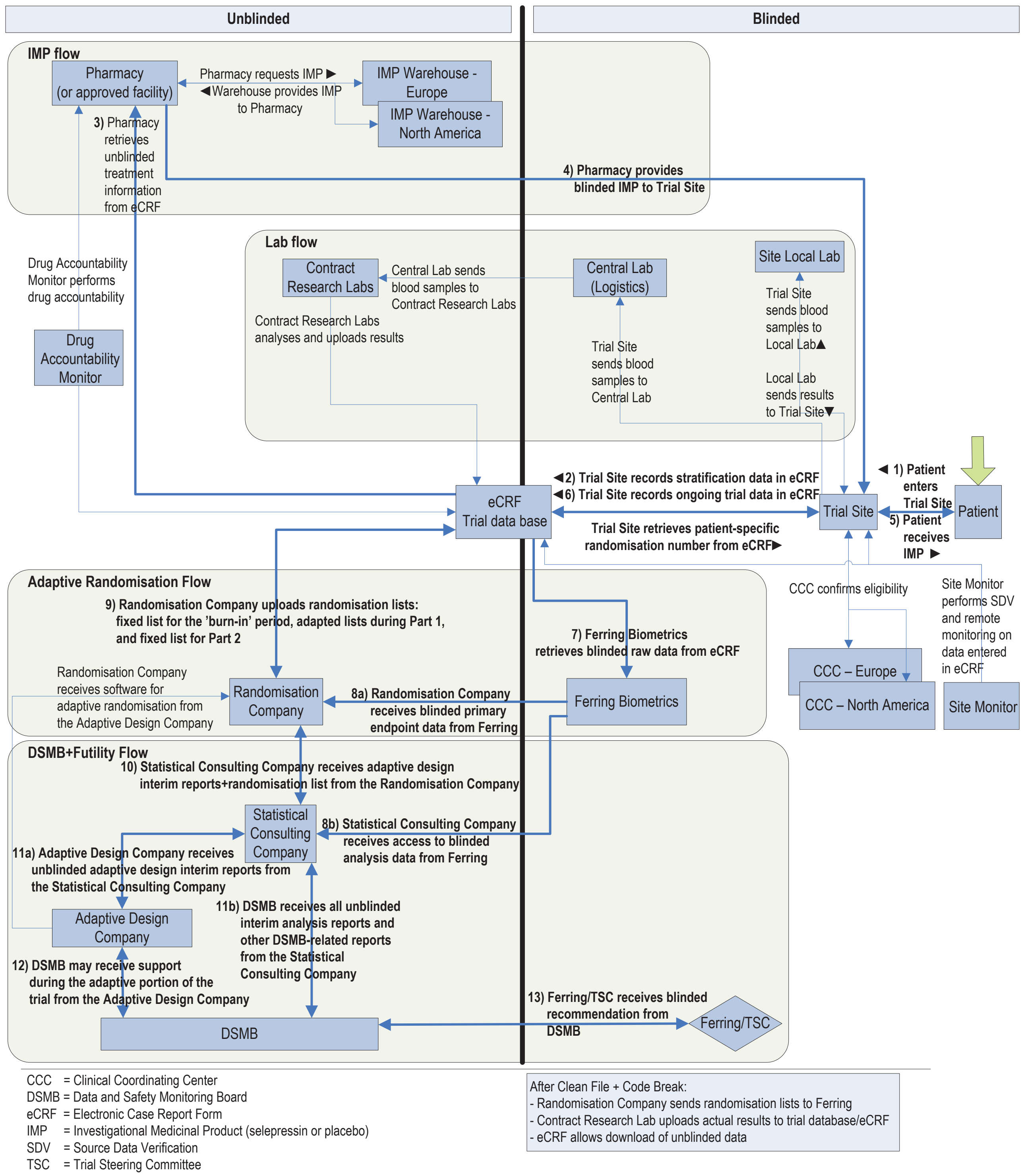


Figure 2 Safeguards to Maintain Blinding during Trial Conduct

5.5.2 Unblinding of Individual Patient Treatment

An emergency decoding possibility (via the eCRF) will be available to the investigator and designated persons at Ferring. Breaking of the blind for individual patients in emergency situations is an investigator responsibility. As far as the emergency permits, the need to break the blind will be discussed with the CCC and communicated to Ferring.

The unblinding in emergency situations is only permitted in case of a suspected unexpected serious adverse reaction (SUSAR) or in case of an important adverse event where the knowledge of the medicinal product in question is required for therapeutic decisions for the management of the patient. It will be documented in the eCRF that the code is broken, when, and by whom. The investigator must record the event of unblinding in the patient’s medical record, including the reason for unblinding, but not the treatment allocation if this can be avoided.

In case of accidental unblinding, the same documentation as for emergency unblinding must be obtained.

It may be necessary to unblind an individual patient’s treatment for the purposes of expedited reporting to the authorities and/or independent ethics committees (IECs) / institutional review boards (IRBs). In that situation, every effort will be made to maintain blinding of personnel involved in data analysis and interpretation. Other personnel may be unblinded for SUSARs, including trial site staff as well as staff acting on behalf of Ferring.

Information on whether the blind has been broken for any patients must be collected before the database is declared clean and released to the trial team statistician.

5.6 Treatment Compliance

The IMPs will only be administered by authorised staff at the trial sites to patients who meet the eligibility criteria and are randomised to a treatment in the trial. The investigator (or his/her designated personnel, e.g. trial nurse) will maintain a drug dispensing log detailing the dates and quantities of IMP administered to, and used by, each patient, as well as the unique batch identifier used in the trial.

The tear-off portion of the labels will be affixed to the drug accountability form. The monitors will verify the drug accountability during the trial.

5.7 Return and Destruction of IMP

All used IMP will be destructed at the trial site in accordance with local requirements after the drug accountability has been finalised, verified by the monitor, and signed off by the investigator. Any material used for preparation of the infusion solution and for the infusion will be destroyed at the trial site immediately after usage according to standard procedures at the trial site.

All unused IMP will be accounted for and must be destructed in a certified way in accordance with trial-specific instructions.

6 TRIAL PROCEDURES

Participating sites should consider all septic shock patients for inclusion in the trial; the main reason for not being eligible will be documented. Refer to Section 4.1 for inclusion, exclusion, and eligibility criteria. One of the assigned CCCs must be contacted to confirm the eligibility of each patient.

The trial consists of a pre-IMP treatment period, an IMP treatment period, and a follow-up period (see Section 6.1).

The pre-IMP treatment period is defined as the time from sepsis-induced hypotension to the start of IMP infusion. To ensure start of IMP treatment without delay, informed consent will be obtained, in compliance with local regulations, as early as possible. During this period, baseline evaluations will be made.

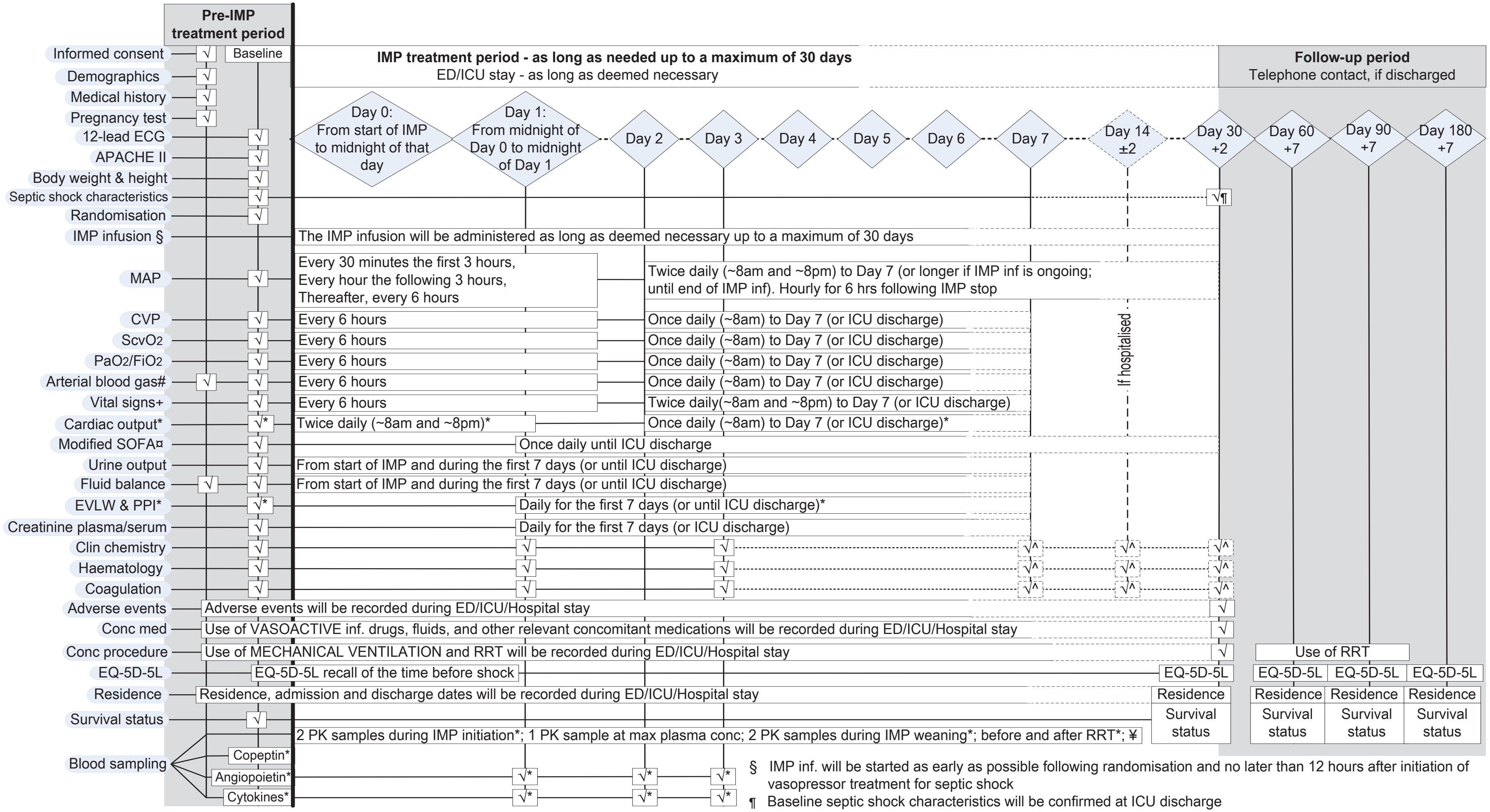
The IMP treatment period begins with Day 0 (which is defined as time from onset of IMP infusion to midnight of that day). The infusion of the IMP (selepressin or placebo) will be started as early as possible following randomisation and no later than 12 hours after initiation of continuous infusion of vasopressor treatment for septic shock. During the course of treatment, the IMP infusion rate will be adjusted as within pre-defined infusion rates to keep the MAP at the target. The IMP administration is described in Section 3.5.5 and the target MAP is defined in Section 7.1.11. A detailed IMP and vasopressor administration guideline will be provided to trial sites. The IMP infusion will continue as long as blood pressure support is deemed necessary with a maximum period of 30 days. A confirmation of the septic shock characteristics recorded at time of enrolment will be recorded in the eCRF at ICU discharge.

The follow-up period includes Days 30, 60, 90, and 180. If a patient has been discharged from the trial hospital, trial site personnel will contact the patient, the patient’s legal representative, or the patient’s health care professional to collect required trial information.

All assessments to be performed and all data to be collected during the trial are summarised in Section 6.1 and further described in Section 7. The majority of the assessments are widely and routinely used clinically and generally regarded as reliable, accurate, and relevant. Collection of data requiring invasive equipment or mechanical ventilation is applicable only if it is measured as part of local clinical practice. Samples collected and assessments performed for clinical purposes in accordance with standard of care after start of fluid resuscitation for hypotension and before start of IMP may be used as baseline values if deemed appropriate. In case several values of the same assessment are available, the recordings obtained closest to the start of IMP treatment will be used as baseline and the recordings obtained closest to a given time-point will be used as post-baseline values (if not otherwise specified).

At pre-selected trial sites, additional data collection will be performed to further evaluate the effect of selepressin.

6.1 Trial Flow Chart



§ IMP inf. will be started as early as possible following randomisation and no later than 12 hours after initiation of vasopressor treatment for septic shock
¶ Baseline septic shock characteristics will be confirmed at ICU discharge
* Only applicable for patients at pre-selected trial sites
^ Only applicable in hospitalised patients
# PaO_2, $PaCO_2$, SaO_2, HCO_3, pH, base excess, and lactate
\+ Diastolic and systolic blood pressure, heart rate, respiratory rate, body temperature
¤ Based on respiratory, cardiovascular, renal, hepatic, and coagulation
¥ A blood sample will be collected if the IMP infusion is prematurely paused or discontinued due to a serious adverse event

7 TRIAL ASSESSMENTS

7.1 Assessments Related to Endpoints

Vasopressor treatment, mechanical ventilation, and mortality are all part of the primary endpoint and it is extremely important to document these variables thoroughly and without delay. If a patient has been discharged from the trial hospital before Day 30, trial site personnel will contact the patient, the patient's legal representative, or the patient's health care professional to collect required information. If the trial site personnel are unable to collect the required information, Ferring should be notified. Completeness of data collection for the primary endpoint will be closely monitored during the conduct of the trial and issues of missing data collection will result in re-training (or termination) of trial sites.

7.1.1 Vasopressors

The integrity of the primary endpoint depends on accurate start and stop times of vasopressor treatment and therefore start and stop time of all vasopressor periods must be recorded in the eCRF. It is key to ensure that vasopressor treatment is neither prolonged longer than necessary nor prematurely stopped leading to episodes of hypotension.

All vasopressor treatment (any intravenous dose of norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, terlipressin, and IMP [selepressin and placebo]) must be thoroughly documented up to Day 30. The infusion rate of IMP will be recorded at baseline and at 1, 3, 6, 12, and 24 hours after start of IMP infusion. After 24 hours, the infusion rate of IMP will be recorded twice daily (around 8:00 a.m. and 8:00 p.m. when the MAP is recorded) until end of IMP treatment. In addition, the IMP infusion rate at the time of pharmacokinetic blood sampling will be recorded in the eCRF. In approximately 200 patients at pre-selected trial sites, the IMP infusion rate at 30 minutes after start IMP infusion will also be recorded (see Section 7.2.2). Furthermore, the total volume of infused IMP within the following time periods will be recorded: 0-12 hours, 12-24 hours, and from 24 hours to regular schedule according to clinical practice at each trial site. Thereafter, daily according to local clinical practice. For all other vasopressors, the dose of each vasopressor will be recorded at baseline and at 1, 3, 6, 12, and 24 hours after start of IMP infusion. After 24 hours, the dose of the individual vasopressors will be recorded twice daily (around 8:00 a.m. and 8:00 p.m. when the MAP is recorded) until end of vasopressor treatment.

7.1.2 Mechanical Ventilation

Respiratory failure, requiring mechanical ventilation, is a common complication of septic shock. In septic shock patients, it is a key goal of management of ventilation to minimise the duration of ventilation because increased duration of mechanical ventilation increases the risk of nosocomial pneumonia, neuromuscular weakness, and death.

The use of mechanical ventilation (as defined in Section 2.2) must be thoroughly documented up to Day 30. Start and stop time of all mechanical ventilation periods must be recorded in the eCRF.

The weaning of mechanical ventilation will be based on daily spontaneous breathing trials as described in a trial-specific weaning guide. The time and outcome (i.e. success or failure) of each spontaneous breathing trial will be documented in patient's medical records to monitor if a successful spontaneous breathing trial is followed by extubation.

7.1.3 Renal Replacement Therapy (RRT) and Renal Function

Acute kidney injury is a common complication of septic shock. Treatment of acute kidney injury is primarily supportive including RRT (continuous RRT, intermittent haemodialysis, or peritoneal dialysis). The decision to initiate RRT in a patient enrolled in this trial will be based on local clinical practice. The use of RRT must be thoroughly documented up to Day 90. The reason for and type of RRT as well as start and stop time will be recorded in the eCRF. If a patient is shifted from continuous RRT to intermittent haemodialysis, the patient is still on RRT until the last hemodialysis has been performed.

Renal function will be assessed, using urine output, plasma/serum creatinine, and creatinine clearance, at baseline and the first 3 days after initiation of IMP infusion and up to 7 days if collected for clinical purposes [or until ICU discharge if the patient leaves the ICU before Day 7]). Creatinine clearance will be determined by estimated glomerular filtration rate (using plasma/serum creatinine, age, and gender as per Cockcroft-Gault equation).

7.1.4 Modified Sequential Organ Failure Assessment (SOFA) Score

SOFA is a scoring system used to track the patient's organ function status during episodes of critical illness (Vincent et al, 1996).

In this trial, a modified version of the SOFA will be used (i.e. SOFA except the Glasgow Coma Scale). As many patients are sedated due to mechanical ventilation a meaningful assessment of the neurological function using the Glasgow Coma Scale cannot be performed. In addition, any dose of IMP, vasopressin, terlipressin, or phenylephrine will attribute 3 points on the cardiovascular scale, and any dose of the positive inotropes milrinone and levosimendan will attribute 2 points on the cardiovascular scale.

The worst value for each individual organ system (i.e. respiratory, cardiovascular, renal, coagulation, and hepatic) components within the past 24 hours (at baseline and once daily until ICU discharge) will be recorded in the eCRF and the eCRF will calculate the overall modified SOFA score.

7.1.5 Mortality Rate and Hospitalisation

The actual date and time for ED, ICU, and hospital admission and discharge as well as time of death (if applicable) will be recorded. Trial site personnel will visit or contact each patient who is still hospitalised and contact each patient or healthcare professional for patients who has left the hospital to determine survival status and current residence.

Functional status and residence of each patient before the infection leading to this episode of septic shock (baseline) as well as functional/survival status and residence throughout the trial will be recorded by using one of the following alternatives:

- Home, receiving no support
- Home, receiving paid professional support
- Home, receiving unpaid support
- Rehabilitation site/skilled (or unskilled) nursing facility
- Other acute care hospital (including long-term acute care)
- Still in (or readmitted to) trial hospital
- Unknown
- Dead

7.1.6 Health-related Quality of Life

The EuroQoL-5-Dimensions (EQ-5D™) will be used to assess patient's overall health. EQ-5D™ is a standardised instrument in two parts for use as a measure of health outcome; it provides a simple, generic measure of health for clinical and economic appraisal (The EuroQol Group; http://www.euroqol.org). The first part includes five dimensions where the patient will indicate which given statements best describe the health state on the day of questionnaire completion. The second part contains a visual analogue scale (VAS) where the patient will indicate how good or bad his or her own health is on the day of questionnaire completion. The VAS scores range from 0 (worst health state) to 100 (best health state).

The five level version of the instrument will be used in this trial (i.e. EQ-5D-5L).

The baseline value refers to a recall of the time before the infection leading to the septic shock episode and the EQ-5D-5L will be completed as soon as possible after the shock state. A telephone interview will be adequate at Days 30, 60, 90, and 180 if the patient has been discharged. The responses will be entered into the eCRF.

The investigator and/or delegated personnel will receive training and instruction in completion of the questionnaire before enrolment of patients.

Each patient must receive proper training and instruction before use. The investigator or a delegated trial team member will instruct the patient to respond to each question without influence from trial team members or accompanying family or friends and explain that there are no right or wrong answers. Nobody will be allowed to answer or interpret questions for the patient.

7.1.7 Fluid balance, Fluids, and Urine Output

In order to investigate selepressin's effect on fluid balance, it is important to document all fluid administered during this episode of severe sepsis/septic shock including the time (i.e. up to 18 hours) before initiation of the IMP infusion (baseline) and during the first 7 days after initiation of IMP infusion [or until ICU discharge if the patient leaves the ICU before Day 7]). The period of Day 0 is from the start of IMP infusion to the time of the first regular daily fluid recording according to local clinical practice. Thereafter, daily fluid recordings according to local clinical practice.

The fluid balance (as calculated in accordance with local clinical practice) and the total amount of intravenous fluid and urine output will be recorded in the eCRF. Urine output will be used for assessing renal function (see Section 7.1.3).

7.1.8 Adverse Events

The procedure for collecting and reporting adverse events is described in Section 8.

7.1.9 Safety Laboratory Variables (Clinical Chemistry, Haematology, and Coagulation)

Standard safety laboratory variables will be analysed using standard equipment in accordance with local clinical practice. Accreditation/certification of the laboratories and reference ranges of the laboratory variables will be kept in the trial files. The investigator must document their review of the laboratory results. Any laboratory abnormality should be assessed by the investigator as to whether it constitutes an adverse event (see Section 8.1).

The baseline levels and the results, of the variables as listed in Table 2, obtained 1 and 3 days after initiation of the IMP infusion will be recorded. The results obtained at Days 7, 14, 30 will also be recorded if the patient is still in trial hospital.

Daily creatinine, bilirubin, and platelets values will be used for the SOFA scores (see Section 7.1.4). Creatinine level will also be used for assessing renal function and calculation of creatinine clearance (see Section 7.1.3).

Table 2 Safety Laboratory Variables

Clinical Chemistry	Haematology	Coagulation
Alanine aminotransferase	Haematocrit	Activated partial thromboplastin time
Albumin	Haemoglobin	
Alkaline phosphatase	Platelet count	Prothrombin time / international normalised ratio
Aspartate aminotransferase	Red blood cell count	
Calcium (free or total)	White blood cell count	
Chloride		
C-reactive protein		
Creatine phosphokinase		
Creatinine (plasma or serum)		
Lactate dehydrogenase		
Phosphate		
Potassium		
Sodium		
Total bilirubin		
Troponin (I or T)		
Urea (blood urea nitrogen or blood urea)		

7.1.10 Diastolic and Systolic Blood Pressure, Heart Rate, Respiratory Rate, and Body Temperature

Diastolic and systolic blood pressure, heart rate, respiratory rate, and body temperature will be monitored up to Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) using standard equipment in accordance with local clinical practice. The baseline values, the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and twice daily (around 8:00 a.m. and 8:00 p.m.) the following days will be recorded in the eCRF. Any abnormality should be assessed by the investigator as to whether it constitutes an adverse event (see Section 8.1).

7.1.11 Mean Arterial Pressure

The MAP will be measured intra-arterially (or non-invasively if an arterial line is not available) using standard equipment in accordance with local clinical practice. The method (intra-arterially or non-invasively) used will be documented in the eCRF.

The infusion rate of the IMP will be adjusted to keep the MAP at the target of 65 mmHg. However, a different target MAP will be accepted, if pre-specified in the eCRF and if appropriate, as judged by the investigator, for the clinical management e.g. in patients with previous hypotension or hypertension (if deemed necessary to maintain adequate organ perfusion). In such case, the target MAP and the reason for why a different target MAP has been chosen will be recorded in the eCRF.

The following MAP values will be recorded in the eCRF:

- Baseline.
- Every 30 minutes during the first 3 hours after initiation of IMP infusion.
- Every hour the following 3 hours.
- Thereafter, every 6 hours up to midnight of Day 1.
- Twice daily (around 8:00 a.m. and 8:00 p.m.) until Day 7 or longer if IMP infusion is ongoing (until end of IMP infusion).
- Every hour for 6 hours following discontinuation of the IMP infusion.

In order to assess that vasopressor treatment is not prematurely stopped leading to episodes of hypotension, the MAP will be recorded hourly for the first 6 hours after complete weaning of IMP and episodes of clinically relevant hypotension (i.e. any period recorded in the hospital/medical source records through routine clinical care where the MAP drops below 60 mmHg for more than one hour) will be recorded in the eCRF detailing the total duration of the episode, the lowest MAP measured during the episode, and the actions taken to correct the hypotension.

MAP is also needed for the SOFA score (see Section 7.1.4).

In addition, in the first patients at each trial site, the MAP should be noted in the patient's medical record at the time when the infusion rate of the IMP or the norepinephrine/noradrenaline is adjusted in order to monitor compliance with the IMP and vasopressor administration guide.

7.1.12 PaO_2/FiO_2 Ratio

PaO_2/FiO_2 ratio will be monitored up to Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) in accordance with local clinical practice. The baseline value, the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and daily (around 8:00 a.m.) the following days will be recorded in the eCRF.

The PaO_2 and FiO_2 are also needed for the SOFA score (see Section 7.1.4).

7.1.13 Extravascular Lung Water and Pulmonary Permeability Index

The extravascular lung water (EVLW) is the amount of water that is contained in the lungs outside the pulmonary vasculature, that is, in the interstitial and alveolar spaces (Jozwiak et al, 2013). The ratio between the EVLW and the pulmonary blood volume is called the pulmonary permeability index (PPI) which is believed to reflect the permeability of the alveolo-capillary barrier (Monnet et al, 2007). Both EVLW and PPI are useful tools to characterise pulmonary oedema.

EVLW and PPI will be collected at pre-selected trial sites who measure these variables as part of clinical practice. The baseline values and the daily (around 8:00 a.m.) values for the first 7 days after initiation of IMP infusion (or until ICU discharge if the patient leaves the ICU before Day 7), as measured using standard equipment in accordance with local clinical practice, will be recorded if available.

7.1.14 Cardiac Output

Cardiac output will be monitored up to Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) at pre-selected trial sites who measure cardiac output as part of clinical practice. The baseline value and the values obtained twice daily (around 8:00 a.m. and 8:00 p.m.) for the first 2 days after initiation of IMP infusion and daily (around 8:00 a.m.) for the following days, as measured using standard equipment in accordance with local clinical practice, will be recorded if available.

7.2 Trial-specific Blood Sampling

7.2.1 Copeptin Levels

In patients at pre-selected trial sites, a blood sample will be collected before initiation of the IMP infusion for measurement of baseline levels of copeptin.

7.2.2 Pharmacokinetics

One blood sample for measurement of maximal plasma concentration of selepressin will be collected from all patients at one of the following time points (the one that occurs first): 1) right before first attempt to wean the IMP infusion, 2) after at least 7 hours of IMP infusion at maximal infusion rate, 3) at time of first pause of IMP, or 4) at time of IMP discontinuation.

Moreover, if the IMP infusion is prematurely paused or discontinued due to a serious adverse event, an additional blood sample will be collected at the time of IMP discontinuation or pause for measurement of plasma concentration of selepressin.

The IMP infusion rate at the time of the blood sampling will be recorded in the eCRF.

In approximately 200 patients at pre-selected trial sites, additional blood sampling for analysis of pharmacokinetic parameters will be conducted twice during the initiation of the IMP infusion (at approximately 1-3 hours and 6-9 hours after start of infusion) and twice during the weaning of the IMP infusion (at approximately 1-2 hours and 2-3 hours after stop of infusion). If these patients are on RRT, additional blood sampling will also be performed before and after the RRT. The IMP infusion rate at 30 minutes after start of IMP infusion will be recorded in the eCRF.

7.2.3 Cytokines

The development of septic shock is associated with elevated levels of proinflammatory cytokines (Fjell et al, 2013). Exposure to inflammatory mediators and interaction with leukocytes causes endothelial activation and damage (Aird et al, 2003). Vasopressin has effects on immunity (Russell et al, 2010) and decreases cytokines more than does norepinephrine/noradrenaline (Russell et al, 2013). Selepressin is a potent $V1_a$ agonist that could also have effects on cytokines. Furthermore, cytokine levels in the blood may be predictive of the response to selepressin compared to placebo in septic shock.

In patients at pre-selected trial sites, a blood sample will be collected at baseline (before initiation of IMP infusion) and at the first 3 days after initiation of IMP infusion for analysis of several cytokines e.g. VEGFA (vascular endothelial growth factor), interleukin (IL)-6, IL-2, CCL (CC chemokine ligand)-22, CCL11, LTA (lymphotoxin alpha), CSF (colony-stimulating factor)-2.

7.2.4 Angiopoietins

Angiopoietin-1 and angiopoietin-2 are circulating proteins with opposing roles on the vascular endothelium, i.e. angiopoietin-1 protects against vascular leakage whereas angiopoietin-2 promotes increased vascular permeability.

In patients at pre-selected trial sites, a blood sample will be collected at baseline (before initiation of IMP infusion) and at the first 3 days after initiation of IMP infusion for measurement of angiopoietin-1 and -2 levels.

7.3 Other Assessments

7.3.1 Central Venous Pressure

If central venous pressure (CVP) is measured for clinical purposes (via a central venous catheter using standard equipment in accordance with local clinical practice), the baseline value and the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and a daily (around 8:00 a.m.) value the following days until Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) will be recorded, if available.

7.3.2 Central Venous Oxygen Saturation

If central venous oxygen saturation ($ScvO_2$) is measured for clinical purposes (via a central venous catheter using standard equipment in accordance with local clinical practice), the baseline value and the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and a daily (around 8:00 a.m.) value the following days until Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) will be recorded, if available.

7.3.3 Arterial Blood Gases and Lactate Levels

If arterial blood gases and acid/base status (arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$), arterial oxygen saturation (SaO_2), arterial pH, bicarbonate (HCO_3), base excess) and lactate levels are available, as measured using standard equipment in accordance with local clinical practice, the baseline values and the values measured every 6 hours after initiation of IMP infusion up to midnight of Day 1 and a daily (around 8:00 am) result the following days until Day 7 (or until ICU discharge if the patient leaves the ICU before Day 7) will be recorded. In addition, the highest lactate level obtained after start of vasopressor treatment but before start of IMP will be recorded. If arterial lactate level has not been measured, the venous value can be recorded if available.

PaO_2 will be used for the SOFA (see Section 7.1.4) and the PaO_2/FiO_2 ratio (see Section 7.1.12).

7.4 Demographics and Other Baseline Assessments

7.4.1 Demographics

Demographic data including date of birth, race, and ethnic origin will be recorded.

7.4.2 Septic Shock Characteristics

Baseline septic shock characteristics (including site of infection, cause of infection, and whether the infection was proven or suspected) will be recorded. In addition, a confirmation of the septic shock characteristics recorded at time of enrolment and whether initial antibiotic treatment and source control was adequate will be recorded at ICU discharge.

7.4.3 APACHE II

APACHE II is a classification system which uses a point score based upon initial values of routine physiologic measurements, age, and previous health status to provide a general measure of severity of disease (Knaus et al, 1985). In this trial, the baseline (within the preceding 24 hours before initiation of the IMP infusion) APACHE II score will be recorded to assess the similarity of illness severity at baseline between treatment arms.

7.4.4 Electrocardiography

A 12-lead baseline ECG obtained prior to start of IMP infusion must be available. ECGs obtained for clinical purposes within 48 hours of starting IMP may be used as baseline ECG, provided there is no indication of any change in the cardiac condition. If there is any reason to suspect a change in cardiac condition, a new 12-lead ECG should be obtained prior to initiation of IMP. The purpose of the baseline ECG is to have a status at entry to which later ECGs may be compared, should this become relevant. The ECG should be obtained in accordance with local clinical practice and ECG recordings should capture at least four QRS complexes, i.e. three evaluable RR intervals.

Continuous ECG monitoring will be performed as medically required in accordance with local clinical practice.

7.4.5 Body Weight and Height

The baseline (usual) body weight and height will be assessed in accordance with local clinical practice. The baseline weight will be used for calculation of the infusion rate of IMP and the norepinephrine/noradrenaline dosing.

7.4.6 Medical History

Information about relevant medical history will be collected. The Charlson Comorbidity Index will be used.

7.4.7 Prior and Concomitant Medication/Procedure

Information about relevant concomitant medications/procedures will be collected (see Section 4.3).

7.4.8 Pregnancy Test

Women of child bearing potential will be tested for pregnancy (in accordance with local clinical practice) before initiation of IMP infusion. Urine dipstick pregnancy tests will be provided to the trial sites.

7.5 Handling of Biological Samples

Copeptin, cytokines, angiopoietin-1, angiopoietin-2, and selepressin levels will be measured by contract research laboratories. A detailed description of the sample collection and shipment procedures will be included in a trial-specific laboratory manual. These blood samples will be maintained in storage after the end of the trial. Destruction will take place within one year after last visit/trial-related contact with the last ongoing patient. Bio bank/data protection will be handled in compliance with the national/local regulations.

Handling of all other biological samples will be in accordance with local clinical practice.

8 ADVERSE EVENTS

8.1 Adverse Event Definition

An adverse event is any untoward medical occurrence in a patient participating in a clinical trial. It includes:

- Any unfavourable and unintended sign, symptom or disease temporally associated with the use of the IMP, whether or not considered to be caused by the IMP.
- Adverse events commonly observed and adverse events anticipated based on the pharmacological effect of the IMP.
- Any laboratory abnormality, vital sign, or finding from physical examination assessed as clinically significant and unanticipated in a setting of septic shock by the investigator. Findings from assessments and examinations done during screening are not adverse events, but are recorded as medical history.
- Accidental injuries, reasons for any change in medication (drug and/or dose), reasons for any medical, nursing or pharmacy consultation, or reasons for admission to hospital or surgical procedures.
- Overdoses and medication errors with and without clinical consequences.

8.2 Collection and Recording of Adverse Events

8.2.1 Collection of Adverse Events

The investigator must monitor the condition of the patient throughout the trial from the time of obtaining informed consent until the last visit/trial-related contact.

The sources of adverse events cover:

- Investigations and examinations where the findings are assessed by the investigator to be clinically significant changes or abnormalities which are unanticipated in the setting of septic shock.
- The patient’s response to questions about his/her health (a standard non-leading question such as "How have you been feeling since your last visit?" is asked at each visit).
- Symptoms spontaneously reported by the patient.
- Other information relating to the patient’s health becoming known to the investigator (e.g. hospitalisation in the follow-up period).

8.2.2 Recording of Adverse Events

The investigator must record all adverse events in the adverse event log provided in each patient's eCRF with information about:

- Adverse event

- Date and time of onset (time can be omitted, if applicable)
- Intensity
- Causal relationship to the IMP
- Action taken to the IMP
- Other action taken
- Date and time of outcome (time can be omitted, if applicable)
- Outcome
- Seriousness

Each of the items in the adverse event log is described in detail in the following sections.

Adverse Event

Adverse events should be recorded as diagnoses, if available. If not, separate signs and symptoms should be recorded. One diagnosis/symptom should be entered per record.

If a patient suffers from the same adverse event more than once and the patient recovers in between the events, the adverse events should be recorded separately.

If an adverse event changes (decreases or increases) in intensity, a worst-case approach should be used when recording the event, i.e. the highest intensity and the longest duration of the event. Exception: if an adverse event with onset before the first administration of the IMP (i.e. a pre-treatment adverse event) changes in intensity after the administration of the IMP, this must be recorded as two separate events. The initial adverse event should be recorded with outcome “not yet recovered” and the date and time of outcome is when the intensity changed. The second adverse event should be recorded with date and time of onset when the intensity changed.

Pre-existing conditions not related to the patient’s current clinical setting of vasopressor-dependent septic shock are not adverse events, but become adverse events if worsening occurs after administration of the IMP during the trial. Pre-existing clinically significant conditions diagnosed or observed as a result of the screening procedures must be recorded as medical history.

Note the following: A procedure is not an adverse event; the reason for conducting the procedure is. However, a procedure may be captured along with the reason for conducting the procedure if the investigator finds it adds value to emphasise the procedure. Hospitalisation is not an adverse event; the reason for hospitalisation is. Death is not an adverse event, but the cause of death is (an exception is sudden death of unknown cause, which is an adverse event).

Overdoses and medication errors with or without clinical consequences are recorded as adverse events. The medication error must be specified. Any clinical consequence must be reported as “xxxx due to overdose/medication error”. In the absence of a clinical consequence this must specified e.g. “overdose with no clinical consequence”.

Date and Time of Onset

The date of onset is the date when the first sign(s) or symptom(s) were first noted. If the adverse event is an abnormal clinically significant laboratory test or outcome of an examination, the onset date is the date the sample was taken or the examination was performed. For pre-existing clinically significant conditions (diagnosed or observed as a result of the screening procedures) becoming worse after administration of the IMP, the date of onset is the date the worsening began. Time is to be recorded if relevant for the adverse event.

Intensity

The intensity of an adverse event must be classified using the following 3-point scale:

- Mild: Awareness of signs or symptoms, but no disruption of usual activity.
- Moderate: Event sufficient to affect usual activity (disturbing).
- Severe: Inability to work or perform usual activities (unacceptable).

Causal Relationship to IMP

The possibility of whether the IMP caused the adverse event must be classified as one of the following:

Reasonable possibility
There is evidence or argument to suggest a causal relationship between the IMP and the adverse event. The adverse event may occur as part of the pharmacological action of the IMP or may be unpredictable in its occurrence. Examples:

- Adverse events that are uncommon but are known to be strongly associated with exposure of the IMP.
- Adverse events that are not commonly associated with exposure of the IMP, but the event occurs in association with other factors strongly suggesting causation, such as a strong temporal association or the event recurs on rechallenge.

No reasonable possibility
There is no reasonable evidence or argument to suggest a causal relationship between the IMP and the adverse event. Examples:

- Known consequences of the underlying disease or condition under investigation.
- Adverse events common in the trial population, which are also anticipated to occur with some frequency during the course of the trial, regardless of exposure of the IMP.

Action Taken to IMP

The action taken to the IMP in response to an adverse event must be classified as one of the following:

- No change (medication schedule maintained or no action taken).

- Discontinued (medication schedule modified through permanent termination of prescribed regimen of medication).
- Paused (medication schedule was modified by temporarily terminating a prescribed regimen of medication).

Other Action Taken

Adverse events requiring therapy must be treated with recognised standards of medical care to protect the health and well-being of the patient. Appropriate resuscitation equipment and medicines must be available to ensure the best possible treatment of an emergency situation.

If medication is administered to treat the adverse event, relevant medication will be entered in the medication forms and the serious adverse event form provided in each patient's eCRF.

Date and Time of Outcome

The date and time (if applicable) the patient recovered or died.

Outcome

The outcome of an adverse event must be classified as one of the following:

- Recovering (the event is improving).
- Recovered (the event has improved i.e. fully recovered or the condition has returned to the level observed at initiation of trial treatment).
- Recovered with sequelae (patient recuperated but retained pathological conditions resulting from the prior disease or injury e.g. resulted in persistent or significant disability / incapacity).
- Not recovered (the event has not improved).
- Fatal (termination of life as result of an adverse event).

8.3 Adverse Events of Special Interest

Some event types are considered especially important to record during this trial as they are considered a crucial part of both septic shock and the safety profile of the IMP. Event types that should always be reported as adverse events are:

- Episodes of atrial fibrillation and other cardiac arrhythmias that require treatment intervention, specifying the type of arrhythmia, treatment and/or intervention, severity and outcome.
- Stroke and other cerebrovascular events.
- Ischaemic events (myocardial ischaemia, peripheral ischaemia or mesenteric ischaemia).

8.4 Pregnancy

Known pregnancy is an exclusion criteria and women of child bearing potential will be tested for pregnancy and excluded if pregnant.

8.5 Serious Adverse Events

8.5.1 Serious Adverse Event Definition

An event is defined as being a serious adverse event if it:	**Guidance:**
Results in **death**	Any event resulting in a fatal outcome must be fully documented and reported, including deaths occurring within four weeks after the treatment ends and irrespective of the causal relationship to the IMP. The death of a patient enrolled in a trial is *per se* not an event, but an outcome (except in case of sudden death from unknown cause).
Is **life-threatening**	The term life-threatening refers to an adverse event in which the patient was at immediate risk of death at the time of the event. It does not refer to an event, which may have caused death if it were more severe.
Requires in-patient **hospitalisation** or prolongation of existing hospitalisation	The term hospitalisation means that the patient was admitted to hospital or that existing hospitalisation was extended as a result of an event. Hospitalisation describes a period of at least 24 hours. Over-night stay for observation, stay at emergency room or treatment on an out-patient basis do not constitute a hospitalisation. However, medical judgement must always be exercised and when in doubt the case should be considered serious (i.e. if case fulfils the criterion for a medically important event). Hospitalisations for administrative or social purposes do not constitute a serious adverse event. Hospital admissions and/or surgical operations planned before trial inclusion are not considered adverse events, if the illness or disease existed before the patient was enrolled in the trial, provided that the condition did not deteriorate during the trial.
Results in persistent or significant **disability/incapacity**	Disability/incapacity means a substantial disruption of a person's ability to conduct normal life functions. In doubt, the decision should be left to medical judgement by the investigator.
Is a **congenital anomaly/birth defect**	Congenital anomaly/birth defect observed in any offspring of the patient conceived during treatment with the IMP.
Is an **important medical event**	Important medical events are events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of important medical events include adverse events that suggest a significant hazard, contraindication or precaution, occurrence of malignancy or development of drug dependency or drug abuse. Medical and scientific judgement should be exercised in deciding whether events qualify as medically important. Important medical events include any suspected transmission of an infectious agent via a medicinal product. Any organism virus or infectious particle (e.g. prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings indicating an infection in a patient exposed to a medicinal product.

8.5.2 Collection, Recording and Reporting of Serious Adverse Events

Serious Adverse Event Reporting by the Investigator

All serious adverse events must be reported **immediately** to Global Pharmacovigilance at Ferring as soon as it becomes known to the investigator and not later than within 24 hours of their knowledge of the occurrence of a serious adverse event.

The investigator is responsible for submitting the completed serious adverse event report form with the fullest possible details **within 3 calendar days** of his/her knowledge of the serious adverse event.

The serious adverse event report form is included in the eCRF system, and must be completed and submitted according to the instructions provided. In case the eCRF cannot be accessed and hence the serious adverse event report form cannot be filled in within the eCRF system, a paper serious adverse event report form should be used and sent to Global Pharmacovigilance at Ferring using the contact details below.

Global Pharmacovigilance, Ferring Pharmaceuticals A/S
E-mail: [redacted]
Fax: [redacted]

Completion of the demographics, adverse events, medical history, and concomitant medication are mandatory for initial reports and for follow-up reports if any relevant changes have been made since the initial report. Data entries must have been made in the eCRF for Ferring Global Pharmacovigilance to access the information.

Additional information relevant to the serious adverse event such as hospital records, results from investigations, e.g. laboratory parameters (that are not already uploaded in the eCRF), invasive procedures, scans and x-rays, and autopsy results can be faxed or scanned and e-mailed to Ferring Global Pharmacovigilance using the contact details in the section above. In any case this information must be supplied by the investigator upon request from Ferring. On any copies provided, details such as patient’s name, address, and hospital identification number should be concealed and instead patient number should be provided.

The investigator will supply Ferring and the IEC/IRB with any additional requested information such as results of post-mortem examinations and hospital records.

Expedited Reporting by Ferring

Ferring will report all adverse events that are **serious, unexpected and with a reasonable possible causality to the IMP** as judged by either the investigator or Ferring to the relevant parties within the stipulated timelines.

Taking into consideration the patient population, fatal outcome of certain events occurring in the trial will be considered expected; a mortality rate of 20-25% has been assumed. Consequently the fatal outcome is not considered to be a reportable event, whereas the investigator must still report the events to Ferring as outlined above. If the cause of death is one of the below MedDRA terms or those medically judged to be similar, the event will not qualify for expedited reporting:

Circulatory collapse, distributive shock, endotoxic shock, septic shock, shock, toxic shock syndrome, acute respiratory failure, cerebral hypoperfusion, hypoperfusion, multi-organ failure.

The expectedness is assessed by Ferring according to the investigator's brochure for selepressin.

Serious adverse events will be considered reportable regardless of whether or not the IMP was used in accordance with the provisions in the protocol and investigator's brochure.

8.6 Follow-up of Adverse Events and Serious Adverse Events

8.6.1 Follow-up of Adverse Events with Onset during the Trial

During the trial, the investigator must follow-up on each adverse event until it is resolved or until the medical condition of the patient is stable.

After the patient's last visit, the investigator must follow-up on any adverse event that occurred during the trial and are classified as serious or considered to have a reasonable possible causality to the IMP with the outcome 'not recovered' until it is resolved or until the medical condition of the patient is stable. All such relevant follow-up information must be reported to Ferring. If the event is a chronic condition, the investigator and Ferring may agree that further follow-up is not required.

8.6.2 Collection of Serious Adverse Events with Onset after End of Trial

If an investigator becomes aware of a serious adverse event after the end of the trial, and he/she assesses the serious adverse event to have a reasonable possible causality to the IMP, the case will have to be reported to Ferring, regardless how long after the end of the trial this takes place.

9 STATISTICAL METHODS

The Global Biometrics department at Ferring will be responsible for the final statistical analyses. External statistical consultancies will be responsible for all interim analyses during the trial. All analyses will be detailed in a separate statistical analysis plan.

The Health Economics & Outcome Research department at Ferring will evaluate the cost-effectiveness of selepressin utilising relevant data recorded in this trial. Details of these analyses will be described in a separate health economic analytical plan.

The department of Experimental Medicine at Ferring will perform an analysis of pharmacokinetic parameters in a subset of patients. Details of this analysis will be described in a separate pharmacokinetic analysis plan.

9.1 Determination of Sample Size

At least 1800 evaluable patients combined for Part 1 and Part 2 are needed for the final analysis. The overall power of obtaining statistical significance based on combined evidence from Part 1 and Part 2 is 91% in situations where all four arms have a true underlying 1.5% lower mortality rate and a 1.5-day higher expected number of P&VFDs for survivors (corresponding to an overall treatment effect of 1.5 P&VFDs) as compared to placebo. If the effect sizes are 2% on mortality and 2 days for P&VFDs in survivors for all four arms (corresponding to an overall treatment effect of 2 P&VFDs) then the overall power is 98%. In this latter case the probability of engaging into Part 2 is ~99%. The four arms are described in Table 1.

9.2 Patient Disposition

A summary table will present, for each part of the trial and overall, the number of patients in the population sets: ‘Screened’, ‘Intention to treat’, Full analysis set’, ‘Per protocol’, ‘Safety’, ‘Completed trial’, ‘Withdrawals’, and ’IMP discontinuations’ with a breakdown of reasons/categories for and trial withdrawals and IMP discontinuations.

The patient disposition table will be broken down by each of the stratification variables [the need for mechanical ventilation (Yes/No), norepinephrine/noradrenaline requirement at baseline (< or ≥30 μg/min) and plasma/serum creatinine (< or ≥150 μmol/L)] and broken down chronologically displaying number of patients ‘completed’ and ‘withdrawn from trial’ at Day 30, Day 90, and Day 180.

The number of patients screened but not randomised/allocated to treatment will be presented with the reason(s) for screen failure in a data listing.

All major protocol deviations (including misrandomisation), based on the full analysis set (FAS), will be summarised for each part of the trial.

Furthermore, 1-Kaplan Meier (KM) plots, based on the intention-to-treat (ITT), will be presented for the time to trial withdrawals/IMP discontinuations (whichever comes first) differentiated by reason for trial withdrawal/IMP discontinuation using cumulative incidence functions. Dropout rates between treatment groups will be evaluated by the log-rank test.

9.3 Protocol Deviations

Protocol deviations will be classified as 'minor' or 'major' by the Ferring clinical trial team on the basis of a blinded review of data before declaration of clean file and lock of database.

Major protocol deviations, such as significant non-compliance or other serious unforeseen violations deemed to invalidate the data and affect the conclusions of the trial, will lead to exclusion of data from the per protocol (PP) analysis set, while data will not be excluded because of minor protocol deviations. All major protocol deviations will be detailed and documented in the clean file document prior to database release. All protocol deviations (minor and major) will be listed in patient data listings.

9.4 Analysis Sets

9.4.1 Intention-to-Treat Analysis Dataset

The ITT analysis set comprises all randomised (as planned) patients.

9.4.2 Full Analysis Set

The FAS comprises data from all randomised (as planned) and dosed patients.

9.4.3 Per Protocol Dataset

Patients in the FAS will be excluded from the PP analysis set if they meet any major protocol deviations as defined in the statistical analysis plan. Data will be used up to the point of protocol deviation.

9.4.4 Safety Dataset

The safety analysis set comprises all treated patients and is analysed according to the actual treatment received.

9.5 Trial Population

9.5.1 Demographics and other Baseline Characteristics

Descriptive statistics of demographics and other baseline characteristics (including vital signs) will be presented for the FAS population by treatment arm and total.

Categorical data will be summarised using numbers and percentages. The percentages are based on the total number of patients with a corresponding assessment. Continuous data will be presented, for example, using the number of patients, mean and standard deviation, median, interquartile range, minimum and maximum. All baseline characteristics will be listed.

9.5.2 Medical History and Prior/Concomitant Medication

Medical history will be summarised by treatment arm and total and presented in patient data listings.

Prior and concomitant medication will be summarised by treatment arm and total and presented in patient data listings.

9.6 Endpoint Assessments

9.6.1 General Considerations

All statistical tests will be performed using a two-sided test at a 5% significance level.

If the trial is stopped prematurely due to e.g. futility, the data will be analysed as planned in accordance with the statistical analysis plan.

The efficacy endpoints will be analysed for the FAS and the PP analysis set, with the FAS being considered as primary and the PP analyses as supportive.

Categorical data will be summarised using counts and percentages, while continuous data will be presented using the number of patients, mean, standard deviation, median, interquartile range, minimum and maximum.

All assessments will be listed in patient data listings.

9.6.2 Primary Endpoint

The primary endpoint of this trial is "vasopressor and mechanical ventilator-free days (P&VFDs) up to Day 30."

This composite endpoint is defined as the number of days (reported to one decimal place (0.0 to 30.0)) from start of treatment with the investigational medicinal product (IMP) [selepressin or placebo] to 30.0 days thereafter during which the patient is: 1) alive; 2) free of treatment with intravenous vasopressors; and 3) free of any invasive mechanical ventilation (see definition below).

Patient Death

By definition, any patient that dies within this 30-day period will be assigned zero P&VFDs, even if there is a period during which the patient is alive and free of both vasopressor treatment and mechanical ventilation.

Definition of "Free of Vasopressors"

Free of vasopressors means less than 60 minutes during any contiguous 24-hour period (regardless of calendar day). If a patient requires periods of vasopressors longer than 60 minutes in total during any 24-hour period, the intervening intervals during which they are free of vasopressors will not be included in the period free of vasopressors in the determination of the number of P&VFDs. Thus, the period free of vasopressors begins at the end of the last use of vasopressors that was either: 1) longer than 60 minutes in duration; or 2) part of greater than 60 minutes of use within a contiguous 24-hour period.

Norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, terlipressin, and IMP (i.e. selepressin and placebo) all constitute a vasopressor for the purpose of the primary analysis.

Vasopressor use due to anaesthesia or procedure-induced hypotension during and up to three hours after a surgery or procedure (including bedside) is exempt from this rule (i.e. such use of vasopressors would not affect the calculation of P&VFDs).

Definition of "Free of Mechanical Ventilation"

Mechanical ventilation is defined as use of endotracheal or tracheostomy tube assisted ventilation (>5 cm H_2O continuous positive airway pressure and >5 cm H_2O of pressure support from the ventilator in tracheostomy patients). End of mechanical ventilation is defined as: 1) extubation of intubated patients or 2) ≤5 cm H_2O continuous positive airway pressure and ≤5 cm H_2O of pressure support from the ventilator in tracheostomy patients. If non-invasive ventilation by mask or bag (>5 cm H_2O of pressure support) is deployed to avoid (re)intubation, it also counts as mechanical ventilation. However, all other uses of non-invasive ventilation such as chronic night-time use of positive airway pressure for COPD or sleep apnea does not count as mechanical ventilation (regardless of pressure). Free of mechanical ventilation means less than 60 minutes during any contiguous 24-hour period (regardless of calendar day). If a patient requires mechanical ventilation for periods longer than 60 minutes in total during any 24-hour period, the intervening intervals during which they are not receiving mechanical ventilation will not be included in the period free of mechanical ventilation in the determination of the number of P&VFDs. Thus, the period free of mechanical ventilation begins at the end of the last use of mechanical ventilation that was either: 1) longer than 60 minutes in duration; or 2) part of greater than 60 minutes of use within a contiguous 24-hour period.

The use of mechanical ventilation associated with anaesthesia or procedural sedation during and up to three hours after a surgery or procedure (including bedside) is exempt from this rule (i.e. such use of mechanical ventilation would not affect the calculation of P&VFDs).

It is important to note that the determination of freedom from vasopressors and freedom from mechanical ventilation are made separately; in other words, periods of vasopressor use and mechanical ventilation are not combined when determining whether 60 minutes of use has occurred within a 24-hour period.

Missing data during the time of hospitalisation will be imputed using a worst case approach taking into account previous and subsequent starting and stopping times of vasopressor administration and mechanical ventilation. If only the stop date but not time is given, the imputed time will be midnight of that date, unless a subsequent starting time was recorded prior to midnight in which case the imputed time would be the start time of the subsequent record. If neither stop date nor time is given, the imputed stop time will be the start date and time of the subsequent recording. Likewise, missing start dates and times would be imputed as worst case scenarios, i.e. is the patient found to be on mechanical ventilation with a date but no time for intubation, the imputed start time would be recorded as 00:01 of that day or the stop date of a preceding recording on that same date, whichever occurs last. If both start date and time is missing, the imputed start time would be the date and time of the preceding stop time recorded. In case of data being completely missing from a certain time point and onwards, the "last status carried forward" imputation will be applied. If a patient was last seen on either ventilator or vasopressors, it is assumed that the patient remained so, and is imputed to a value of 0 (zero) P&VFDs. If the patient was last seen off ventilator and vasopressors, it is also assumed that the patient remained so in the remaining 30-day period. If the patient was last seen (alive) on e.g. day 10 and at that point had been off both ventilator and vasopressors for three days, a value of 23 P&VFDs is imputed.

The primary endpoint, P&VFDs, will be analysed using a van Elteren test, stratified by need for ventilation (Yes/No), time from onset of shock (onset of any vasopressor) to start of IMP treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min).

The primary analysis will compare all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) to all patients on the placebo arm from both parts of the trial. The primary analysis will be a test of superiority using a two-sided 5% significance level test. This test, within the trial, controls the type 1 error at a two-sided 5% level. Further details are provided in the statistical analysis plan.

Treatment effects will be estimated assuming a negative binomial distribution (to allow for possible overdispersion in a Poisson distribution) for the quantity (30 minus P&VFDs) for survivors, and a binomial distribution to model the probability of surviving. Both models adjusted for need for ventilation (Yes/No), time from onset of shock to start of IMP treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min). Further details are provided in the statistical analysis plan.

Furthermore, P&VFDs will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically by histograms and cumulative distributions functions.

The success (statistical/clinical significance) of the trial will be based upon the comparison of the analysis above (all patients on all selepressin dosing regimens from both parts of the trial [pooled together and treated as a single arm] compared to all patients on the placebo arm from both parts of the trial).

9.6.2.1 Sensitivity Analyses of the Primary Endpoint

As the adaptations of the trial provide a conservative estimate of the p-value, sensitivity p-values will be provided using post-simulation bootstrap calculations.

In order to check for consistency, the primary endpoint treatment differences will, as a minimum, be estimated and presented by forest plots for the following subgroups:

- Region
- Age
- Gender
- Race/ethnicity

Furthermore, the primary endpoint will be stratified by severity of the patients, with risk of dying as indicator of severity (see Figure 3). Mortality (the risk of dying) will be analysed by a logistic regression model, with relevant baseline characteristics as covariates (e.g. the individual SOFA scores and age). The model used to generate the predicted risk (for all patients) will be based on patients in the placebo arm only, as the risk of dying should reflect the severity in the absence of selepressin. Stratified by the risk of dying (intervals of 20% if suitable, based on the mortality rates in the covariate categories in the model), the treatment effect of the primary endpoint will be presented graphically, in order to visually inspect whether the average treatment effect is distributed evenly across the severity of patients.

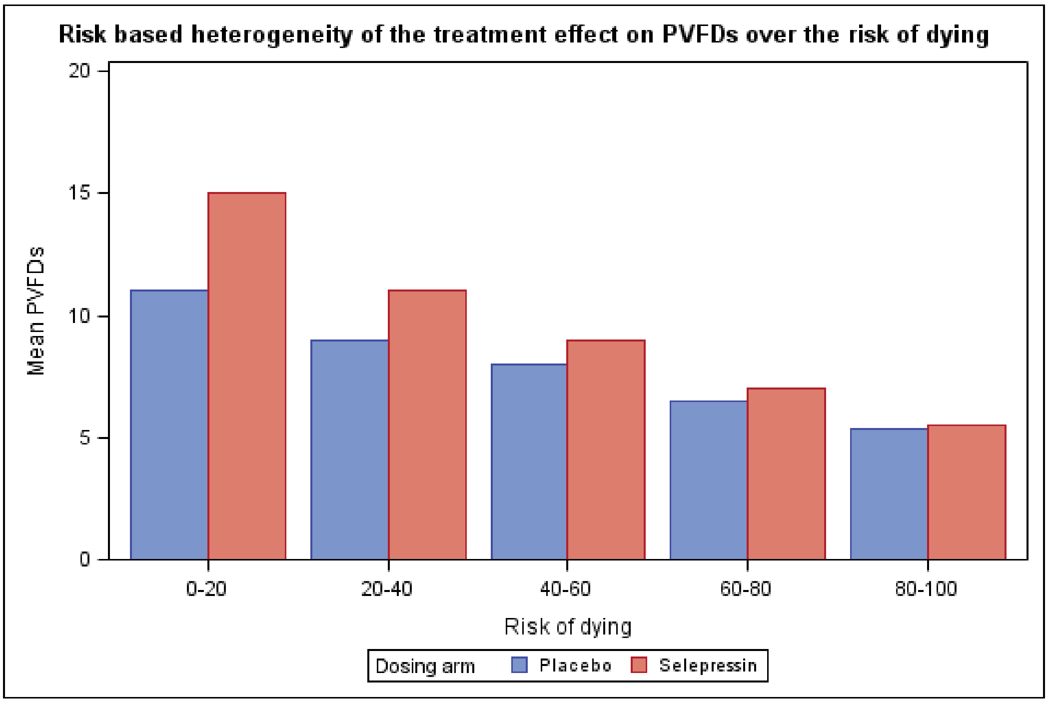


Figure 3 Risk-based Heterogeneity of the Treatment Effect on P&VFDs over the Risk of Dying (an example)

The impact and robustness of the imputation of missing data will be checked by analysing data in the following ways

- excluding all patients with missing/imputed data
- imputing the 30-day P&VFD status for patients lost to follow up or otherwise withdrawn from trial using the observed ratio of P&VFDs at time of lost to follow up or time of withdrawal, to the same proportion for a 30-day status

For this analysis the 30-day P&VFD status for patients lost to follow up or otherwise withdrawn from trial will be imputed so that the 30-day ratio of P&VFDs is equal to the ratio of P&VFDs at time of lost to follow up or time of withdrawal. E.g. a patient being lost to follow up at Day 15 with 4 P&VFDs (a ratio of 4/15 P&VFDs per days observed) will be imputed to 8 P&VFDs at Day 30 (equivalent ratio 8/30 = 4/15). Patients having zero P&VFDs at time of lost to follow up will be imputed to a value of zero P&VFDs.

- tipping point analysis

The tipping point analysis will compare all possible combinations of 'best case' and 'worst case' scenarios between placebo and selepressin for patients lost to follow up or otherwise withdrawn from trial. Best case being an imputation assuming the remaining days off ventilator and vasopressors, and worst case being an imputation of zero P&VFDs. N_P and N_S will be the number of patients in the placebo and selepressin arms with missing data. The tipping point analysis will compare all combinations (from 0 to N_P) of X patients on placebo imputed best case and $N_P – X$ imputed worst case, to Y patients on selepressin imputed best case and $N_S – Y$ imputed worst case. In other words, all N_P +1 times N_S +1 combinations will be analysed for the primary endpoint. Since the 'best case' is not the same for all patients (depending on when they were last seen off both ventilator and vasopressors), there are multiple outcomes within each combination. For each combination, the average p-value of the multiple outcomes will be plotted in the tipping point analysis.

Below is an example of a tipping point analysis of 25 placebo versus 40 selepressin patients with imputed values. The x- and y-axis displays the number of patients with the 'best case' imputed. In the example in Figure 4, the red area displays the non-significant p-values, indicating that one would have to impute almost all placebo patients to a 'best case' and almost all selepressin to a 'worst case' in order to get non-significant p-values, and hence 'proving' the robustness of the imputation method.

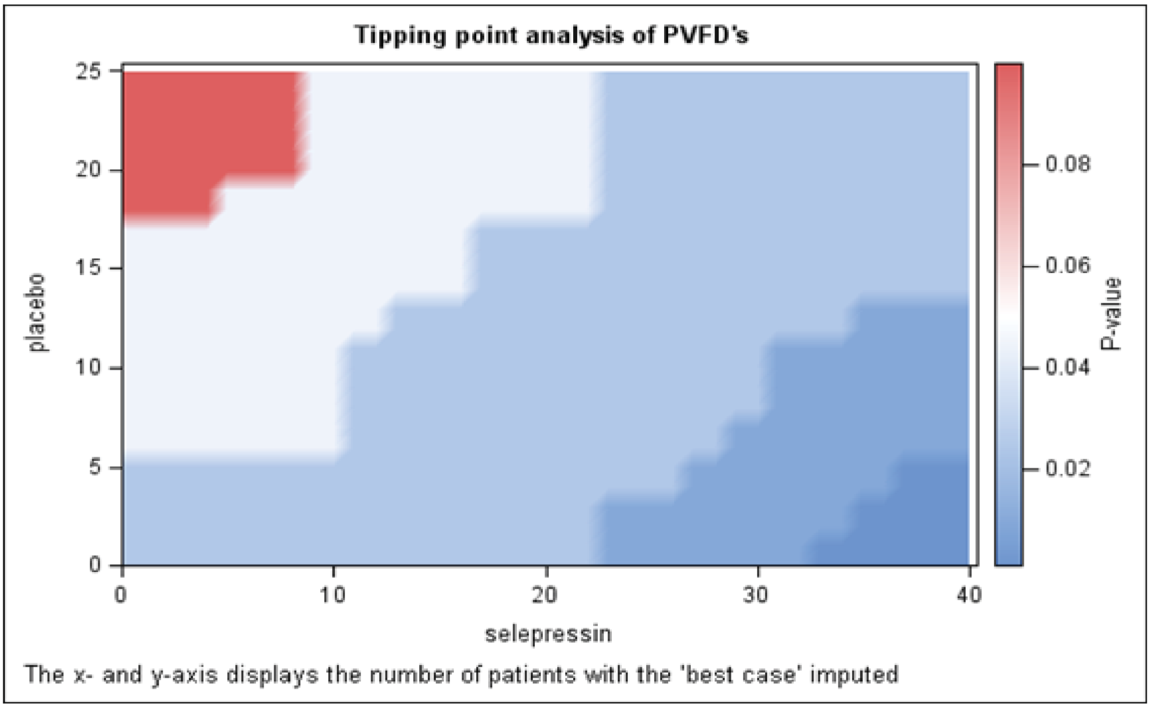


Figure 4 Tipping Point Analysis of P&VFDs (an example)

Also, to make sure that the use of vasopressor in each group is not simply being replaced by an increased use of inotropic agents, there will be a sensitivity analysis of the primary endpoint in which the use of inotropic agents will count as vasopressor use.

9.6.2.2 Additional Analyses

The primary analysis will be repeated for:

- The selected dose only, i.e. comparing all patients on the selected dose (from Part 1) from both parts of the trial (pooled), to all patients on the placebo arm from both parts of the trial.
- Data from Part 2 only, i.e. comparing the selected dose to placebo on data from Part 2 only.

9.6.3 Secondary Endpoints

For the purpose of a possible label inclusion, the Hochberg procedure for adjustment on multiplicity will be implemented to selected key secondary endpoints. Only if the primary efficacy analysis leads to a statistically significant result at the (one-sided) 2.5% level, then the Hochberg procedure, which is described below, is applied to the selected key secondary analyses. If the primary efficacy analysis does not result in statistical significance at the (one-sided) 2.5% level, then statistical significance (for the purpose of a possible label inclusion only) will not be declared for any of the key secondary analyses, regardless of their p-values.

The selected key secondary endpoints aimed at further demonstrating treatment effect are:

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause) at Day 90
- RRT-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)
- ICU-free days up to Day 30

In this application of the Hochberg procedure there are three hypothesis tests of superiority for each of the selected secondary endpoints. The target alpha level is (one-sided) 2.5%. The Hochberg procedure is as follows:

- Order the p-values from the smallest to the largest value, p(1) < p(2) < p(3), with corresponding null hypothesis $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$.
- Start with the highest p-value. If p(3) < 2.5% (one-sided), then stop and declare all three comparisons significant at the 2.5% (one-sided) level (i.e. reject $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$). Otherwise, accept $H_{(3)}$ for the endpoint related to p(3), and go to p(2) the second highest p-value.
- If p(2) < 2.5/2 = 1.25% (one-sided), then stop and declare significance for $H_{(1)}$ and $H_{(2)}$. Otherwise, accept $H_{(2)}$, for the endpoint related to p(2), and go to p(1) the lowest p-value.
- If p(1) < 2.5/3 = 0.833% (one-sided), then stop and declare significance for $H_{(1)}$. Otherwise, accept $H_{(1)}$, for the endpoint related to p(1).

Regardless of the statistical significance declared according to the Hochberg procedure, all analyses will be included and presented in the statistical report.

As for the primary analysis, the primary comparison (which determines the success, i.e. statistical and clinical significance) for the secondary efficacy endpoints is between all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) and all patients on the placebo arm from both parts of the trial.

As an additional analysis, all secondary efficacy analyses will, as for the primary, be repeated for:

- The selected dose only, i.e. comparing all patients on the selected dose (from Part 1) from both parts of the trial (pooled), to all patients on the placebo arm from both parts of the trial.
- Data from Part 2 only, i.e. comparing the selected dose to placebo on data from Part 2 only.

All free-days endpoints will be reported to one decimal place.

The following secondary endpoints will be defined and analysed in a similar manner as the primary endpoint:

- ***Vasopressor-free Days up to Day 30***
- ***Mechanical ventilator-free Days up to Day 30***

- ***RRT-free Days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)***
- ***ICU-free Days up to Day 30***

Incidence of RRT up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation) is defined as any form of renal replacement therapy defined as continuous renal replacement therapy, intermittent haemodialysis, or peritoneal dialysis. In order to ensure that any reduction in incidence of RRT is not caused by an increase in mortality, all patients dying within the 30-day period will be counted as on RRT. For patients withdrawn prior to Day 30, incidence of RRT will be based on the data available up until the time of withdrawal.

Incidence of RRT will be analysed by a logistic regression model with time from onset of shock to start of treatment, baseline creatinine and norepinephrine/noradrenaline requirement at baseline (µg/kg/min), as covariates, and treatment, and need for ventilation as factors. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method. Patients already on RRT at time of inclusion will be excluded from the analysis of incidence of RRT.

Non-inferiority will be claimed if the upper limit of the two-sided 95% CI of the adjusted difference in proportions is less than 20% of the estimated incidence of RRT in the placebo group. Superiority can be claimed if the upper limit is less than 0.

I.e., let $\widehat{p_S}$ and $\widehat{p_P}$ be the estimated incidences of RRT in the combined selepressin groups and the placebo group respectively. Non-inferiority will then be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0.2 * \widehat{p_P}$$

and superiority will be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0$$

Furthermore, incidence of RRT will be tabulated by treatment arm (including pooled active treatment arms).

A subgroup analysis will be performed on patients without acute RRT at baseline.

Duration of septic shock up to Day 30. Shock is defined as the cumulative periods (>1 hour) from start of IMP until Day 30, on IMP or vasopressors. Vasopressor use due to anaesthesia / procedure-induced hypotension during - and up to three hours after - surgery / procedure (including bedside) is exempt from this rule. For patients withdrawn (in the survivors analysis) or dying (in the non-survivors analysis) while still in septic shock, the duration will be based on the data available up until the time of withdrawal or death.

Duration of septic shock will be analysed separately for survivors, non-survivors (within the first 30 days) and overall, comparing treatment arms by an analysis of covariance (ANCOVA) model with time from onset of shock to start of treatment and norepinephrine/noradrenaline requirement at baseline (µg/kg/min) as covariates, and treatment and need for ventilation (Yes/No) as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented.

Some patients will get out of shock prior to Day 30 (and stay alive until Day 30), some will get out of shock and die later on (prior to Day 30), others will die while still in shock (prior to Day 30), and the remaining few will not get out of shock prior to Day 30. This means that if mortality rates vary between treatment arms, the results of the analysis for the overall population will be influenced by the skewed mortality rates. Hence, for the overall population, the distribution of duration of shock (time to out of shock), will be presented graphically as competing risks between 'time to out of shock' and 'dying while in shock'. Further, a KM (sub)-graph on 'time to death' will be presented for those getting out of shock (for which some will die later on, prior to Day 30). This is done in order to elucidate any skewness in mortality rates, influencing the results of the analysis. The duration of septic shock will also be tabulated by treatment arm (including pooled active treatment arms).

The following secondary endpoints will be defined and analysed in a similar manner as for duration of septic shock:

- ***Duration of mechanical ventilation up to Day 30***
- ***Duration of RRT up to Day 90 (excluding patients on RRT for chronic renal failure at time of randomisation)***
- ***ICU length of stay up to Day 30***

Daily overall (modified) and individual organ scores of the SOFA will be compared between treatment arms up until Day 7 using a repeated measures ANCOVA model with baseline SOFA score as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented.

Last observation carried forward (LOCF) will be used for missing SOFA scores on Days 2-7. No LOCF for Day 1 (as previous value is baseline). Patients dying will be imputed with a worst possible outcome, i.e. a value of 4 for each individual SOFA score.

Daily overall (modified) and individual SOFA scores will be tabulated by treatment arm (including pooled active treatment arms).

Incidence of new organ dysfunction and new organ failure. New organ failure is defined as an increase (i.e. worsening) in any of the individual SOFA scores from (0, 1, 2) at baseline to (3, 4) post baseline up until the end of the period (Days 7 or 30) (if the SOFA scores goes from [0, 1, 2] to [3, 4] and back to [0, 1, 2] again within the period, that will still count as a new organ failure). If a patient dies within the period, he/she is considered to fail on all organs, and the number of new organ failures will be all organs except those already failed at baseline. Patients discontinued within the period will be evaluated based on the data available at time of discontinuation.

Incidence of new organ dysfunction is defined as an increase ≥1 from baseline to post baseline up until the end of the period (e.g. going from 1 to 2) in any of the individual SOFA scores. Patients with an individual SOFA score of 4 at baseline can per default not have a new organ dysfunction. If a patient dies within the period, he/she is considered to have dysfunction on all organs, and the number of new organ dysfunctions will be all organs except those already having a score of 4 at baseline. Patients withdrawn within the period will be evaluated based on the data available at time of withdrawal.

Incidence of at least one new organ failure will be analysed for any new organ failure (across all organ systems) and by individual organ systems, and compared between treatment arms using a logistic regression model with age, modified SOFA score, and norepinephrine/noradrenaline requirement at baseline (µg/kg/min) as covariates and gender and treatment arm as factors, presenting odds ratios with 95% confidence intervals.

Incidence of at least one new organ dysfunction will be analysed for any new organ dysfunction (across all organ systems) and by individual organ systems, and will be analysed as above for new organ failures.

The number of new organ dysfunctions and new organ failures will be compared between treatment arms using a negative binominal model with age, modified SOFA score, and norepinephrine/noradrenaline requirement at baseline (µg/kg/min) as covariates, and gender and treatment as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method.

Incidence of any new organ dysfunction and any new organ failure and the number of new organ dysfunctions and new organ failures will be tabulated by treatment arm (including pooled active treatment arms).

All-cause mortality is defined as the fraction of patients that have died, regardless of cause, by the end of Day 30, Day 90, and Day 180 and will be analysed and compared between treatment arms using a logistic regression model with the individual SOFA scores and age as covariates and treatment arm as factor. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method as for incidence of RRT. There will be no imputations for mortality.

Non-inferiority will be claimed if the upper limit of the two-sided 95% CI of the adjusted difference in proportions is less than 30% of the estimated incidence of mortality in the placebo group. Superiority can be claimed if the upper limit is less than 0.

Assuming an observed 30-day mortality rate of 20-25% in the placebo group, a non-inferiority limit of 30% corresponds to a maximum observed mortality rate of 2-3% in the combined selepressin groups in order for selepressin to be non-inferior to placebo.

Furthermore, mortality will be tabulated by treatment arm (including pooled active treatment arms), and the time to death presented graphically by a Kaplan-Meier plot.

EQ-5D-5L will be analysed by the index value, the overall quality-adjusted life years (QALY) at Day 30 and 180, and the VAS score. The QALY scores will NOT be adjusted to e.g. a half yearly time scale at Day 180. As the QALY is not defined for patients with all remaining values missing, and hence also not defined for those who die, the analyses will automatically only be analysed for those surviving up until Day 30 and Day 180, respectively.

For patients with missing baseline index value, the QALY score will also be set to missing. For robustness, a sensitivity analyses will be performed, imputing the missing baseline scores with the overall mean of the baseline health index. Baseline is the timing prior to acute admission.

The QALY at Day 30 and Day 180 will be compared between treatment arms using an ANCOVA model with baseline health index as covariate, and treatment as factor. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

The index value and VAS scores will be analysed separately for survivors and non-survivors at Day 180 (since all non-survivors will have non-random missing values, and hence would artificially inflate the mean estimates if survivors and non-survivors were analysed together) and will be compared between treatment arms using a repeated measures ANCOVA model with baseline health index/VAS score as covariate, treatment, time and treatment by time interaction as factors, and subject as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented for Day 30, Day 60, Day 90, and Day 180. There will be no imputations for missing values.

The QALY, index value, and VAS scores will be tabulated by treatment arm (including pooled active treatment arms), and the index value and VAS scores will be presented graphically.

Daily and cumulative fluid balance (for 7 days or until ICU discharge). Fluid overload is defined as fluid balance as a percentage of baseline weight (e.g. if a patient weighs 90 kg at baseline and has a fluid balance of 9 litres, fluid overload is then 100% * 9 litres / 90 kg = 10%.

Fluid balance and cumulative fluid balance will be presented both unadjusted and adjusted for weight.

Daily and cumulative fluid balance as well as daily and cumulative fluid overload will all be compared between treatment arms using a repeated measures ANCOVA model with baseline (baseline fluid balance or baseline fluid overload) as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

The absolute values and change from baseline will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

All analyses will be presented for 'all patients' and for 'patients in ICU throughout Day 0-7'.

Daily and cumulative Urine Output (for 7 days or until ICU discharge) will be analysed as for fluid balance.

9.6.4 Other Efficacy Endpoints

Other assessments include:

- Hospital-free Days up to Day 90

Will be defined and analysed in a similar manner as for the primary endpoint.

- Hospital length of stay up to Day 90

Will be defined and analysed in a similar manner as for duration of septic shock.

- Norepinephrine/noradrenaline and other vasopressor doses

The dose of norepinephrine/noradrenaline administered (adjusted for baseline weight) will be compared between treatment arms using a repeated measures ANCOVA model with baseline dose of norepinephrine/noradrenaline as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented. If a patient has missing values and the patient is still in the trial (not dead or withdrawn) it will be assumed that the specific vasopressor was not given and a value of zero will be imputed, unless there is an interval in the timing log covering the exact time point (8 AM and 8 PM is the assumed time point for missing morning and evening collection time points). In that case LOCF will be used, but only within the time interval.

The mean dose administered will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

The same analysis (adjusted for baseline dose of norepinephrine/noradrenaline) will be performed for the following endpoints:

- Catecholamines (defined as the sum of doses of norepinephrine/noradrenaline, epinephrine/adrenaline, dopamine, and phenylephrine)
- Catecholamines excluding norepinephrine/noradrenaline
- Vasopressin

For the sum of catecholamine doses we define 100 µg dopamine, 1 µg epinephrine, and 2.2 µg phenylephrine all equivalent to 1 µg norepinephrine.

Also, the number of patients receiving terlipressin will be summarised.

- Patient residence at Day 30, Day 60, Day 90, and Day 180
- MAP
- Arterial blood gases and acid/base status (PaO_2, $PaCO_2$, SaO_2, pH, bicarbonate, base excess) and lactate levels
- $ScvO_2$
- PaO_2/FiO_2 (in a subset of patients)
- EVLW and PPI (in a subset of patients)
- Cardiac output (in a subset of patients)
- Cytokines, angiopoietin-1 and -2 levels (in a subset of patients)

These assessments will be presented by descriptive statistics, i.e. the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point. Baseline value will be the value obtained at the last assessment prior to the infusion start of the IMP.

- Creatinine clearance

Creatinine clearance will be analysed as for fluid balance with baseline creatinine clearance as a covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit.

9.7 Extent of Exposure and Treatment Compliance

The total amount (adjusted by weight [µg/kg]) of selepressin administered and the number of (decimal) days treated with selepressin will be summarised by (active) treatment arm and total (active treatment arms).

Furthermore, the mean cumulative amount administered and the mean infusion rate will be tabulated by treatment arm and presented graphically (also by treatment arm and total).

9.8 Safety

9.8.1 General Considerations

Safety parameters will be evaluated for the safety analysis data set. All safety summaries will be tabulated by treatment arm (including pooled active treatment arms).

9.8.2 Adverse Events

Adverse events will be classified according to the medical dictionary for regulatory activities (MedDRA). The MedDRA version will be documented.

A pre-treatment adverse event is any adverse event occurring after informed consent and before administration of the IMP.

A treatment-emergent adverse event is any adverse event occurring after the administration of the IMP and within the time of residual drug effect (i.e. 12 hours), or a pre-treatment adverse event or pre-existing medical condition that worsens in intensity after start of IMP and within the time of residual drug effect. The time of residual drug effect is the estimated time after the end of the administration of the IMP, where the effect of the product is still considered to be present based on pharmacokinetic, pharmacodynamic, or other substance characteristics. A generally accepted time for residual drug effect is 5 half-lives. The terminal half-life of selepressin is expected to be not more than 1.8 hours, and hence, a treatment-emergent adverse event is defined as any adverse event occurring after the start of IMP infusion and within 12 hours after the IMP infusion is stopped.

A post-treatment adverse event is any adverse event occurring after the residual drug effect period.

Missing values will be treated as missing, except for causality, intensity, seriousness, and outcome of adverse events. A "worst case" approach will be used: if causality is missing, the adverse event will be regarded as related to the IMP; if the intensity of an adverse event is missing, the adverse event will be regarded as severe; if seriousness is missing the adverse event will be regarded as serious; if start date is missing or incomplete, worst case will be assumed and the adverse event will be regarded as treatment-emergent (only if the incomplete start date is not compromised). If start date is completely missing, start date will be set as same day as start of treatment. If start date is incomplete, the date closest to start of treatment will be assumed, without compromising the incomplete data available on the start date; if outcome is missing and no date of outcome is present the outcome is regarded as 'not recovered'.

Adverse event overview summary tables will be prepared for treatment-emergent AEs and all AEs (treatment-emergent and non treatment-emergent) including the number of patients reporting an adverse event, the percentage of patients with an adverse event, and the number of events reported, for the following categories:

- Adverse events
- Deaths
- Serious adverse events
- Adverse events leading to discontinuation of IMP
- Severe and life-threatening adverse events
- Adverse drug reactions

Adverse events will be summarised in a table by SOC (sorted alphabetically) and PT (sorted in decreasing frequency of occurrence) using MedDRA. The table will display the total number of patients reporting an adverse event, the percentage of patients with an adverse event, and the number of events reported.

For both treatment-emergent AEs and all AEs (treatment-emergent and non treatment-emergent) during the treatment period, summary tables will be prepared for:

- All adverse events
- Adverse events with an incidence ≥5% of patients in any treatment arm
- Non-serious adverse events with an incidence ≥5% of patients in any treatment arm
- Critical adverse events, details are provided in the statistical analysis plan
- Adverse events by causality (related/unrelated)
- Adverse events leading to death
- Adverse events by intensity
- Serious adverse events
- Adverse events leading to discontinuation of IMP (related/unrelated)

Supporting data listings will be provided for:

- All adverse events sorted by trial site and patient number
- All adverse events sorted by MedDRA PT
- Serious adverse events
- Adverse events leading to death
- Adverse events leading to discontinuation of IMP (related/unrelated)
- Post-treatment adverse events

9.8.3 Safety Laboratory Variables

Safety laboratory variables will be grouped under ‘Clinical Chemistry’, ‘Haematology’, and ‘Coagulation’.

Baseline for all safety laboratory variables will be the values obtained at the last assessment prior to the infusion start of the IMP. End of treatment period will include the last post-baseline observation during the trial up until Day 30.

Mean change and mean percentage change from baseline at end of treatment period will be presented for each laboratory variable. In addition, descriptive statistics, i.e. the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each laboratory variable.

Also, summary tables will be presented, displaying by time and laboratory parameter, the number of patients with a clinically significant result which is unanticipated in the setting of septic shock.

Furthermore, a summary table will be prepared for selected laboratory variables that display the number and percentage of patients in each treatment arm with X% increments (increase or decrease) from baseline at each time-point. The selected laboratory variables and categories are detailed in the statistical analysis plan.

9.8.4 Vital Signs and Central Venous Pressure

Baseline for all vital signs variables and CVP will be the values obtained at the last assessment prior to the IMP infusion start. End of treatment period will include the last post-baseline observation during the trial up until Day 30.

Mean change and mean percentage change from baseline at end of treatment period will be presented for each variable. In addition, descriptive statistics, i.e. the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each variable.

Also, summary tables will be presented, displaying by time and vital signs parameter, the number of patients with a clinically significant result which is unanticipated in the setting of septic shock.

Furthermore, a summary table will be prepared for each variable that display the number and percentage of patients in each treatment arm with X% increments (increase or decrease) from baseline at each time-point and end of treatment period. The variables and categories are detailed in the statistical analysis plan.

9.8.5 Episodes of Hypotension

Descriptive statistics of number of patients with episodes of hypotension and the total length of periods with hypotension will be summarised by treatment arm.

The total length of periods with hypotension will be summarised for both all patients, and patients having one or more episodes of hypotension.

9.9 Interim Analyses

There will be no interim analyses with the potential to stop the trial early for treatment efficacy. However, once the “burn-in” period in Part 1 is completed (first 200 treated patients), interim analyses will be conducted regularly to improve the efficiency of dose selection and to allow early termination of the part or the trial for futility or for successful dose selection. The following steps will be considered at each interim analysis:

- When 200 patients are treated, the allocation probabilities for the active treatment arms are changed using response-adaptive randomisation (with placebo still 1/3). For the two-thirds of patients assigned to the active arms, the probability that a given active arm is assigned to a patient is proportional to the probability that that arm is the arm with the largest expected number of P&VFD.
- Potentially stopping the trial for futility during Part 1. This occurs if no active arm has better than a 5% predictive probability of a significant result in Part 2 if it were to start

immediately. This decision can occur at any interim during Part 1 after 200 evaluable patients.

- Potentially ending Part 1 and selecting an active treatment arm to continue to Part 2. This decision can occur at any interim analysis between 300 evaluable and 800 treated patients, and it occurs if some arm has a predictive probability of a successful trial of at least 90% before 800 treated patients, and the threshold drops to 25% for the final Part 1 interim analysis at 800 treated patients. The selected arm is the arm with the largest posterior predictive probability of trial success. This will generally be the best-performing active arm, but if multiple arms are performing equally well, it will be the arm with the lowest dosing level. If Part 1 ends after N patients, then Part 2 will consist of up to 1800 - N evaluable patients.
- If the trial is not stopped for futility or proceeding to Part 2 and active treatment Arm 4 has not yet been approved for assignment of patients, the decision can be made to open up Arm 4. Arm 4 is only opened between 200 evaluable and 600 treated patients and if there is at least a 50% probability that Arm 3 has a higher expected P&VFD than Arm 2 and if data from the lower dosing levels do not suggest any significant safety signals.
- If Part 1 reaches its maximum of 800 treated patients and no arm has a predictive probability of Part 2 success of more than 25%, the trial stops with an inconclusive result and will be interpreted as a standalone Phase 2b trial.

During Part 2, interim analyses will be conducted regularly (until 1600 patients have been treated) to allow early termination of the trial for futility. This occurs if the predictive probability of an overall significant result is less than 5%. In addition, if the predictive probability of observing a more than 2% higher mortality in the active arms compared to placebo is greater than 90% then the trial will stop for futility.

10 DATA HANDLING

10.1 Source Data and Source Documents

Source Data – ICH Definition

Source data are defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

Source Documents - ICH Definition

Source documents are defined as original documents, data, and records (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, patient files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial).

Trial-specific Source Data Requirements – Ferring

The investigators must maintain patient records. For each randomised patient, the investigators will indicate in the hospital/medical source records that the patient participates in this trial and the date of obtaining the informed consent. The records will include data on the condition of the patient at the time the patient is enrolled in the trial in order to document (and enable verification of) eligibility. Signed and dated informed consent forms will be stored and archived in accordance with local requirements.

The following information, as a minimum, has to be recorded in the hospital/medical source records for each patient:

- Documentation of informed consent obtained
- Trial identification
- Screening/patient (randomisation) number
- Patient's name
- Demographic data including date of birth, race, and ethnic origin
- Diagnosis, septic shock characteristics
- Relevant medical history
- Relevant concomitant medications/procedures
- Body weight and height
- Eligibility for participation in the trial (documenting all inclusion/exclusion/eligibility criteria)

- Details of the administration of IMP
- Details of the administration of norepinephrine/noradrenaline and other vasopressors
- Details of the MAP during the IMP infusion
- Details of mechanical ventilation (including spontaneous breathing trials) and RRT
- Functional/survival status of the patient throughout the trial
- ED/ICU/hospital admission and discharge dates and times
- Details and results of all other examinations and tests performed
- Date of each visit/contact
- Details of adverse events
- Reason for discontinuation/withdrawal, if applicable

Information included in the patient’s hospital records may be subject to local regulations. If there is a discrepancy between local requirements and the trial protocol, local regulations should be followed. The identification of source data for each variable may then be described in a separate document.

Documents collected during the trial (e.g. health-related quality of life questionnaires, laboratory reports, print-outs of MAP and ECG) should be stored and archived together with the patient’s hospital/medical records or in the investigator file as agreed upon prior to the trial start at each trial site.

No specific protocol data can be recorded directly in the eCRF without prior written or electronic record.

10.2 Electronic Case Report Form (eCRF)

An eCRF system provided by an independent third-party contract research organisation will be used for data capture. Contact details of the contract research organisation are provided in a trial-specific contact list. The system is validated and access at all levels to the system is granted/revoked following Ferring and vendor procedures, in accordance with regulatory and system requirements.

Data should be entered into the system within a reasonable time after source data is collected.

The investigator will approve/authorise the eCRF entries for each patient with an electronic signature which is equivalent to a handwritten signature.

The eCRF system and the database will be hosted at and administered by the independent third party contract research organisation. After the trial database is declared clean and released to the statistician, a final copy of the database will be stored at Ferring. The investigator will also receive a copy of the trial site’s final and locked data (including audit trail, electronic signature and queries) as write-protected PDF-files produced and distributed by the independent third party contract research organisation. The PDF-files will be stored on a CD and will be provided to the investigator before read access to the eCRF is revoked.

Modification of data entered into the eCRF will be captured in an electronic audit trail detailing the date and time of the correction and the user name of the person making the correction. Only site coordinator and investigator have privileges to modify data.

10.3 Data Management

A data management plan will be created under the responsibility of the Global Biometrics department at Ferring. The data management plan will be issued before data collection begins and will describe all functions, processes, and specifications for data collection, cleaning, and validation.

The data management plan will describe capture methods, who is authorised to enter the data, decisions about ownership of data, source data storage, which data will be transferred (including timing of transfers), the origin and destination of the data, and who will have access to the data at all times.

10.4 Provision of Additional Information

On request, the investigators will provide Ferring with additional data relating to the trial, duly anonymised and protected in accordance with applicable requirements.

11 MONITORING PROCEDURES

11.1 Periodic Monitoring

The monitors will contact and visit the investigators periodically to ensure adherence to the protocol, International Conference of Harmonisation-Good Clinical Practice (ICH-GCP), standard operating procedures and applicable regulatory requirements, maintenance of trial-related source records, completeness, accuracy and verifiability of eCRF entries compared to source data, verification of drug accountability, and compliance to safety reporting instructions. The investigators will permit the monitors direct access to all source data, including electronic medical records, and/or documents in order to facilitate data verification. The investigators will co-operate with the monitors to ensure that any discrepancies that may be identified are resolved. The investigators are expected to be able to meet the monitors during these visits. The first on-site monitoring visit will take place shortly after randomisation of the first patient. The frequency of the on-site monitoring visits is dependent on the number of enrolled patients at the trial site.

The source data verification process and definition of key variables to be monitored will be described in the trial-specific monitoring plan.

11.2 Audit and Inspection

The investigators will make all the trial-related source data and records available at any time to quality assurance auditor(s) mandated by Ferring, or to domestic/foreign regulatory inspector(s) or representative(s) from IECs/IRBs who may audit/inspect the trial.

The main purposes of an audit or inspection are to assess compliance with the trial protocol and the principles of ICH-GCP including the Declaration of Helsinki and all other relevant regulations.

The patients must be informed by the investigators and in the informed consent documents that authorised Ferring representatives and representatives from regulatory authorities and IECs/IRBs may wish to inspect their medical records. During audits/inspections the auditors/inspectors may copy relevant parts of the medical records. No personal identification apart from the screening/ randomisation number will appear on these copies.

The investigators should notify Ferring without any delay of any inspection by a regulatory authority or IEC/IRB.

11.3 Confidentiality of Patient Data

The investigators will ensure that the confidentiality of the patients' data will be preserved. In the eCRF or any other documents submitted to Ferring, the patients will not be identified by their names, but by an identification system, which consists of an assigned number in the trial. Documents that are not for submission to Ferring, e.g. the confidential patient identification code and the signed informed consent documents, will be maintained by the investigators in strict confidence.

12 CHANGES IN THE CONDUCT OF THE TRIAL

12.1 Protocol Amendments

Any change to this protocol will be documented in a protocol amendment, issued by Ferring, and agreed upon by the TSC, investigators, and Ferring prior to its implementation. Amendments may be submitted for consideration to the approving IECs/IRBs and regulatory authorities, in accordance with local regulations. Changes to the protocol to eliminate immediate hazard(s) to trial patients may be implemented prior to IECs/IRBs approval/favourable opinion.

12.2 Deviations from the Protocol

The investigators must inform the monitor if deviations from the protocol occur and the implications of the deviation must be reviewed and discussed. Any deviation must be documented, either as an answer to a query in the eCRF, in a protocol deviation report, or a combination of both. A log of protocol deviation reports will be maintained by Ferring. Protocol deviation reports and supporting documentation must be kept in the investigator's file and the trial master file.

12.3 Premature Trial Termination

Both the investigators (with regard to his/her participation) and Ferring reserve the right to terminate the trial at any time. Should this become necessary, the procedures will be agreed upon after consultation between the two parties. In terminating the trial, Ferring and the investigators will ensure that adequate consideration is given to the protection of the best interests of the patients. Regulatory authorities and IECs/IRBs will be informed.

In addition, Ferring reserves the right to terminate the participation of individual trial sites. Conditions that may warrant termination include, but are not limited to, insufficient adherence to protocol requirements and failure to enter patients at an acceptable rate.

13 REPORTING AND PUBLICATION

13.1 Clinical Trial Report

The data and information collected during this trial will be reported in a clinical trial report prepared by Ferring and submitted for comments and signature to the signatory investigators.

13.2 Confidentiality and Ownership of Trial Data

Any confidential information relating to the IMP or the trial, including any data and results from the trial will be the exclusive property of Ferring. The investigators and any other persons involved in the trial will protect the confidentiality of this proprietary information belonging to Ferring.

13.3 Publications and Public Disclosure

13.3.1 Publication Policy

Sponsor recognises and accepts that investigators may have a meaningful right to publish research results of the trial. Investigators must agree that the first publication of trial results is to be a joint publication covering all trial sites, and that subsequent publications will reference that primary publication. At the end of the trial, one or more manuscripts (including manuscripts, presentation, abstracts, posters etc.) for joint publication may be prepared in collaboration between the investigators and the TSC and Ferring, and the criteria for such publication shall be coordinated through the TSC. As the trial is a multi-centre trial, all publications shall be joint publications covering all trial sites unless specific written permission is obtained in advance from the TSC. However, if a joint manuscript has not been submitted for publication within 18 months of completion or termination of the trial, the investigators shall be free to publish separately. Any publication of results must acknowledge all trial sites.

Under the coordination of the TSC, authorship is granted based on the International Committee of Medicinal Journal Editors (ICMJE) criteria (see current official version: http/www.ICMJE.org).

Any external contract research organisation or laboratory involved in the conduct of this trial has no publication rights regarding this trial.

Any publication, whether joint or independent, on the results of the trial must be submitted in writing to the TSC and Ferring for comment prior to submission at least 60 days in advance of the submission of such proposed publication to the applicable journal or other forum in which the publication or presentation may be published or presented. At Ferring's request, the respective institution and/or investigator shall arrange for an additional delay in publication or presentation, not to exceed an additional 60 days, to enable Ferring to request deletion of Ferring Confidential Information and to arrange for filing of patent applications or other intellectual property protection. This statement does not give Ferring editorial rights over the content of a publication, other than to restrict the disclosure of Ferring's intellectual property. If the matter considered for publication is deemed patentable by Ferring, scientific publication will not be allowed until after a filed patent application is published. Under such conditions the publication will be modified or delayed to allow sufficient time for Ferring to seek patent protection of the invention.

13.3.2 Public Disclosure Policy

ICMJE member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public, clinical trials registry. Thus, it is the responsibility of Ferring to register the trial in an appropriate public registry, i.e. www.ClinicalTrials.gov which is a website maintained by the National Library of Medicine at the U.S. National Institutes of Health. The trial will also be made publicly available at the EU Clinical Trials Register at www.clinicaltrialsregister.eu. Trial registration may occur in other registries in accordance with local regulatory requirements. A summary of the trial results is made publicly available in accordance with applicable regulatory requirements.

14 ETHICAL AND REGULATORY ASPECTS

14.1 Independent Ethics Committees or Institutional Review Boards

Independent ethics committees/institutional review boards will review the protocol and any amendments. The IECs/IRBs will review the patient information sheet and the informed consent form, their updates (if any), and any written materials given to the patients. A list of all IECs/IRBs to which the protocol has been submitted will be included in the clinical trial report.

14.2 Regulatory Authorities Authorisation / Approval / Notification

The regulatory permission to perform the trial will be obtained in accordance with applicable regulatory requirements. All ethical and regulatory approvals must be available before a patient is exposed to any trial-related procedure, including screening tests for eligibility.

14.3 End-of-Trial and End-of-Trial Notification

End of-trial is defined as the date of the last trial-related contact with the last patient ongoing in the trial. At the end-of-trial, Ferring shall notify the regulatory authorities and the IECs/IRBs in the participating countries about the completion of the clinical trial in accordance with national/local regulations.

14.4 Ethical Conduct of the Trial

This trial will be conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki (World Medical Association, 2013), in compliance with the approved protocol, ICH-GCP, and applicable regulatory requirements.

14.5 Patient Information and Consent

Critically ill patients receiving care in the EDs/ICUs represent a highly vulnerable population with regard to informed consent as these patients are often not capable of participating in the consent process. Because of this, proxies are often required to provide consent and other health decisions for impaired patients.

The informed consent process in this trial will be obtained in accordance with national/local regulations.

The investigator (or the person delegated by the investigator) will obtain a freely given written consent in accordance with national/local regulations from each patient or his/her legally acceptable representative after an appropriate explanation of the aims, methods, anticipated benefits, potential hazards, and any other aspects of the trial which are relevant to the patient’s decision to participate. The patient or the legal representative should be given ample time to consider participation in the trial, before the consent is obtained. The patient (and the legal representative, if applicable) will receive a copy of the patient information and the signed informed consent form.

The investigator (or the person delegated by the investigator) will explain that it is completely free to refuse to enter the trial or to withdraw from trial at any time, without any consequences for the patient's further care and without the need to justify the decision.

The investigator (or the person delegated by the investigator) will inform that the monitor(s) and quality assurance auditor(s) mandated by Ferring, IRB/IEC representatives, or regulatory authority inspector(s), in accordance with applicable regulatory requirements, may review the patient's source records and data. Data protection will be handled in compliance with the national/local regulations.

If new information becomes available that may be relevant to the willingness to continue participation in the trial, a new patient information and informed consent form will be forwarded to the IECs/IRBs and the regulatory authorities, if required. The trial patients (and the legal representatives, if applicable) will be informed about this new information and re-consent will be obtained.

14.6 Patient Information Card

If required by local regulations, the patient will be provided with a patient information card bearing required trial-related information.

Each patient's primary care physician will be notified of their participation in the trial by the investigator, if the patient agrees.

14.7 Compliance Reference Documents

The Helsinki Declaration, the consolidated ICH-GCP, the European Union Clinical Trials Directive, 21 CFR Part 312, and other national laws in the countries where the trial takes place shall constitute the main reference guidelines for ethical and regulatory conduct.

15 LIABILITIES AND INSURANCE

15.1 ICH-GCP Responsibilities

The responsibilities of Ferring, the monitors, and the investigators are defined in the ICH-GCP consolidated guideline, and applicable regulatory requirements in the country where the trial takes place. The investigators are responsible for adhering to the ICH-GCP responsibilities of investigators, for dispensing the IMP in accordance with the approved protocol or an approved amendment, and for its secure storage and safe handling throughout the trial.

15.2 Liabilities and Insurance

In case of any damage or injury occurring to a patient in association with the IMP or the participation in the trial, Ferring has contracted an insurance which covers the liability of Ferring, the investigators, and other persons involved in the trial in compliance with the laws in the countries involved.

16 ARCHIVING

16.1 Investigator File

The investigator is responsible for maintaining all the records, which enable the conduct of the trial at the site to be fully understood, in compliance with ICH-GCP. The trial documentation including all the relevant correspondence should be kept by the investigator for at least 15 years (or longer if so required by local law) after the completion or discontinuation of the trial, if no further instructions are given by Ferring.

The investigator is responsible for the completion and maintenance of the confidential patient identification code which provides the sole link between named patient source records and anonymous eCRF data for Ferring. The investigator must arrange for the retention of this patient identification log and signed informed consent documents for at least 15 years (or longer if so required by local law) after the completion or discontinuation of the trial.

No trial site document may be destroyed without prior written agreement between the investigator and Ferring. Should the investigator elect to assign the trial documents to another party, or move them to another location, Ferring must be notified. If the investigator retires and the documents can no longer be archived by the site, Ferring can arrange having the investigator file archived at an external archive.

16.2 Trial Master File

Ferring will archive the trial master file in accordance with ICH-GCP and applicable regulatory requirements.

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STATISTICAL ANALYSIS PLAN

A Double-blind, Randomised, Placebo-controlled Phase 2b/3 Adaptive Clinical Trial Investigating the Efficacy and Safety of Selepressin as Treatment for Patients with Vasopressor-dependent Septic Shock

000133

Investigational Product:	Selepressin; concentrate for solution for infusion Placebo; sterile 0.9% sodium chloride solution
Indication:	Vasopressor-dependent Septic Shock
Phase:	2b/3
Author:	[redacted]
Date of issue:	December 17th - 2014
Version:	Final

Change log

Version No.	**Effective Date**	**Reason for the Change / Revision**	**Supersedes**
1	Dec 17-2014	Original SAP	Not applicable

Signed agreement on Statistical Analysis Plan

This analysis plan was reviewed by,

- [redacted] Global Biometrics, Ferring Pharmaceuticals A/S
- [redacted] Clinical R&D, Selepressin, Ferring Pharmaceuticals A/S
- [redacted] Global Pharmacovigilance, Ferring Pharmaceuticals A/S
- [redacted] Clinical R&D, Selepressin, Ferring Pharmaceuticals A/S
- [redacted] Health Economics & Outcomes Research, Clinical and Non-Clinical Development, Ferring Pharmaceuticals A/S

And reviewed and approved (signed electronically) by,

- [redacted] Biometrics, Ferring Pharmaceuticals A/S

TABLE OF CONTENTS

1 Introduction

This document describes the planned statistical analyses for selepressin (FE202158) 000133 (protocol dated December 17th 2014).

1.1 Definitions/ Abbreviations

1.1.1 Definition of Terms

Terms	**Definitions**
Randomised	Patient randomised to trial treatment
Screened	Patient who enters the screening phase
Selepressin	FE 202158
1-KM curve	1 minus Kaplan Meier curve

1.1.2 Abbreviations

Abbreviations	**Meaning of abbreviations in document**
AE	Adverse Event
ANCOVA	Analysis of covariance
CVP	Central venous pressure
EVLW	Extra-vascular lung water
EQ-5D-5L	EuroQol – 5 Dimensions – 5 Levels
FAS	Full-Analysis Set
ICU	Intensive care unit
IMP	Investigational Medicinal Product
ITT	Intention-to-treat
LOCF	Last observation carried forward
MAP	Mean Arterial Pressure
MedDRA	Medical Dictionary for Regulatory Activities
NE	Norepinephrine
PK	Pharmacokinetic
PP	Per-Protocol
PPI	Pulmonary permeability index
PT	Preferred term
P&VFD	Pressor and ventilator free days
QALY	Quality adjusted life years
RAR	Response adaptive randomisation
RRT	Renal replacement therapy

Abbreviations	**Meaning of abbreviations in document**
ScvO2	Oxygen Saturation in Vena Cava Superior
SOFA	Sequential Organ Failure Assessment score
SOC	System Organ Class
WBC	White blood cells

2 Trial Objectives and Endpoints

2.1 Objectives

Primary Objective

- To demonstrate superiority of selepressin plus standard care versus placebo plus standard care in the number of vasopressor- and ventilation-free days (with penalty for mortality) in patients with vasopressor-dependent septic shock

Secondary Objectives

- To determine the efficacy of selepressin on:
    - Organ dysfunction
    - Morbidity and mortality
    - Fluid balance
    - Health-related quality of life
- To determine the safety profile of selepressin
- To determine the pharmacokinetics of selepressin
- To determine the health economics of selepressin
- To further evaluate a range of biomarkers in relation to the mode of action of selepressin

2.2 Endpoints

2.2.1 Primary Endpoint

- Pressor- and ventilator-free days (P&VFDs) up to day 30

2.2.2 Key Secondary Endpoints

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause, by the end of Day 90)
- Renal replacement therapy (RRT)-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)
- Intensive care unit (ICU)-free days up to Day 30

2.2.3 Secondary Endpoints

Organ dysfunction

- Vasopressor-free days up to Day 30

- Ventilator-free days up to Day 30
- Duration of septic shock up to Day 30
- Duration of mechanical ventilation up to Day 30
- Incidence of RRT up to Day 30 (counting patients who die as on RRT and excluding patients on RRT for chronic renal failure at time of randomisation)
- Duration of RRT up to Day 90 (excluding patients on RRT for chronic renal failure at time of randomisation)
- Daily overall and individual organ (cardiovascular, respiratory, renal, hepatic, coagulation) scores using the modified Sequential Organ Failure Assessment (SOFA) scores until ICU discharge
- Incidence of new organ dysfunctions and new organ failures (based on the SOFA score) up to Days 7 and 30

Morbidity/mortality
- ICU length of stay up to Day 30
- All-cause mortality (defined as the fraction of patients that have died, regardless of cause, by the end of Days 30 and 180)

Fluid balance
- Daily and cumulative fluid balance until ICU discharge (for a maximum of 7 days)
- Daily and cumulative urinary output until ICU discharge (for a maximum of 7 days)

Health-related quality of life
- Change in utility, based on EQ-5D-5L, up to Day 180

2.2.4 Safety Endpoints
- Incidence of adverse events (type, frequency, and intensity) with specific emphasis on:
  - Ischaemic events (e.g. myocardial, skin, cerebral, mesenteric, and limb ischaemia)
  - Changes in vital signs assessed as unanticipated in the setting of septic shock
  - Changes in safety laboratory variables assessed as unanticipated in the setting of septic shock
- Changes in vital signs and safety laboratory variables

2.2.5 Additional Endpoints
- Hospital-free days up to Day 90
- Hospital length of stay up to Day 90
- Patient residence at Day 30, Day 60, Day 90, and Day 180

- Mean arterial pressure (MAP) daily until ICU discharge (for a maximum of 7 days)
- Cumulative dose over 7 days and infusion rates of norepinephrine/noradrenaline
- Pharmacokinetic response (in a subset of approximately 200 patients) - to be reported separately according to a pre-specified pharmacokinetic analysis plan (incl Plasma level of selepressin at the first attempt to wean the IMP infusion)
- Health economic evaluation – to be reported separately according to a pre-specified health economic analytical plan
- Creatinine Clearance
- PaO2/FiO2 ratio
- Extravascular Lung Water and Pulmonary Permeability Index (in a subset of 100-350 patients)
- Cardiac output (in a subset of 100-350 patients)
- Cytokines (in a subset of 100-350 patients)
- Angiopoietin 1 and 2 levels (in a subset of 100-350 patients)

2.2.6 Other Assessments

- Central Venous Pressure
- Central Venous Oxygen Saturation
- Arterial Blood Gases (PaO2, PaCO2, SaO2, pH, HCO3, base excess) and Lactate

3 Trial design

3.1 General Design Considerations

The overall adaptive design is a Phase 2b/3 trial, in which dose-ranging with response-adaptive randomization (RAR) (see Appendix 5 Statistical Model for Adaptive Design Decisions for details) is utilized in a first part (the Phase 2b part – Part 1), followed by a traditional 1:1 randomised comparison of selepressin to placebo in the second part (the Phase 3 part – Part 2). The final analysis uses patients from both parts of the trial. The entire trial, combining both parts, represents an adequate and well-controlled comparison of selepressin and placebo.

In Part 1 of the trial, up to four dosing regimens will be investigated.
Arm 1: Starting dose at 1.7 ng/kg/min, and a max. dose of 2.5 ng/kg/min
Arm 2: Starting dose at 2.5 ng/kg/min, and a max. dose of 3.75 ng/kg/min
Arm 3: Starting dose at 3.5 ng/kg/min, and a max. dose of 5.25 ng/kg/min
Arm 4: Starting dose at 5 ng/kg/min, and a max. dose of 7.5 ng/kg/min

Part 1 comprises a minimum of 300 patients and a maximum of 800. During Part 1, patients will be randomised to placebo or selepressin (Arms 1 to 3). Arm 4 will only be opened between 200 - 600 patients and if there is at least a 50% probability that Arm 3 has a higher expected P&VFD than Arm 2 and if data from the lower dosing levels do not suggest any significant safety signals. To minimize the risk of imbalance between treatment arms, randomisation will be stratified based on trial site, the need for mechanical ventilation (Yes/No), norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and serum creatinine (< or ≥150 µmol/L) (See Appendix 7 Randomisation Plan for details).

Part 1 will begin with a 200-patient "burn-in" period during which fixed randomisation across the treatment arms will be used (one-third of the patients randomised to placebo and two-ninths of the patients to each of the selepressin arms [Arms 1 to 3]). The factors described above will be used to stratify the randomisation.

After completion of the burn-in period, Part 1 will utilize response-adaptive randomisation to preferentially place patients into the arms that appear to have the maximum benefit with respect to the primary endpoint. A fixed fraction (one third) of patients will be randomised to placebo throughout Part 1 to ensure contemporaneous control patients are enrolled throughout the trial.

If Part 1 culminates in the decision to run Part 2, Part 2 will be a 1:1 comparison of placebo to the best-performing active treatment arm. The best-performing active treatment arm will be identified at the end of Part 1. Part 2 will utilize a fixed 1:1 randomisation proportion, with stratified randomisation as described for Part 1. Part 2 can begin after any interim analysis after 300-800 patients in Part 1 and the size of Part 2 will include enough patients to bring the total number of patients in Part 1 and Part 2 up to 1800, ensuring a minimum sample size of Part 2 of 1000 patients.

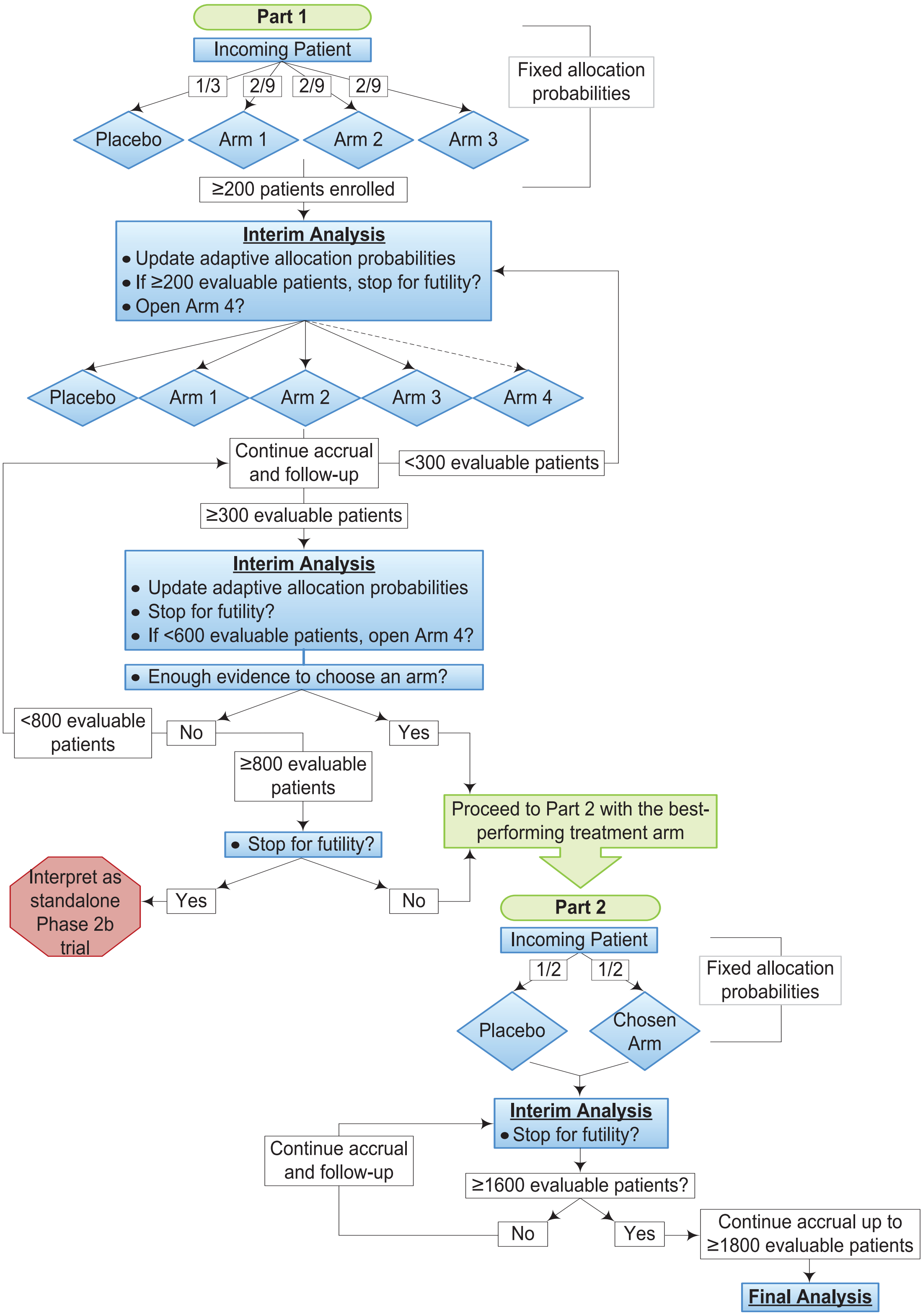

Figure 1: Design Flow Chart

3.2 Determination of Sample Size

At least 1800 evaluable patients combined for Part 1 and Part 2 are needed for the final analysis. The overall power of obtaining statistical significance based on combined evidence from Part 1 and Part 2 is 91% in situations where all 4 arms have a true underlying 1.5% lower mortality rate and a 1.5-day higher expected number of P&VFDs for survivors as compared to placebo (corresponding to an overall treatment effect of 1.5 P&VFDs). If the effect sizes are 2% on mortality and 2 days for P&VFDs in survivors for all 4 arms (corresponding to an overall treatment effect of 2 P&VFDs) then the overall power is 98%. In this latter case the probability of engaging into Part 2 is ~99%.

4 Patient Disposition

A summary table will present, for each part of the trial and overall, the number of patients in the population sets: ‘Screened’, ‘Intention to treat’, Full analysis set’, ‘Per protocol’, ‘Safety’, ‘Completed trial’, ‘Withdrawals/IMP discontinuations (whichever comes first)’ with a breakdown of reasons/categories for and trial withdrawals/IMP discontinuations.

The patient disposition table will be broken down by each of the stratification variables [the need for mechanical ventilation (Yes/No), norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and serum creatinine (< or ≥150 µmol/L)] and broken down chronologically displaying number of patients ‘completed’ and ‘withdrawn from trial/discontinued from IMP’ at Day 30, Day 90 and Day 180.

The number of patients screened but not randomised/allocated to treatment will be presented with the reason(s) for screen failure in a data listing.

All major protocol violations (including misrandomisations), based on the Full analysis set, will be summarised for each part of the trial.

Furthermore 1-KM plots, based on the ITT, will be presented for the time to trial withdrawals/IMP discontinuations (whichever comes first) differentiated by reason of trial withdrawal/IMP discontinuation using cumulative incidence functions. Dropout rates between treatment groups will be evaluated by the log-rank test.

5 Protocol Deviations

Patients in the full analysis set (FAS) will be excluded from the per-protocol (PP) analysis set if they meet any of the following criteria:

- Patients assigned to the wrong treatment arm according to randomisation
- More than 16 hours from onset of vasopressor treatment to start of IMP
- Violation of exclusion criteria 2: Primary cause of hypotension not due to sepsis (e.g., major trauma including traumatic brain injury, hemorrhage, burns, or congestive heart failure/cardiogenic shock)
- Violation of exclusion criteria 6: Chronic mechanical ventilation for any reason OR severe chronic obstructive pulmonary disease (COPD) requiring either continuous daily oxygen use during the preceding 30 days or mechanical ventilation (for acute exacerbation of COPD) during the preceding 30 days
- Violation of exclusion criteria 8: Decision to limit full care
- Violation of exclusion criteria 9: Use of vasopressin in the past 12 hours prior to start of IMP infusion or use of terlipressin within 7 days prior to start of IMP infusion
- Violation of exclusion criteria 11: Prior use of an investigational medicinal product within the last month OR planned or concurrent participation in a clinical trial for any investigational drug or investigational device
- Violation of inclusion criteria 3: Septic shock defined as hypotension (systolic blood pressure less than 90 mmHg OR MAP less than 65 mmHg) requiring vasopressor treatment (i.e. any dose of norepinephrine / noradrenaline greater than 5 µg/min) despite adequate fluid resuscitation (at least one litre for hypotension).

Furthermore, any other major protocol violations, such as serious unforeseen violations deemed to invalidate the data and affect the conclusions of the study will lead to exclusion of data from the PP analysis set.

Major protocol deviations will lead to exclusion of data from the PP analysis, while data will not be excluded because of minor protocol deviations. The list of major protocol deviations will be detailed and documented in the clean file document prior to database release.

All protocol deviations (minor and major) will be listed in patient data listings.

6 Analysis sets

6.1 Intention-To-Treat Analysis Set

The intention-to-treat (ITT) analysis set comprises of all randomised (as planned) patients.

6.2 Full-Analysis Set

The FAS comprises data from all randomised (as planned) and dosed patients.

6.3 Per Protocol Analysis Set

Patients in the FAS will be excluded from the PP analysis set if they meet any major protocol violations defined in (Section 5). Data will be used up to the point of protocol violation.

6.4 Safety Analysis Set

The safety analysis set comprises all treated patients and are analysed according to the actual treatment received.

7 Trial population

7.1 Demographics and Other Baseline Characteristics

Categorical data will be summarised using numbers and percentages. The percentages are based on the total number of patients with a corresponding assessment. Continuous data will be presented, for example, using the number of patients (N), mean and standard deviation, median, interquartile range, minimum and maximum. All baseline characteristics will be listed.

Demographics and baseline characteristics of the study population will be summarised for the FAS.

7.1.1 Demographics

Descriptive statistics of baseline demographics variables will be summarized by treatment arm and total.

7.1.2 Vital Signs at Baseline

Baseline vital signs will be summarised by treatment arm and total.

7.1.3 SOFA Score, APACHE II Score and Septic Shock Characteristics

Baseline SOFA score (modified), APACHE II score and information on septic shock (infection proven/suspected, primary infection type and location will be summarised by treatment arm and total.

7.2 Medical and Surgical History

Medical and surgical history recorded at screening visit will be summarised by treatment arm and total.

Furthermore, medical and surgical history will be presented in patient data listings.

7.3 Prior and Concomitant Medication

Prior and concomitant medication will be summarised by treatment arm and total.

Furthermore, concomitant medication will be presented in patient data listings.

8 Exposure and Treatment Compliance

8.1.1 Extent of Exposure

The total amount (adjusted by weight (µg/kg)) of selepressin administered and the number of (decimal) days treated with selepressin will be summarised by (active) treatment arm and total (active treatment arms).

Furthermore, the mean cumulative amount administered and the mean infusion rate will be tabulated by treatment arm and presented graphically (also by treatment arm and total).

9 Efficacy

9.1 General Considerations

All statistical tests will be performed using a two-sided test at a 5% significance level.

If the trial is stopped prematurely due to e.g. futility, the data will be analysed as planned in this statistical analysis plan.

The efficacy endpoints will be analysed for the FAS and the PP analysis set, with the FAS being considered as primary and the PP analyses as supportive.

Categorical data will be summarised using counts and percentages, while continuous data will be presented using the number of patients (N), mean, standard deviation, median, interquartile range, minimum and maximum. All efficacy endpoints will be listed in patient data listings.

9.2 Primary Endpoint

- Pressor- and ventilator-free days (P&VFDs) up to day 30

P&VFDs is defined as the length of period (counted as decimal days) from start of treatment with the investigational medicinal product (IMP) [selepressin or placebo] to 30 days thereafter during which the patient is: 1) alive; 2) free of treatment with intravenous vasopressors (norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, and IMP [selepressin or placebo]); and 3) free of any invasive mechanical ventilation (i.e. via a tube in the trachea, see definition below). By definition, any patient that dies within this 30-day period (thirty complete 24 hour periods from start of dosing) will be assigned zero P&VFDs, even if there is a period during which the patient is alive and free of both vasopressor treatment and mechanical ventilation.

Free of vasopressors means no use or less than one hour collectively during any 24-hour period. If a patient requires periods of vasopressors longer than one hour collectively during any 24-hour period or mechanical ventilation multiple times during the 30-day period, the intervening intervals during which they are free of vasopressors and mechanical ventilation will not be included in the periods free of vasopressors or ventilation in the determination of the number of P&VFDs.

For calculation of ventilator-free days, mechanical ventilation is defined as use of endotracheal or tracheostomy tube assisted ventilation (but excluding non-invasive ventilation by mask). End of mechanical ventilation is defined as: 1) extubation of intubated patients or 2) ≤5 cm H_2O continuous positive airway pressure and ≤5 cm H_2O of pressure support from the ventilator in tracheostomy patients.

Vasopressor use due to anaesthesia / procedure-induced hypotension during - and up to three hours after - surgery / procedure (including bedside) is exempt from this rule (i.e. does not reset the calculation of P&VFDs). Likewise, mechanical ventilation during - and up to three hours after - surgery / procedure is exempt from this rule

For patients with missing data on the primary endpoint, a 'last status carried forward' approach will be applied. If a patient was last seen on either ventilator or vasopressors, it is assumed that the patient remained so, and is imputed to a value of 0 (zero) P&VFDs. If the patient was last seen off ventilator and vasopressors, it is also assumed that the patient remained so in the remaining 30-day period. If the patient was last seen (alive) on e.g. day 10 and at that point had been off both ventilator and vasopressors for three days, a value of 23 P&VFDs is imputed.

9.2.1 Primary Variable Analysis

The primary endpoint, P&VFDs, will be analyzed using a van Elteren test, stratified by need for ventilation (Yes/No), time from onset of shock (onset of vasopressor (i.e. norepinephrine/noradrenaline)) to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min).

The primary analysis will compare all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) to all patients on the placebo arm from both parts of the trial (see Appendix 8 Bias on Treatment Estimate for P&VFDs for a discussion on treatment estimate bias).

The primary analysis will be a test of superiority using a two-sided 5% significance level test.

Treatment effects will be estimated assuming a negative binomial distribution (to allow for possible overdispersion in a Poisson distribution) for the quantity (30 minus P&VFDs) for survivors, and a binomial distribution to model the probability of surviving. Both models adjusted for need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) (see Appendix 3 Estimation of Treatment Effects for P&VFDs for details). For completeness, the proportion of patients dying, and the P&VFDs for survivors will also be presented.

Furthermore, P&VFDs will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically by histograms and cumulative distributions functions.

The success (statistical/clinical significance) of the trial will be based upon the comparison of the analysis above (all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) compared to all patients on the placebo arm from both parts of the trial).

9.2.2 Sensitivity Analyses

In order to check for consistency the primary endpoint treatment differences will, as a minimum, be estimated and presented by forest plots for the following subgroups

- region (US/Canada vs. Europe)
- age (<65, 65-74, 75-84, >85)
- gender
- race/ethnicity

Furthermore, the primary endpoint will be stratified by severity of the patients, with risk of dying as indicator of severity (Figure 2). Mortality (the risk of dying) will be predicted by a logistic regression model, with relevant baseline characteristics as covariates (e.g. the individual SOFA scores and age). The model used to generate the predicted risk (for all patients) will be based on patients in the placebo arm only, as the risk of dying should reflect the severity in the absence of selepressin. Stratified by the risk of dying (intervals of 20% if suitable, based on the mortality rates in the covariate categories in the model), the treatment effect of the primary endpoint will be presented graphically, in order to visually inspect whether the average treatment effect is distributed evenly across the severity of patients.

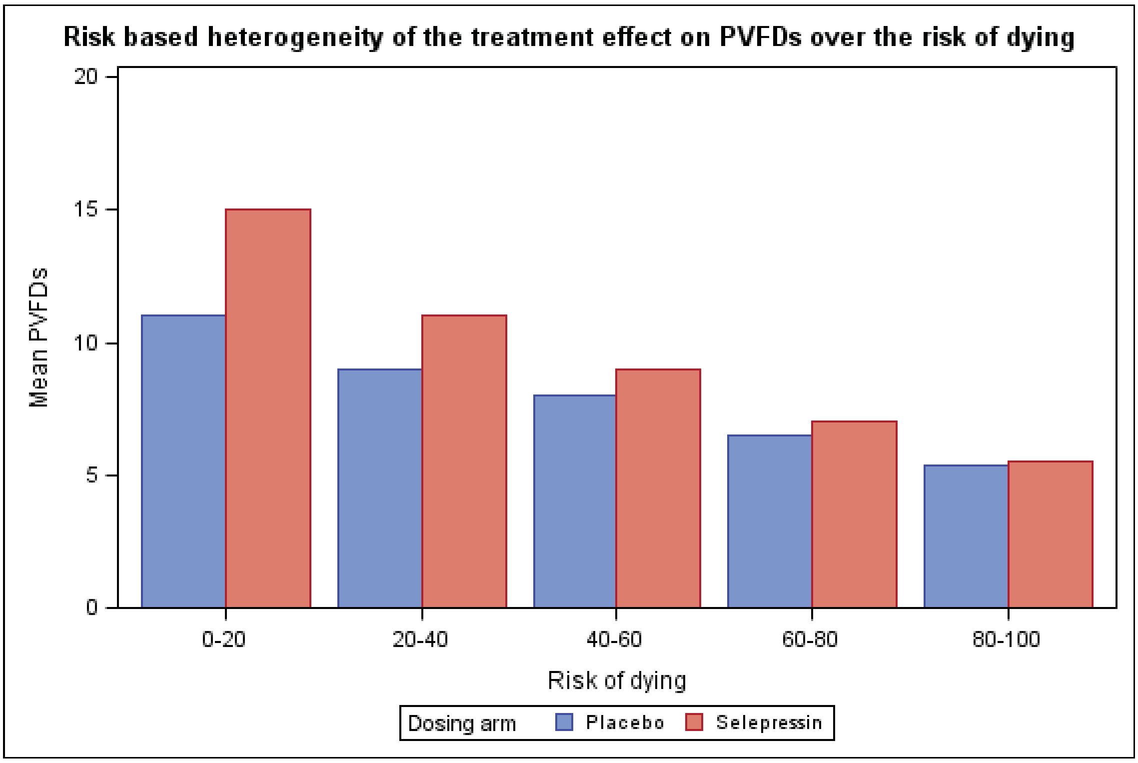

Figure 2: Risk Based Heterogeneity of the Treatment Effect on P&VFDs over the Risk of Dying (an Example)

The impact and robustness of the imputation of missing data will be checked by analysing data excluding all missing/imputed data, and by a tipping point analysis. The tipping point analysis will compare all possible combinations of 'best case' and 'worst case' scenarios between placebo and selepressin (Figure 3). Best case being an imputation assuming the remaining days off ventilator and vasopressors, and worst case being an imputation of 0 P&VFDs. Let N_p and N_s be the number of patients in the placebo and selepressin arms with missing data. The tipping point analysis will compare all combinations (from 0 to N_p) of X patients on placebo imputed best case and $N_p - X$ imputed worst case, to Y patients on selepressin imputed best case and $N_s - Y$ imputed worst case. I.e all $N_p + 1$ times $N_s + 1$ combinations will be analysed for the primary endpoint. Since the 'best case' is not the same for all patients (depending on when they were last seen off both ventilator and vasopressors) there are multiple outcomes within each combination. For each combination, the average P-value of the multiple outcomes will be plotted in the tipping point analysis. Below is an example of a tipping point analysis of 25 placebo patients vs. 40 selepressin patients with imputed values. The x- and y-axis displays the number of patients with the 'best case' imputed. In the example below the red area displays the non-significant p-values, indicating that one would have to impute almost all placebo patients to a 'best case' and almost all selepressin to a 'worst case' in order to get non-significant p-values, and hence 'proving' the robustness of the imputation method.

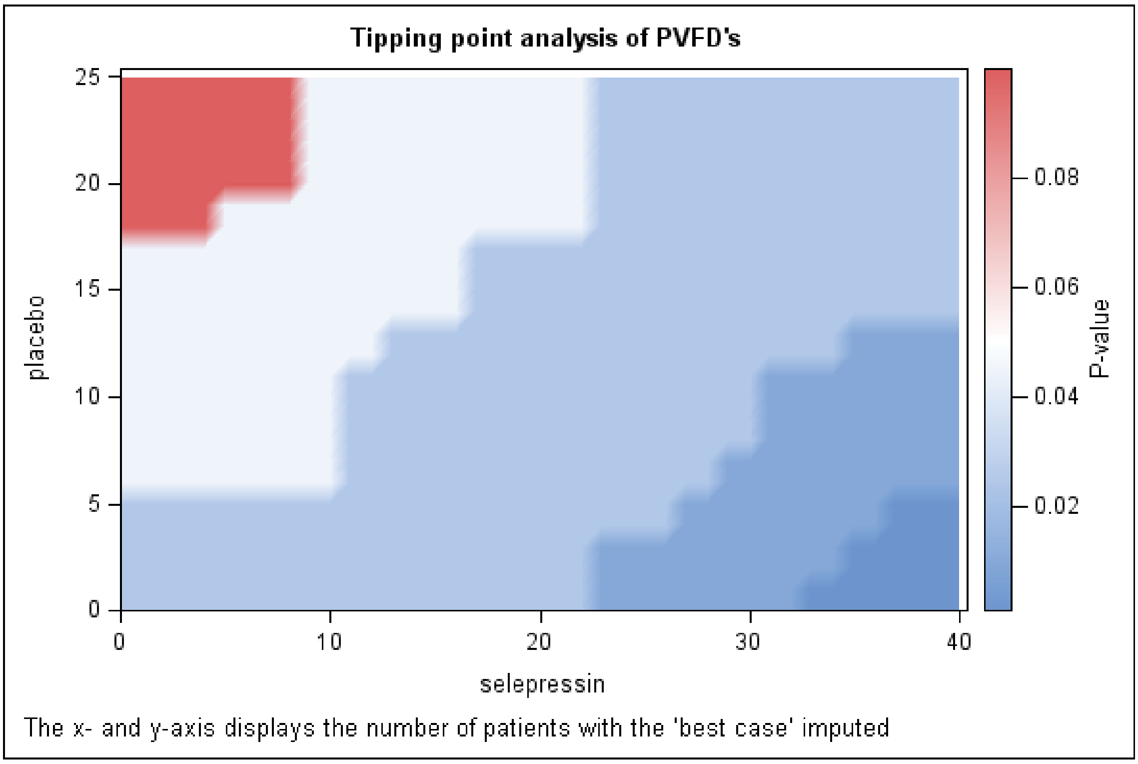

Figure 3: Tipping Point Analysis of P&VFDs (an Example)

Also, to make sure that the use of vasopressor in each group is not simply being replaced by an increased use of inotropic agents (e.g. dobutamine, milrinone, levosimendan, and amrinone), there will be a sensitivity analysis of the primary endpoint in which the use of inotropic agents will count as vasopressor use.

9.2.3 Additional Analyses

The primary analysis will be repeated for:

- the selected arm only, i.e. comparing all patients on the selected arm (from part 1) from both parts of the trial (pooled), to all patients on the placebo arm from both parts of the trial.
- data from part 2 only, i.e. comparing the selected arm to placebo on data from part 2 only

9.3 Secondary Endpoints

For the purpose of a possible label inclusion, the Hochberg procedure [4] for adjustment on multiplicity will be implemented to selected key secondary endpoints. Only if the primary efficacy analysis leads to a statistically significant result at the (one-sided) 2.5% level, then the Hochberg procedure which is described below is applied to selected key secondary analyses. If the primary efficacy analysis does not result in statistical significance at the (one-sided) 2.5% level, then statistical significance (for the purpose of a possible label inclusion only) will not be declared for any of the key secondary analyses, regardless of their p-values.

The selected key secondary endpoints aimed at further demonstrating treatment effect are:

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause, by the end of Day 90)
- Renal replacement therapy-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)
- ICU-free days up to Day 30

In this application of the Hochberg procedure there are three hypothesis tests of superiority for each of the selected secondary endpoints. The target alpha level is (one-sided) 2.5%. The Hochberg procedure is as follows:

- Order the p-values from the smallest to the largest value, p(1) < p(2) < p(3), with corresponding null hypothesis $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$.

- Start with the highest p-value. If p(3) < 2.5% (one-sided), then stop and declare all three comparisons significant at the 2.5% (one-sided) level (i.e. reject $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$). Otherwise, accept $H_{(3)}$ for the endpoint related to p(3), and go to p(2) the second highest p-value.

- If p(2) < 2.5/2 = 1.25% (one-sided), then stop and declare significance for $H_{(1)}$ and $H_{(2)}$. Otherwise, accept $H_{(2)}$, for the endpoint related to p(2), and go to p(1) the lowest p-value.

- If p(1) < 2.5/3 = 0.833% (one-sided), then stop and declare significance for $H_{(1)}$. Otherwise, accept $H_{(1)}$, for the endpoint related to p(1).

Regardless of the statistical significance declared according to the Hochberg procedure, all analysis will be included and presented in the statistical report.

As for the primary analysis, the primary comparison (which determines the success, i.e. statistical and clinical significance) for the secondary efficacy endpoints is between all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) and all patients on the placebo arm from both parts of the trial.

As an additional analysis, all secondary efficacy analyses will, as for the primary, be repeated for:

- the selected arm only, i.e. comparing all patients on the selected arm (from part 1) from both parts of the trial (pooled), to all patients on the placebo arm from both parts of the trial.

- data from part 2 only, i.e. comparing the selected arm to placebo on data from part 2 only

All free-days endpoints will be counted in decimal days.

9.3.1 Organ Dysfunction

9.3.1.1 Vasopressor-free Days up to Day 30

Vasopressor-free Days up to Day 30 will be defined and analyzed in a similar manner as the primary endpoint, with the van Elteren test stratified by need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 μg/min).

9.3.1.2 Ventilator-free Days up to Day 30

Ventilator -free Days up to Day 30 will be defined and analyzed in a similar manner as the primary endpoint, with the van Elteren test stratified by need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min).

9.3.1.3 Duration of Septic Shock up to Day 30

Duration of septic shock is defined as the cumulative periods (>1 hour) from start of IMP until Day 30, on IMP or vasopressors. Vasopressor use during surgery for anesthesia-induced hypotension is exempt from this rule.

For patients withdrawn (in the survivors analysis) or dying (in the non-survivors analysis) while still in septic shock, the duration will be based on the data available up until the time of withdrawal or death.

Duration of septic shock will be analyzed separately for survivors, non-survivors (within the first 30 days) and overall, comparing treatment arms by an ANCOVA model with time from onset of shock to start of treatment and norepinephrine/noradrenaline requirement at baseline as covariates, and treatment and need for ventilation (Yes/No) as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented.

Some patients will get out of shock prior to Day 30 (and stay alive until Day 30), some will get out of shock and die later on (prior to Day 30), others will die while still in shock (prior to Day 30), and the remaining few will not get out of shock prior to Day 30. This means that if mortality rates vary between treatment arms, the results of the analysis for the overall population will be influenced by the skewed mortality rates. Hence, for the overall population, the distribution of duration of shock (time to out of shock), will be presented graphically as competing risks between ‘time to out of shock’ and ‘dying while in shock’. Further, a Kaplan-Meier (sub)-graph on ‘time to death’ will be presented for those getting out of shock (for which some will die later on, prior to Day 30). This is done in order to elucidate any skewness in mortality rates, influencing the results of the analysis.

Furthermore, duration of septic shock will be tabulated by treatment arm (including pooled active treatment arms).

9.3.1.4 Duration of Mechanical Ventilation up to Day 30

Duration of mechanical ventilation is defined as the cumulative periods (>1 hour) from start of IMP until Day 30, on mechanical ventilation.

For patients withdrawn or dying while still on mechanical ventilation, the duration will be based on the data available up until the time of withdrawal or death.

Duration of mechanical ventilation will be analyzed as for duration of septic shock in (Section 9.3.1.3) with time from onset of shock to start of treatment, and norepinephrine/noradrenaline requirement at baseline as covariates, and treatment and need for ventilation as factors.

9.3.1.5 Daily Overall and Individual Organ (Cardiovascular, Respiratory, Renal, Hepatic, Coagulation) Scores using the Modified Sequential Organ Failure Assessment (SOFA) Scores Until ICU Discharge

Last observation carried forward (LOCF) will be used for missing SOFA scores on Days 2-7. No LOCF for Day 1 (as previous value is baseline). Patients dying will be imputed with a worst possible outcome, i.e. a value of 4 for each individual SOFA score.

Daily overall (modified) and individual SOFA scores will be compared between treatment arms up until Day 7 using a repeated measures ANCOVA model with baseline SOFA score as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented.

Furthermore, daily overall and individual SOFA scores will be tabulated by treatment arm (including pooled active treatment arms).

9.3.1.6 Incidence of New Organ Dysfunctions and New Organ Failures (Based on the SOFA score) up to Days 7 and 30

Incidence of new organ failures is defined as a change in any of the individual SOFA scores from (0,1,2) at baseline to (3,4) post baseline up until the end of the period (Day 7 or 30) (if the SOFA scores goes from (0,1,2) to (3,4) and back to (0,1,2) again within the period, that will still count as a new organ failure). If a patient dies within the period, he is considered to fail on all organs, and the number of new organ failures will be all organs except those already failed at baseline. Patients withdrawn within the period will be evaluated based on the data available at time of withdrawal.

Incidence of new organ dysfunction is defined as an increase ≥ 1 from baseline to post baseline up until the end of the period (e.g. going from 1 to 2) in any of the individual SOFA scores. Patients with an individual SOFA score of 4 at baseline can per default not have a new organ dysfunction. If a patient dies within the period, he is considered to have dysfunction on all organs, and the number of new organ dysfunctions will be all organs except those already having a score of 4 at baseline. Patients withdrawn within the period will be evaluated based on the data available at time of withdrawal.

As the SOFA score is only collected for patients still in the ICU, it is assumed that as soon as the patient leaves the ICU, the patient will not experience any new organ dysfunctions or organ failures. Unless of course the patient dies.

Incidence of at least one new organ failure will be analyzed for any new organ failure (across all organ systems) and by individual organ systems, and compared between treatment arms using a logistic regression model with age, modified SOFA score and norepinephrine/noradrenaline requirement at baseline as covariates and gender and treatment arm as factors, presenting odds ratios with 95% confidence intervals.

Incidence of at least one new organ dysfunction will be analyzed for any new organ dysfunction (across all organ systems) and by individual organ systems, and will be analyzed as above for new organ failures.

The number of new organ failures and the number of new organ dysfunctions will be compared between treatment arms using an ANCOVA model with age, modified SOFA score and norepinephrine/noradrenaline requirement at baseline as covariates, and gender and treatment as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented.

Furthermore, incidence of any new organ failure and any new organ dysfunction, and the number of new organ failures and new organ dysfunctions will be tabulated by treatment arm (including pooled active treatment arms).

9.3.1.7 Renal Replacement Therapy (RRT)-free Days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)

RRT-free Days is defined as for the primary endpoint with free of treatment with any form of renal replacement therapy defined as continuous renal replacement therapy, intermittent hemodialysis or peritoneal dialysis.

RRT-free Days will be analyzed excluding patients on RRT for chronic renal failure at time of randomisation.

RRT-free Days up to Day 30 will be analyzed in a similar manner as the primary endpoint, with the van Elteren test stratified by need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and baseline creatinine (< or ≥150 µmol/L).

9.3.1.8 Incidence of RRT up to Day 30 (counting patients who die as on RRT and excluding patients on RRT for chronic renal failure at time of randomisation)

RRT is defined as any form of renal replacement therapy defined as continuous renal replacement therapy, intermittent hemodialysis or peritoneal dialysis. In order to ensure that any reduction in incidence of RRT is not caused by an increase in mortality, all patients dying within the 30-day period will be counted as on RRT. For patients withdrawn prior to Day 30, incidence of RRT will be based on the data available up until the time of withdrawal.

Incidence of RRT will be analysed by a logistic regression model with time from onset of shock to start of treatment, baseline creatinine and norepinephrine/noradrenaline requirement at baseline, as covariates, and treatment and need for ventilation as factors. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method (see Appendix 4 Estimation of Treatment Effects for Difference of Proportions in Incidence of RRT for details). Patients already on RRT for chronic renal failure at time of inclusion will be excluded from the analysis of incidence of RRT.

Non-inferiority will be claimed if the upper limit of the two-sided 95% CI of the adjusted difference in proportions is less than 20% of the estimated incidence of RRT in the placebo group. Superiority can be claimed[5] if the upper limit is less than 0.

I.e., let $\widehat{p_S}$ and $\widehat{p_P}$ be the estimated incidences of RRT in the combined selepressin groups and the placebo group respectively. Non-inferiority will then be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0.2 * \widehat{p_P}$$

and superiority will be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0$$

Furthermore, incidence of RRT will be tabulated by treatment arm (including pooled active treatment arms).

A subgroup analysis will be performed on patients with acute RRT at baseline.

9.3.1.9 Duration of RRT up to Day 90 (excluding patients on RRT for chronic renal failure at time of randomisation)

Duration of RRT is defined as the cumulative periods with RRT (continuous renal replacement therapy, intermittent hemodialysis or peritoneal dialysis) and will be analyzed excluding patients on RRT for chronic renal failure at time of randomisation.

For patients withdrawn or dying while still on RRT, the duration will be based on the data available up until the time of withdrawal or death.

Duration of RRT will be analyzed as for duration of septic shock in (Section 9.3.1.3) with time from onset of shock to start of treatment, baseline creatinine and norepinephrine/noradrenaline requirement at baseline, as covariates, and treatment and need for ventilation as factors.

9.3.2 Morbidity/Mortality

9.3.2.1 Intensive Care Unit (ICU)-free Days up to Day 30

ICU -free Days up to Day 30 will be defined and analyzed in a similar manner as the primary endpoint, with the van Elteren test stratified by need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and baseline creatinine (< or ≥150 µmol/L).

9.3.2.2 ICU Length of Stay up to Day 30

ICU length of stay is defined as the cumulative periods spent in ICU from start of IMP to 30 days after.

For patients withdrawn or dying while still in ICU up to Day 30, the duration will be based on the data available up until the time of withdrawal or death.

ICU length of stay will be analyzed as for duration of septic shock in (Section 9.3.1.3), with time from onset of shock to start of treatment, baseline creatinine and norepinephrine/noradrenaline requirement at baseline as covariates, and treatment and need for ventilation as factors.

9.3.2.3 All-cause Mortality (Defined as the Fraction of Patients That Have Died, Regardless of Cause, by the end of Day 30, Day 90, and Day 180)

Mortality will be analysed by a logistic regression model with the individual SOFA scores and age as covariates and treatment arm as factor. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method as for incidence of RRT (section 9.3.1.8). There will be no imputations for mortality.

Non-inferiority will be claimed if the upper limit of the two-sided 95% CI of the adjusted difference in proportions is less than 30% of the estimated incidence of mortality in the placebo group. Superiority can be claimed[5] if the upper limit is less than 0.

I.e., let $\widehat{p_S}$ and $\widehat{p_P}$ be the estimated incidences of mortality in the combined selepressin groups and the placebo group respectively. Non-inferiority will then be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0.3 * \widehat{p_P}$$

and superiority will be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0$$

Assuming observed mortality rates of 20-25% in the placebo group, a non-inferiority limit of 30% corresponds to a maximum observed mortality rate of 2-3% in the combined selepressin groups in order for selepressin to be non-inferior to placebo.

Furthermore, mortality will be tabulated by treatment arm (including pooled active treatment arms), and the time to death presented graphically by a Kaplan-Meier plot.

9.3.3 Health-Related Quality of Life

9.3.3.1 Change in utility, based on EQ-5D-5L, up to Day 180

EQ-5D-5L will be analyzed by the index value, the overall QALY (Quality-Adjusted Life Years) at Day 30 and 180 (see Appendix 1 EQ-5D-5L Quality Adjusted Life Year (QALY) for details), and the VAS score.

The QALY scores will NOT be adjusted to e.g. a half yearly time scale at Day 180.

As the QALY is not defined for patients with all remaining values missing, and hence also not defined for those dead, the analyses will automatically only be analyzed for those surviving up until Day 30 and 180 respectively.

For patients with missing baseline index value, the QALY score will also be set to missing. For robustness, a sensitivity analyses will be performed, imputing the missing baseline scores with the overall mean of the baseline health index.

The QALY at Day 30 and 180 will be compared between treatment arms using an ANCOVA model with baseline health index as covariate, and treatment as factor. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

The index value and VAS scores will be analysed separately for survivors and non-survivors at Day 180 (since all non-survivors will have non-random missing values, and hence would artificially inflate the mean estimates if survivors and non-survivors were analysed together) and will be compared between treatment arms using a repeated measures ANCOVA model with baseline health index/VAS score as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented for Day 30, 60, 90 and 180. There will be no imputations for missing values.

Furthermore, the QALY, index value and VAS scores will be tabulated treatment arm (including pooled active treatment arms), and the index value and VAS scores will be presented graphically.

9.3.4 Fluid Balance

9.3.4.1 Daily and Cumulative Fluid Balance Until ICU Discharge (for a Maximum of 7 Days)

Fluid overload is defined as fluid balance as a percentage of baseline weight. E.g. if a patient weighs 90 kg at baseline and has a fluid balance of 9 liters, fluid overload is then 100% * 9L / 90kg = 10%.

Fluid balance and cumulative fluid balance will be presented both unadjusted and adjusted for weight.

Fluid balance, cumulative fluid balance, fluid overload and cumulative fluid overload will all be compared between treatment arms using a repeated measures ANCOVA model with baseline (baseline fluid balance or baseline fluid overload) as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

Fluid overload and balance will be based on linear interpolation between actual sampling time points on Day 0, 1, 2, etc. The measurement at Day 0 will be their respective time points of IMP start. For Days 1, 2, etc. sampling time points will be aligned at 12:00 pm (noon) by linear interpolation. For consistency between centres, perspiration is not included in the derivation of fluid balance. There will be no imputations of missing values for any of the fluid balance parameters.

Endpoints (absolute values and change from baseline) will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

9.3.4.2 Daily and Cumulative Urinary Output Until ICU Discharge (for a Maximum of 7 Days)

Urinary output and cumulative urinary output (absolute values) will all be will be compared between treatment arms using a repeated measures ANCOVA model with baseline urinary output as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

Urinary output and cumulative urinary output will be presented both unadjusted and adjusted for weight.

Urinary output will be based on linear interpolation between actual sampling time points on Day 0, 1, 2, etc. The measurement at Day 0 will be their respective time points of IMP start. For Days 1, 2, etc. sampling time points will be aligned at 12:00 pm (noon) by linear interpolation. There will be no imputations of missing values for any of the urinary output parameters.

Endpoints (absolute values and change from baseline) will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

9.4 Other Endpoints/Assessments

9.4.1.1 Hospital-free Days up to Day 90

Hospital -free Days up to Day 90 will be defined and analyzed in a similar manner as the primary endpoint, with the van Elteren test stratified by need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and baseline creatinine (< or ≥150 µmol/L).

9.4.1.2 Hospital Length of Stay up to Day 90

Hospital length of stay is defined as the cumulative periods spent in hospital (including ICU and other wards) from start of IMP to 90 days after.

For patients withdrawn or dying while still in hospital up to Day 90, the duration will be based on the data available up until the time of withdrawal or death.

Hospital length of stay will be analyzed as for duration of septic shock in (Section 9.3.1.3), with time from onset of shock to start of treatment, baseline creatinine and norepinephrine requirement at baseline as covariates, and treatment and need for ventilation as factors.

9.4.1.3 Patient Residence at Day 30, Day 60, Day 90, and Day 180

Patient location at Day 30, 60, 90 and 180 will be summarized by treatment arm (including pooled active treatment arms).

Shift tables will be presented at each time point to assess whether patients have returned to their location at enrollment. There will be no imputations of missing values.

9.4.1.4 Mean Arterial Pressure Daily Until ICU Discharge (for a Maximum of 7 Days)

Mean arterial pressure will be tabulated by treatment arm (including pooled active treatment arms) and presented graphically (at pre-specified time points, i.e. not when NE/NA infusion changes, and only until ICU discharge/day 7) for both MAP alone, and the difference from MAP to target MAP (which is 65, unless the investigator judges it to be otherwise). There will be no imputations of missing values.

9.4.1.5 Cumulative Dose over 7 Days and Infusion Rates of Norepinephrine/Noradrenaline

The amount of norepinephrine/noradrenaline administered, both in terms of rates and cumulative amount administered (both measures adjusted for baseline weight) will be compared between treatment arms using a repeated measures ANCOVA model with baseline rate of norepinephrine/noradrenaline (same baseline covariate for both models as the cumulative amount at baseline is not recorded) as covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented. There will be no imputations of missing values.

The mean cumulative amount administered and mean infusion rate will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

9.4.1.6 Pulmonary Function (PaO2/FiO2)

Baseline for PaO2/FiO2 will be the values obtained at the last assessment prior to the first dose of IMP. There will be no imputations of missing values.

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point by treatment arm (including pooled active treatment arms).

9.4.1.7 Arterial Blood Gases and Acid/Base Status (PaO2, PaCO2, pH, SaO2, Bicarbonate, Base Excess), Lactate and Oxygen Saturation in Vena Cava Superior (ScvO2)

Baseline for arterial blood gases, lactate and ScvO2 will be the values obtained at the last assessment prior to the first dose of IMP. There will be no imputations of missing values.

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point by treatment arm (including pooled active treatment arms).

9.4.1.8 Cytokines, ANG-1 and ANG-2 (in a Subset of 100-350 Patients)

Baseline values will be the values obtained at the last assessment prior to the first dose of IMP. There will be no imputations of missing values.

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point by treatment arm (including pooled active treatment arms).

9.4.1.9 EVLW and PPI (in a Subset of 100-350 Patients)

Baseline for EVLW and PPI will be the values obtained at the last assessment prior to the first dose of IMP. There will be no imputations of missing values.

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point by treatment arm (including pooled active treatment arms).

9.4.1.10 Cardiac Output (in a Subset of 100-350 Patients)

Baseline for cardiac output values will be the values obtained at the last assessment prior to the first dose of IMP. There will be no imputations of missing values.

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point by treatment arm (including pooled active treatment arms).

9.4.1.11 Creatinine Clearance

Creatinine clearance is determined by estimated glomular filtration rate (using serum creatinine, age, and gender as per Cockcroft-Gault).
Cockcroft-Gault equation: creatinine clearance (mL/min) = ((140 - age in years) x weight (kg)) / serum creatinine (µmol/L) for women. For men, multiply result by 1.2.

Creatinine clearance will be analyzed up until day 3 as for fluid balance in (Section 9.3.4.1), with baseline creatinine as a covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit.

10 Safety

10.1 General Considerations

Safety parameters will be evaluated for the safety analysis data set.

All safety summaries will be tabulated by treatment arm (including pooled active treatment arms).

10.2 Adverse Events

Adverse events (AEs) are classified according to the Medical Dictionary for Regulatory Activities (MedDRA). The version of MedDRA will be documented in the clinical report.

Written narratives will be issued for all serious AEs (including deaths) and AEs leading to withdrawal.

A pre-treatment adverse event is any untoward medical occurrence arising or observed between informed consent and administration of the IMP.

A treatment emergent adverse event is any adverse event occurring after the administration of the IMP and within the time of residual drug effect, or a pre-treatment adverse event or pre-existing medical condition that worsens in intensity after start of IMP and within the time of residual drug effect.

The time of residual drug effect is the estimated period of time after the end of the administration of the IMP, where the effect of the product is still considered to be present based on pharmacokinetic, pharmacodynamic or other substance characteristics. A generally accepted time for residual drug effect is 5 half-lives. The terminal half-life of the IMP is expected to be not more than 1.8 hours, and treatment-emergent AEs are defined as AEs occurring after the start of study drug infusion to within 12 hours after study drug infusion is stopped.

A post-treatment adverse event is any adverse event occurring after the residual drug effect period. Missing values will be treated as missing, except for causality, intensity, seriousness, and outcome of adverse events. A “worst case” approach will be used: if causality is missing, the adverse event will be regarded as related to the IMP; if the intensity of an adverse event is missing, the adverse event will be regarded as severe; if seriousness is missing the adverse event will be regarded as serious; if start date is missing or incomplete, worst case will be assumed and the AE will be regarded as treatment emergent (only if the incomplete start date is not compromised). If start date is completely missing, start date will be set as same day as start of treatment. If start date is incomplete, the date closest to start of treatment will be assumed, without compromising the incomplete data available on the start date; if outcome is missing and no date of outcome is present the outcome is regarded as ‘not recovered’.

10.2.1 Overview of Treatment-Emergent Adverse Events

An AE overview summary table will be prepared including the number of patients reporting an AE, the percentage of patients (%) with an AE, and the number of events (E) reported, for the following categories:

- Treatment-emergent adverse events
- Deaths
- Serious adverse events
- Adverse events leading to discontinuation of IMP
- Adverse events leading to withdrawal from the trial
- Severe and life threatening adverse events
- Adverse drug reactions

10.2.2 Incidence of Adverse Events

Treatment-emergent adverse events will be summarised in a Table by SOC and PT for MedDRA. The Table will display the total number of patients reporting an AE, the percentage of patients (%) with an AE, and the number of events (E) reported. AEs will be presented by system organ class (SOC) sorted alphabetically and preferred term (PT) sorted in decreasing frequency of occurrence.

Summary tables will be prepared for:

- All adverse events
- Adverse events with an incidence ≥ 5% of patients in any treatment arm
- Non-serious adverse events with an incidence ≥ 5% of patients in any treatment arm
- Critical adverse events (see Appendix 2 Critical Adverse Events and Classification of Adverse Events Based on Changes in Safety Laboratory Variables
- for details)
- Adverse events based on changes in vital signs assessed as unanticipated in the setting of septic shock (see Appendix 2 Critical Adverse Events and Classification of Adverse Events Based on Changes in Safety Laboratory Variables
- for details)
- Adverse events based on changes in safety laboratory variables assessed as unanticipated in the setting of septic shock (see Appendix 2 Critical Adverse Events and Classification of Adverse Events Based on Changes in Safety Laboratory Variables
- for details)
- Adverse events by causality (related/unrelated)
- Adverse events leading to death
- Adverse events by intensity
- Serious adverse events
- Adverse events leading to discontinuation of IMP (related/unrelated)

- Adverse events leading to withdrawal from trial (related/unrelated)

Supporting data listings will be provided for:

- All adverse events sorted by centre and patient no.
- All adverse events sorted by MedDRA Preferred Term
- Serious adverse events
- Adverse events leading to death
- Adverse events leading to discontinuation of IMP (related/unrelated)
- Adverse events leading to withdrawal from trial (related/unrelated)
- Post treatment-emergent adverse events.

10.3 Safety Laboratory Variables

Baseline for all laboratory analyses will be the values obtained at the last assessment prior to the first dose of the investigational medicinal product (IMP). End of treatment period will include the last post-baseline observation during the trial up until Day 30.

Laboratory variables will be grouped under “Haematology”, “Clinical Chemistry” or “Coagulation”.

10.3.1 Summary Statistics

Mean change and mean percentage (%) change from baseline at end of treatment period will be presented for each laboratory variable. In addition, descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each laboratory variable.

Furthermore, a summary table will be prepared for selected laboratory variables that display the number and percentage of patients in each treatment arm with X% increments (increase or decrease) from baseline at Day 1, 3, 7, 14, 30 and end of treatment period. The following categories for summary tables are defined:

- ≤ -3*X%: Values with more than 3*X% decrease from baseline
- > -3*X% - -2*X%: Values with 2-3*X% decrease from baseline
- > -2*X% - -1*X%: Values with 1-2*X% decrease from baseline
- > -1*X% - 0*X%: Values with 0-1*X% decrease from baseline
- < 0*X%- < 1*X%: Values with 0-1*X% increase from baseline
- 1*X%- < 2*X%: Values with 1-2*X% increase from baseline
- 2*X%- < 3*X%: Values with 2-3*X% increase from baseline
- ≥ 3*X%: Values with more than 3*X% increase from baseline

The following key laboratory variables will be summarised:

- Hemoglobin: 20% change
- WBC: 50% change
- Platelets: 50% change
- Creatinine: 20% change
- Sodium: 5% change
- Lactate: 50% change

10.3.2 Data Listings

Data listings will be prepared by centre, treatment arm, patient and time-point (including baseline) displaying all laboratory values for all patients.

10.4 Vital Signs (including CVP)

Baseline for all vital signs analyses will be the values obtained at the last assessment prior to the first dose of IMP.

10.4.1.1 Summary Statistics

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each vital signs variable.

Furthermore, a summary table will be prepared for each vital signs variable that display the number and percentage of patients in each treatment arm with X% increments (increase or decrease) from baseline. The following categories for summary tables are defined:

- ≤ -3*X%: Values with more than 3*X% decrease from baseline
- > -3*X% - -2*X%: Values with 2-3*X% decrease from baseline
- > -2*X% - -1*X%: Values with 1-2*X% decrease from baseline
- > -1*X% - 0*X%: Values with 0-1*X% decrease from baseline
- < 0*X%- < 1*X%: Values with 0-1*X% increase from baseline
- 1*X%- < 2*X%: Values with 1-2*X% increase from baseline
- 2*X%- < 3*X%: Values with 2-3*X% increase from baseline
- ≥ 3*X%: Values with more than 3*X% increase from baseline

The following % changes will be summarised:

- Heart rate: 50% change
- Blood pressure: 25% change

10.4.1.2 Data Listings

Data listings will be prepared by centre, treatment arm, patients and time-point (including baseline) displaying all vital signs values for all patients with an indication of abnormal values.

11 Interim analyses

There will be no interim analyses with the potential to stop the trial early for treatment efficacy. However, once the "burn-in" period in Part 1 (first 200 patients) is completed, regular interim analyses will be conducted to improve the efficiency of dose selection and to allow early termination of the part or the trial for futility or for successful dose selection. The following steps will be considered at each interim analysis:

- Potentially stopping the trial for futility. This occurs if no active arm has better than a 5% predictive probability of a significant result in Part 2 if it were to start immediately.
- Potentially ending Part 1 and selecting an active treatment arm to continue to Part 2. This decision can occur at any interim analysis between 300 and 800 patients, and it occurs if some arm has a predictive probability of a successful trial of at least 90% before 800 patients, and the threshold drops to 25% for the final Part 1 interim analysis at 800 patients. The selected arm is the arm with the largest posterior predictive probability of trial success. This will generally be the best-performing active arm, but if multiple arms are performing equally well, it will be the arm with the lowest dosing level. If Part 1 ends after *N* patients, then Part 2 will consist of up to 1800 - *N* patients.
- If the trial is not stopped for futility or proceeding to Part 2 and active treatment Arm 4 has not yet been approved for assignment of patients, the decision can be made to open up Arm 4. Arm 4 is only opened between 200 and 600 patients and if there is at least a 50% probability that Arm 3 has a higher expected P&VFD than Arm 2 and if data from the lower dosing levels do not suggest any significant safety signals.
- If the trial is not stopped for futility and does not proceed to Part 2, the allocation probabilities for the active treatment arms are changed using response-adaptive randomisation (with placebo still 1/3). For the two-thirds of patients assigned to active arms, the probability that a given active arm is assigned to a patient is proportional to the probability that that arm is the arm with the largest expected number of P&VFD.
- If Part 1 reaches its maximum of 800 evaluable patients and no arm has a predictive probability of Part 2 success of more than 25%, the trial stops with an inconclusive result and will be interpreted as a standalone Phase 2b trial.

During Part 2, interim analyses will be conducted regularly (until data is available for 1600 evaluable patients) to allow early termination of the trial for futility. This occurs if the predictive probability of an overall significant result is less than 5%. In addition, if the predictive probability of observing a more than 2% higher mortality in the active arms compared to placebo is greater than 90% then the trial will stop for futility.

12 Deviations from protocol analysis

NA.

13 References

[1] The EuroQol Group (1990). EuroQol-a new facility for the measurement of health-related quality of life. *Health Policy* 16(3): 199-208

[2] EuroQoL Home page: http://www.euroqol.org/home.html

[3] Mehta, C.R. and Pocock, S.J. (2011) Adaptive increase in sample size when interim results are promising: A practical guide with examples. *Statistics in Medicine*, 30, 3267-3284.

[4] Hochberg (1988). A sharper Bonferroni procedure for multiple tests of significance. *Biometrika* 75(4):800-802

[5] EMEA. (2000). Points to consider on switching between superiority and non-inferiority. CPMP/EWP/482/99.

14 Tables, Listings and Figures

The document with tables, figures and listings (TLF) shells will be presented in a separate document.

Appendix 1 EQ-5D-5L Quality Adjusted Life Year (QALY)

We calculate Quality Adjusted Life Year (QALY)[1][2] at Day 30 and 180 in three steps:
(1) A unique EQ-5D-5L health state is defined by combining 1 level from each of the 5 dimensions of EQ-5D-5L. Each health state is referred to in terms of a 5 digit code. For example, state 12345 indicates no problems with mobility, slight problems with washing or dressing, moderate problems with doing usual activities, severe pain or discomfort and extreme anxiety or depression.
(2) Convert each EQ-5D-5L health state into a single EQ-5D-5L index value. The index values are country specific and we will use the value sets for US and apply these values to all patients in this trial.
(3) QALY for a patient is then defined to be the area under the curve (AUC) for a Time (with unit of Year) versus index values. AUC will be calculated by the linear trapezoidal method. See below for a schematic presentation where the y-axis is the index value with y_0, y_1 and y_2, etc. represent the index values at baseline, Day 30, 60, 90 and 180. The x-axis is Time (in Years) and the t_0 is the start of treatment period, i.e. baseline, and t_1 and t_2 are time of the actual Day 30 and 60, respectively, and so on.

If the index value at baseline is missing then we set QALYs at Day 30 and 180 to missing. No LOCF imputation will be used. However, linear interpolation will be used between data points with missing data in between (e.g. t_0 to t_2, if t_1 is missing).

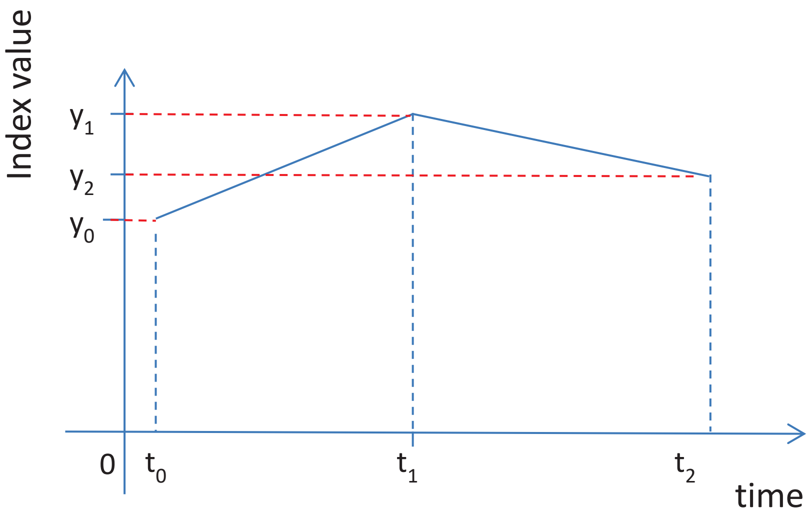

Figure 4: EQ-5D-5L QALY Calculation

Appendix 2 Critical Adverse Events and Classification of Adverse Events Based on Changes in Safety Laboratory Variables

Table 1: Critical Adverse Events

Critical adverse event	**MedDRA terms or SMQ used for search**
Potential hypersensitivity reactions	Anaphylactic reaction (SMQ) (Narrow Scope) Angioedema (SMQ) (Narrow Scope)
Myocardial infarction / ischemia	Ischaemic heart disease (SMQ) (Broad scope)
Cardiac arrest	Cardiac Arrythmias (SMQ) (Broad scope)
Tachyarrythmia	Cardiac Arrythmias (SMQ) (Broad scope)
Bradyarrythmia	Cardiac Arrythmias (SMQ) (Broad scope)
AV-block type II	Cardiac Arrythmias (SMQ) (Broad scope)
Cerebrovascular accident	Haemorrhagic cerebrovascular conditions (SMQ) (Broad Scope) Ischaemic cerebrovascular conditions (SMQ) (Broad Scope) Central nervous system haemorrhages and cerebrovascular accidents (SMQ) (Broad scope)
Digital ischemia	Peripheral ischaemia Pallor Peripheral coldness
Renal failure	Acute Renal failure (SMQ)
Mesenteric ischemia	Ischaemic colitis (SMQ) (Narrow scope)
Hepato-biliary adverse events	Drug related hepatic disorders - severe events only (SMQ) (Broad Scope)

Adverse events based on changes in safety laboratory variables assessed as unanticipated in the setting of septic shock will be categorized as in (Table 2) below.

Table 2: Classification of Adverse Events Based on Changes in Safety Laboratory Variables

Clinical Chemistry	**Classification**
Alanine aminotransferase Aspartate aminotransferase Alkaline phosphatase Lactate dehydrogenase Total bilirubin	Liver
Albumin Uric acid	General
C-reactive protein	Acute phase reactant
Calcium Chloride Phosphate Potassium	Mineral and electrolyte

Clinical Chemistry	Classification
Sodium	
Creatine phosphokinase	Skeletal and cardiac muscle
Creatinine Urea (blood urea nitrogen)	Renal
Haematocrit Haemoglobin Platelet count Red blood cell count White blood cell count	Haematology
Activated partial thromboplastin time Prothrombin time / international normalised ratio	Coagulation

Adverse events based on changes in vital signs assessed as unanticipated in the setting of septic shock will be categorized as in (Table 3) below.

Table 3: Classification of Adverse Events Based on Changes in Vital Signs

Vital Signs Parameter	Classification
Diastolic BP Systolic BP Mean arterial pressure Heart rate	Cardiac
Respiratory rate	Lungs
Body temperature	General

Appendix 3 Estimation of Treatment Effects for P&VFDs

Let Y be the number of P&VFDs for both survivors and non-survivors, let p be the probability of surviving, and say that for survivors (30-Y) has a negative binomial distribution (with for Y=0 consuming all values ≥30).

$$P(Y=y) = \begin{cases} (1-p) + p * \sum_{k\geq 30} \frac{\Gamma(d^{-1}+k)}{\Gamma(d^{-1})k!}\left(\frac{\mu*d}{1+\mu*d}\right)^{k}\left(\frac{1}{1+\mu*d}\right)^{d^{-1}} & for\ y=0 \\ p * \frac{\Gamma(d^{-1}+30-y)}{\Gamma(d^{-1})(30-y)!}\left(\frac{\mu*d}{1+\mu*d}\right)^{(30-y)}\left(\frac{1}{1+\mu*d}\right)^{d^{-1}} & for\ y>0 \end{cases}$$

In the trial it is expected we have a mean of around 5-8 days on pressors and ventilation for those surviving.
Therefore, the probability of getting zero P&VFDs for those surviving $\sum_{k\geq 30} \frac{\Gamma(d^{-1}+k)}{\Gamma(d^{-1})k!}\left(\frac{\mu*d}{1+\mu*d}\right)^{k}\left(\frac{1}{1+\mu*d}\right)^{d^{-1}}$ is negligible (and can be omitted from the model),

For d=1/16

μ	5	6	7	8	9	10	11
Var =μ+ μ^2d	6.56	8.25	10.06	12	14.06	16.25	18.56
P(K≥30)	$1.4*10^{-9}$	$4.2*10^{-8}$	$6*10^{-7}$	$5*10^{-6}$	$2.8*10^{-5}$	$1.2*10^{-4}$	$4*10^{-4}$

μ	12	13	14	15
Var =μ+ μ^2d	21	23.56	26.25	26.09
P(K≥30)	$1.1*10^{-3}$	$2.5*10^{-3}$	$5.4*10^{-3}$	$1*10^{-2}$

For $Y_1,\ldots,Y_N$ we then get two likelihood functions,

$$L_1 = \prod_{y_i=0}(1-p)$$

and,

$$L_2 = \prod_{y_i>0} p * \frac{\Gamma(d^{-1}+30-y)}{\Gamma(d^{-1})(30-y)!}\left(\frac{\mu*d}{1+\mu*d}\right)^{(30-y)}\left(\frac{1}{1+\mu*d}\right)^{d^{-1}}$$

Assuming that p is modeled as $\frac{e^{\beta_1}}{1+e^{\beta_1}}$, and μ as e^{β_2}, the two log-likelihoods then become,

$$l_1 = \sum_{y_i=0} \ln\left(\frac{1}{1+e^{\beta_1}}\right) = \sum_{y_i=0} -\ln(1+e^{\beta_1})$$

and,

$$l_2 = \sum_{y_i > 0} \beta_1 - \ln(1 + e^{\beta_1}) + \ln\left(\Gamma(d^{-1} + 30 - y_i)\right) - \ln\left(\Gamma(d^{-1})\right) - \ln\left((30 - y_i)!\right) + (30 - y_i)\left(\beta_2 + \ln\left(1 + e^{\beta_2}\right)\right) - d^{-1} * \ln\left(1 + e^{\beta_2}\right)$$

Maximizing the full log-likelihood is thus maximizing $l_1 + l_2$
And as can be clearly seen, the maximization of l_1 and l_2 wrt. β_1 does not depend on β_2 and vice-versa. Hence, β_1 and β_2 can be estimated separately from two independent models, i.e. a logistic regression for the probability of surviving, and a poisson regression (or negative binomial to allow for overdispersion) for the distribution of days on pressors and ventilation for those surviving.

In practice, and with the model adjusted for A, B and C, this means that the probability of surviving will be estimated from a logistic regression adjusted for A, B and C and treatment arm. From each treatment arm we can do an LSMEANS and get an 'overall' β_1 along with the standard error of β_1.
For a given treatment arm the mean probability of surviving is given as:

$$\frac{e^{\beta_1}}{1 + e^{\beta_1}}$$

The distribution of days on pressors and ventilation (30- P&VFDs) for those surviving will be estimated from a negative binomial regression (poisson with a potential overdispersion), also adjusted for A, B and C and treatment arm. From each treatment arm we can do an LSMEANS and get an 'overall' β_2 along with the standard error of β_2.
For a given treatment arm the mean P&VFDs for survivors is given as:

$$(30 - e^{\beta_2})$$

The mean P&VFDs for all patients (survivors and non-survivors) is then given as:

$$\frac{e^{\beta_1}}{1 + e^{\beta_1}} * (30 - e^{\beta_2})$$

Let

$$f(\beta_1, \beta_2) = \frac{e^{\beta_1}}{1 + e^{\beta_1}} * (30 - e^{\beta_2})$$

Using the delta method we can calculate the variance of f(β_1, β_2):

$$f'(\beta_1, \beta_2) = \left((30 - e^{\beta_2}) * \frac{e^{\beta_1}}{(1 + e^{\beta_1})^2}, \frac{e^{\beta_1}}{(1 + e^{\beta_1})} * (-e^{\beta_2}) \right)$$

Hence,

$$var[f(\beta_1,\beta_2)] = f'(\beta_1,\beta_2)\begin{pmatrix}\sigma^2_{\beta_1} & 0 \\ 0 & \sigma^2_{\beta_2}\end{pmatrix}f'(\beta_1,\beta_2)^t$$

$$= \left(\sigma^2_{\beta_1} * (30 - e^{\beta_2}) * \frac{e^{\beta_1}}{(1+e^{\beta_1})^2} \quad \sigma^2_{\beta_2} * \frac{e^{\beta_1}}{(1+e^{\beta_1})} * (-e^{\beta_2})\right)\begin{pmatrix}(30 - e^{\beta_2}) * \frac{e^{\beta_1}}{(1+e^{\beta_1})^2} \\ \frac{e^{\beta_1}}{(1+e^{\beta_1})} * (-e^{\beta_2})\end{pmatrix}$$

$$= \sigma^2_{\beta_1} * (30 - e^{\beta_2})^2 * \left(\frac{e^{\beta_1}}{(1+e^{\beta_1})^2}\right)^2 + \sigma^2_{\beta_2} * \left(\frac{e^{\beta_1}}{(1+e^{\beta_1})}\right)^2 * e^{2\beta_2}$$

The 95% CI for the mean PVFDs then become

$$\frac{e^{\widehat{\beta_1}}}{1+e^{\widehat{\beta_1}}} * (30 - e^{\widehat{\beta_2}}) \pm 1.96 * \sqrt{var[f(\widehat{\beta_1},\widehat{\beta_2})]}$$

Let s indicate selepressin and p indicate placebo, the estimated treatment difference then becomes,

$$\frac{e^{\widehat{\beta_{1s}}}}{1+e^{\widehat{\beta_{1s}}}} * (30 - e^{\widehat{\beta_{2s}}}) - \frac{e^{\widehat{\beta_{1p}}}}{1+e^{\widehat{\beta_{1p}}}} * (30 - e^{\widehat{\beta_{2p}}})$$

With 95% CI,

$$\frac{e^{\widehat{\beta_{1s}}}}{1+e^{\widehat{\beta_{1s}}}} * (30 - e^{\widehat{\beta_{2s}}}) - \frac{e^{\widehat{\beta_{1p}}}}{1+e^{\widehat{\beta_{1p}}}} * (30 - e^{\widehat{\beta_{2p}}}) \pm 1.96 * \sqrt{var[f(\widehat{\beta_{1s}},\widehat{\beta_{2s}})] + var[f(\widehat{\beta_{1p}},\widehat{\beta_{2p}})]}$$

In the above reasoning, it is assumed that P&VFDs can only take on integer values between 0 and 30. In practice, P&VFDs will be analysed with one decimal (with values in the range of 0, 0.1, ..., 29.9, 30.0) and hence the distribution of days on pressors and ventilation for those surviving will be estimated from a negative binomial regression model scaled up to 300 (transforming the P&VFDs from 0.0, 0.1, ..., 29.9, 30.0 to 0, 1, ..., 299, 300), and later scaled back to one decimal.

Let X = 10*Y (scaling Y from 0 to 300) and δ_1 and δ_2 the corresponding estimated parameters from the logistic regression and negative binomial regression models based on the modeling of X.

Transforming back to Y, the 95% CI for the mean P&VFDs (E(Y)) then become

$$\frac{e^{\widehat{\delta_1}}}{1+e^{\widehat{\delta_1}}} * \left(30 - \frac{e^{\widehat{\delta_2}}}{10}\right) \pm 1.96 * \sqrt{\frac{var[f(\widehat{\delta_1},\widehat{\delta_2})]}{100}}$$

Again with s indicating selepressin and p indicating placebo, the estimated treatment difference then becomes,

$$\frac{e^{\widehat{\delta_{1s}}}}{1+e^{\widehat{\delta_{1s}}}} * \left(30 - \frac{e^{\widehat{\delta_{2s}}}}{10}\right) - \frac{e^{\widehat{\delta_{1p}}}}{1+e^{\widehat{\delta_{1p}}}} * (30 - \frac{e^{\widehat{\delta_{2p}}}}{10})$$

With 95% CI,

$$\frac{e^{\widehat{\delta_{1s}}}}{1+e^{\widehat{\delta_{1s}}}} * \left(30 - \frac{e^{\widehat{\delta_{2s}}}}{10}\right) - \frac{e^{\widehat{\delta_{1p}}}}{1+e^{\widehat{\delta_{1p}}}} * \left(30 - \frac{e^{\widehat{\delta_{2p}}}}{10}\right) \pm 1.96 * \sqrt{\frac{var\left[f\left(\widehat{\delta_{1s}}, \widehat{\delta_{2s}}\right)\right]}{100} + \frac{var\left[f\left(\widehat{\delta_{1p}}, \widehat{\delta_{2p}}\right)\right]}{100}}$$

Appendix 4 Estimation of Treatment Effects for Difference of Proportions in Incidence of RRT

Let Y be the incidence of RRT (and/or death).

Y can then be modelled using a logistic regression, adjusted for various factors and covariates.

In practice, and with the model adjusted for A, B and C, this means that the probability of RRT incidence will be estimated from a logistic regression (PROC GENMOD preferred over PROC LOGISTIC as estimates can be subtracted by ODS output) adjusted for A, B and C and treatment group. From each treatment group we can do an LSMEANS and get an 'overall'(adjusted) β_1 along with the standard error of β_1.

For a given treatment group the mean probability of RRT incidence is given as:

$$\frac{e^{\beta_1}}{1+e^{\beta_1}}$$

The difference in proportions of RRT incidence is then given as:

$$\frac{e^{\beta_1}}{1+e^{\beta_1}} - \frac{e^{\beta_2}}{1+e^{\beta_2}}$$

Using the delta method we can calculate the variance of f(β_1, β_2):

$$f'(\beta_1, \beta_2\,) = \left(\frac{e^{\beta_1}}{(1+e^{\beta_1})^2}\,, -\frac{e^{\beta_2}}{(1+e^{\beta_2})^2}\right)$$

Hence,

$$var[f(\beta_1, \beta_2\,)] = \; f'(\beta_1, \beta_2\,)\begin{pmatrix}\sigma^2_{\beta_1} & \sigma^2_{\beta_1\beta_2}\\ \sigma^2_{\beta_1\beta_2} & \sigma^2_{\beta_2}\end{pmatrix} f'(\beta_1, \beta_2\,)^t$$

$$= \left(\sigma^2_{\beta_1} * \frac{e^{\beta_1}}{(1+e^{\beta_1})^2} - \sigma^2_{\beta_1\beta_2} * \frac{e^{\beta_2}}{(1+e^{\beta_2})^2},\quad -\sigma^2_{\beta_2} * \frac{e^{\beta_2}}{(1+e^{\beta_2})^2} + \sigma^2_{\beta_1\beta_2} * \frac{e^{\beta_1}}{(1+e^{\beta_1})^2}\right)\begin{pmatrix}\frac{e^{\beta_1}}{(1+e^{\beta_1})^2}\\ \frac{-e^{\beta_2}}{(1+e^{\beta_2})^2}\end{pmatrix}$$

$$= \sigma^2_{\beta_1} * \left(\frac{e^{\beta_1}}{(1+e^{\beta_1})^2}\right)^2 + \sigma^2_{\beta_2} * \left(\frac{e^{\beta_2}}{(1+e^{\beta_2})}\right)^2 - 2 * \sigma^2_{\beta_1\beta_2} * \frac{e^{\beta_1}}{(1+e^{\beta_1})^2} * \frac{e^{\beta_2}}{(1+e^{\beta_2})^2}$$

The 95% CI for the difference in proportions of RRT incidence then become

$$\frac{e^{\widehat{\beta_1}}}{1+e^{\widehat{\beta_1}}} - \frac{e^{\widehat{\beta_2}}}{1+e^{\widehat{\beta_2}}} \pm 1.96 * \sqrt{var\left[f\left(\widehat{\beta_1}, \widehat{\beta_2}\,\right)\right]}$$

Appendix 5 Statistical Model for Adaptive Design Decisions

An Example Trial

This section presents the results of an example trial in order to illustrate how the design behaves. The selected scenario includes defining the probability distributions that describe how patients given placebo behave, and also defining the effects of all four selepressin dosing regimens.

(Figure 5) shows the data available at the first interim analysis, and the results of the statistical analyses performed using those data that are then used to make decisions. The leftmost of the three plots shows the raw data for each of the five arms, with mortality data in the legend and P&VFD data for survivors in the barplots. Red represents the placebo arm, and the shades of blue and black represent the active arms, with darker colors indicating larger maximum doses. The curves added to the plot are naïve density estimates that are not related to the statistical models used in the trial, scaled so that they have the same maximum. Active arm 2 has observed the highest mortality rate, while active arms 1 and 3 have seen tentative improvements compared to placebo. Active arm 3 has had the more surviving patients with small numbers of P&VFDs than the other arms. Active arm 4 is not yet allowed to accept patients. The middle plot displays estimates of mean P&VFDs (with fatalities scored as zero P&VFDs), together with 95% uncertainty intervals. The placebo arm is estimated to be the least effective, and uncertainties are smallest for the intermediate doses. The rightmost plot displays predictive probabilities and updated allocation probabilities. The circles show the allocation probabilities for the next 30 days: the probability for placebo remains at 1/3 for the duration of Part 1, and active arm 3 is assigned the lowest probability for any active arm. The rightmost plot also features squares showing the predictive probability that this trial would be successful if Part 1 were to terminate and Part 2 were to begin with each of the four arms as the chosen arm (It is too early to choose an arm: this can only happen when final data have been observed for at least 300 patients). If all four active arms had predictive probabilities less than 0.05 the trial would terminate for futility. Also, if the predictive probability were at least 90% that the final data set would show an observed increase in mortality of greater than 2%, regardless of which of the four active arms were selected, the trial would stop for futility for that reason, but these predictive probabilities are not shown in the figures.

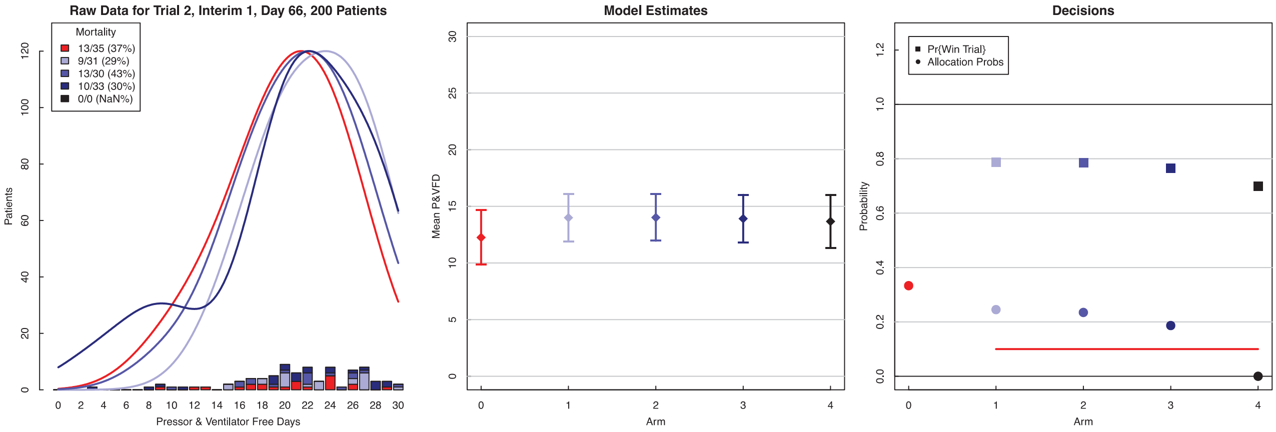

Figure 5: **data and interim analysis results at time of first interim analysis in an example trial.**

Moving ahead to the second interim analysis shown in (Figure 6), when we have observed final data for 199 patients. Mortality has remained high for active arm 2. For survivors, the frequency of small numbers of P&VFDs is lowest for the placebo arm, but all three active arms have seen more patients with very large numbers of P&VFDs than has placebo. According to the rightmost plot, predictive probabilities for trial success are around 40% for all active arms. For the next 30 days, arm 3 will receive the highest allocation probability among the active arms, and since (not shown) the probability is at least 50% that arm 3’s expected P&VFDs is higher than for arm 2, arm 4 becomes eligible for patient allocation to explore whether the apparent increasing trend in efficacy continues.

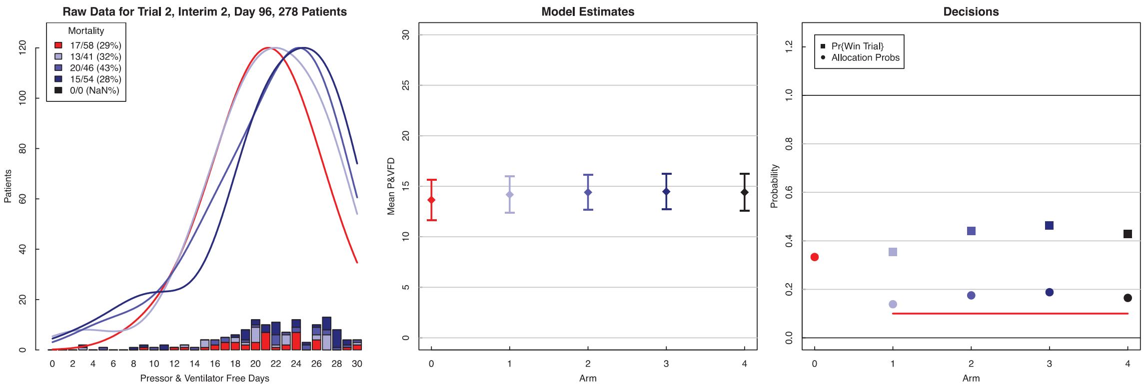

Figure 6: **data and analysis results for second interim analysis in example trial.**

We skip ahead to the fourth interim analysis, shown in (Figure 7), which is the first interim analysis after final data are available for at least 300 patients, so this is the first opportunity to choose a dose and move on to Part 2. The first data from arm 4 have come in, and arms 1, 3, and 4 are all achieving mortality rates no worse than placebo. The placebo arm continues to have the smallest probability of large numbers of P&VFDs among survivors. Since the predictive probability of trial success is largest for arm 3 and its value exceeds 90% (shown by the light green line), the trial elects to terminate Part 1 and proceed to Part 2 with arm 3. The allocation probabilities change to 50% each for placebo and active arm 3. Unless the trial stops earlier for futility, Part 2 will consist of 1430 patients since 370 are in the trial currently. The decision to select arm 3 occurs with only 11 patients allocated to arm 4, and with arm 4 looking promising thus far, but arm 3 has a high enough probability of a successful trial that the trial advances to Part 2.

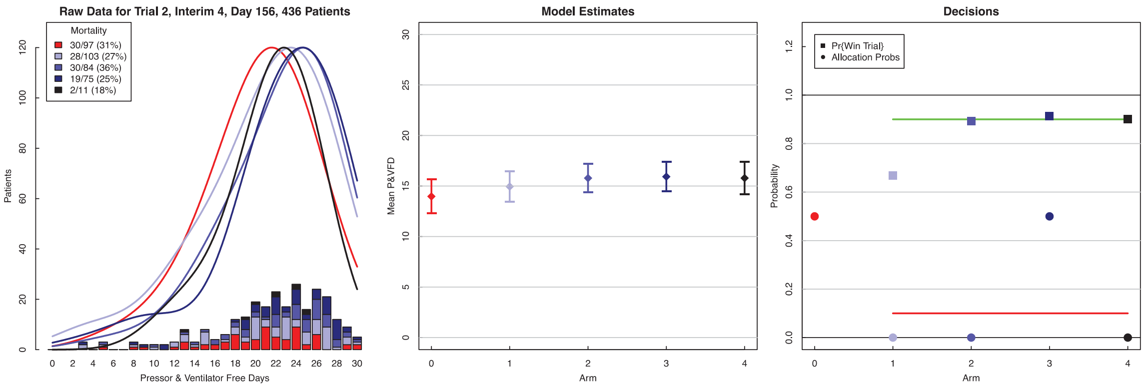

Figure 7: **data and analysis results for fourth interim analysis in example trial.**
During this interim analysis, Part 1 terminates and the trial proceeds to Part 2 with active arm #3.

The first interim analysis of Part 2 takes place after 63 more patients are allocated to arm 3 or placebo. Final data continue to come in for the other active arms as well. Since data are relatively favourable for the placebo arm in this month, the predictive probability of a successful trial at 1800 patients drops to about 0.80, which is much larger than the 5% futility boundary for Part 2 of the trial.

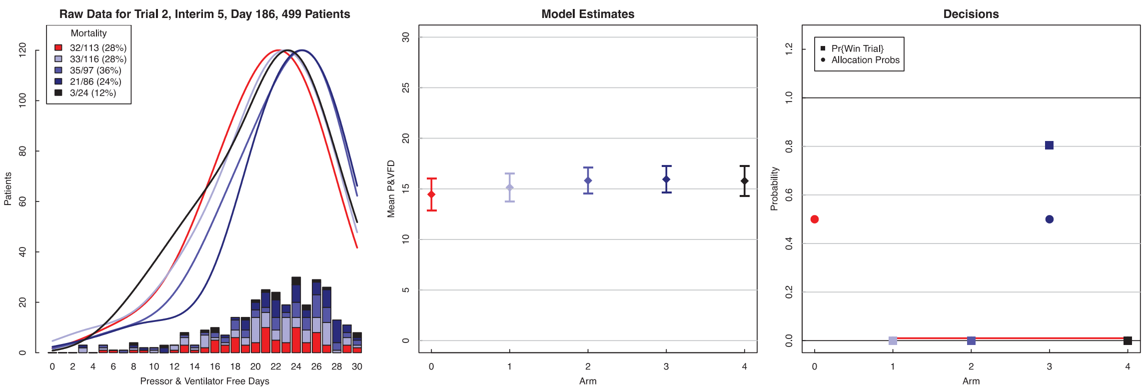

Figure 8: **first interim analysis during Part 2.**

Interim analyses continue every 30 days (frequency chosen for this example), with the only available decision being whether or not to stop for futility. The predictive probability of trial success never approaches the 5% value that would trigger a futility stop. The final data at the end of the trial are shown in (Figure 8). Observed mortality was 2% lower for active arm 3 than for placebo, and the final analysis will be based on a smaller difference than this due to the inclusion of the data from other active arms. The simulation assumed true mortalities and expected P&VFD distributions for survivors are shown in the middle plot: the doses increase in effectiveness at both preventing mortality and increasing P&VFDs for survivors, with arms 3 and 4 each reducing mortality by 1.5% and increasing expected P&VFDs by 1.5 days for survivors. The rightmost plot shows the final p-value for the Wilcoxon test: the p-value for the comparison between patients given placebo and patients given any active arm is lower than 0.025, so this is a successful trial.

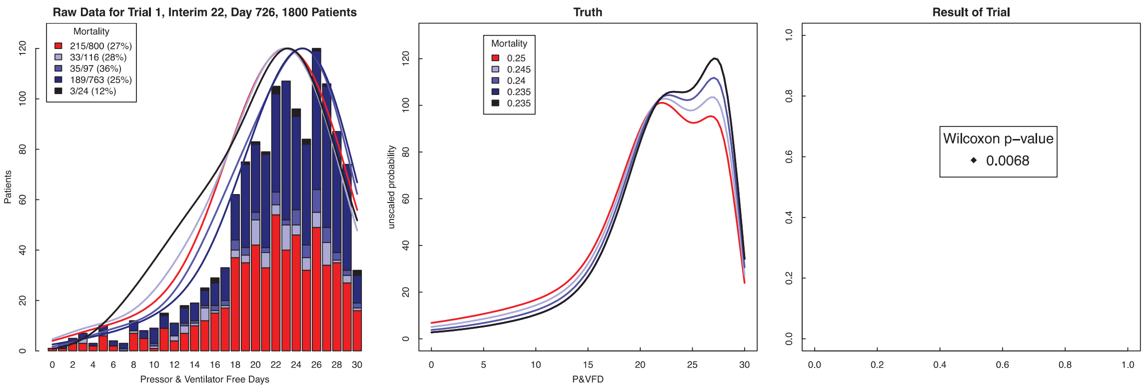

Figure 9: **results of final analysis. The trial is successful, with a p-value of 0.0068.**

Statistical Model for Adaptive Design Decisions

A patient's outcome can be either death or a number of pressor- and ventilator-free days between 0 and 30. Label d_i as the dose regimen assigned to patient i, and denote by s_i the stratum to which the patient belongs ($s_i \in \{0,1,\ldots,7\}$). First, define the distribution of these potential outcomes for patients treated with placebo ($d_i = 0$). If the i'th patient dies, write $X_i = 1$, otherwise $X_i = 0$. So,

$$\Pr\{X_i = 1 | \, d_i = 0, s_i = 0\} = \Delta.$$

Intuitive parameterization of the P&VFD model

To motivate the strategy for modeling the distribution of the number of P&VFD given placebo and survival, we first define a version of the model that is more intuitive but less computationally convenient and is asymmetric with respect to the strata. The version actually recommended is defined below in the section "*Symmetric, computational parameterization of the P&VFD model* ". The distribution can be modeled nonparametrically:

$$\Pr\{Y_i = k | d_i = 0, s_i = 0, X_i = 0\} = \pi_k, \text{ with } \sum_{j=0}^{30} \pi_j = 1.$$

Note that patients who die are modelled separately from patients who survive, but nevertheless accumulate zero P&VFD, although these patients are handled together in the final analysis. We model the treatment effect for a given dose d with two parts: the effect on mortality ϕ_d and the effect on P&VFD given survival, θ_d. The differences between the strata are modeled similarly to the effects of the different doses, with a stratum effect on mortality denoted by ψ_s and a stratum effect on P&VFD given survival denoted by ω_s. By definition $\psi_0 = \omega_0 = 0$. We model the effect on mortality, ϕ_d, on the log-odds scale:

$$\Pr\{X_i = 1|d_i = d, s_i = s\} = \frac{\Delta}{\{\Delta + (1 - \Delta)\exp(\phi_d + \psi_s)\}},$$

where we have defined the effect so that larger values of ϕ_d or ψ_s are beneficial (decrease mortality). We model the effects of the dose arm and the stratum on the number of P&VFDs for survivors using an exponential family whose sufficient statistic is the number of P&VFD:

$$\Pr\{Y_i = k|d_i = d, s_i = s, X_i = 0\} = \frac{\pi_k e^{k(\theta_d + \omega_s)}}{\sum_{j=0}^{30} \pi_j e^{j(\theta_d + \omega_s)}}.$$

In particular, given a dose arm, a sample of P&VFD, and a probability vector π, the maximum likelihood estimators of θ_d and ω_s set the expected values of P&VFD equal to their sample means. In the final analysis, however, patients who died are treated in the same way as patients who survived but who had 0 P&VFD.

For the simpler statistical model with no consideration of stratum effects, the ψ_s and ω_s are omitted.

Symmetric, computational parameterization of the P&VFD model

The version of the statistical model we will actually use is as follows. There are still parameters $\pi_0, \dots, \pi_{30}$, but they do not correspond directly to any stratum and do not have a clear interpretation of their own. Instead of assuming that $\psi_0 = \omega_0 = 0$, we use another choice of identifiability assumptions: $\sum_{s=1}^{8} \psi_s = 0$ and $\sum_{s=1}^{8} \omega_s = 0$. Now we have

$$\Pr\{Y_i = k|d_i = d, s_i = s, X_i = 0\} = \frac{\pi_k e^{k(\theta_d + \omega_s)}}{\sum_{j=0}^{30} \pi_j e^{j(\theta_d + \omega_s)}}$$

for all d and s, where we have defined the placebo parameter $\theta_0 = 0$. This parameterization facilitates the definition of prior distributions for the ψ_s and ω_s, and ensures that the results of the analysis do not depend on which stratum is identified with $s = 0$, etc. We assume hierarchical models on the stratum parameters, with $\psi_s \sim N(0, \tau_\psi^2)$ and $\omega_s \sim N(0, \tau_\omega^2)$ (conditionally on observing the identifiability assumptions), and with $\tau_\psi \sim$Uniform(0,1) and $\tau_\omega \sim$Uniform(0,1).

Inverted-U dose-response models for the dose effects ϕ and θ.

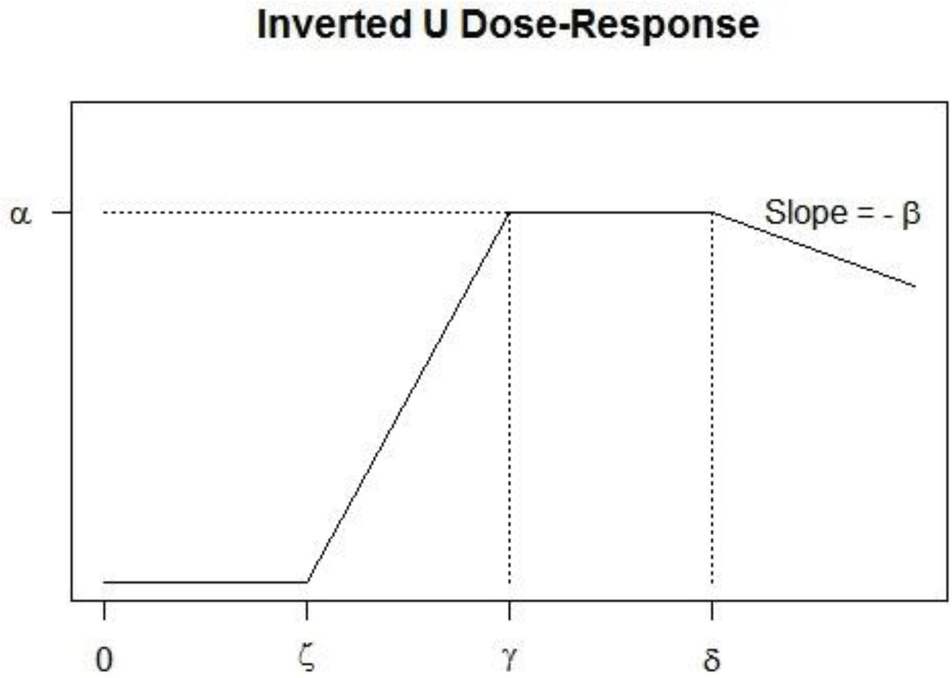

Figure 10: Inverted-U dose-response model. The x-axis represents dose regimen (which is interpreted as maximum dose in the selepressin trial), and the y-axis represents the treatment effect. The model parameters ζ, γ, δ define theoretical special doses (e.g. γ is the smallest dose that gives maximal effect), while α denotes the maximum achievable treatment effect.

An inverted-U dose-response model is used for the effects on both mortality and P&VFD. This is a flexible family that allows for the possibilities that small doses have no effect at all (if those doses are smaller than ζ), and that large doses can start to become less effective than smaller doses (if those doses are larger than δ). Most importantly for this trial, the model allows all doses to be equally effective, since, for a given patient, any assigned maximum dose could be titrated to a similar delivered treatment. In the parameterization, shown in (Figure 9), γ is the smallest dose that delivers maximal effect, δ is the largest dose that delivers maximal effect, ζ is the largest dose that delivers no effect, β is the rate at which performance degrades beyond dose δ, and α is the size of the largest effect ($\alpha \leq 0$ is also allowed and there is a prior distribution for α which is symmetric about zero. To maintain an inverted U shape, β is constrained to be positive). Care must be taken to avoid important identifiability problems: in particular, it is insisted that the interval (γ, δ) contains at least one dose for which data can potentially be obtained, otherwise the largest treatment effect is not uniquely defined. However, γ can be smaller than any active dose, in which case the data contain no information about ζ, and δ can be larger than any active dose, in which case the data contain no information about β, but neither of these nonidentifiabilities are serious problems since they do not impact the ability to predict future data.

In the case where the candidate doses are 1, 2, 3, and 4, a prior distribution is assumed in which

$$\delta - \gamma \sim \text{Uniform}(1,5),$$

$$\gamma|\delta \sim \text{Uniform}(0, \delta - 1), \text{ and}$$

$$\zeta|\gamma \sim \text{Uniform}(0, \gamma).$$

It is assumed that $\phi_d = IU(d|\alpha_1, \zeta_1, \gamma_1, \delta_1, \beta_1)$ and $\theta_d = IU(d|\alpha_2, \zeta_2, \gamma_2, \delta_2, \beta_2)$ for $d = 1,2,3,4$, where the inverted-U function is defined as

$$IU(d|\alpha, \zeta, \gamma, \delta, \beta) = \alpha\{(1 - \left[1 - \frac{(d-\zeta)_+}{(\gamma-\zeta)}\right]_+\Big)_+ - \beta(d-\delta)_+\}, \text{ where } x_+ = \max(x, 0).$$

There is no assumed relationship between the θ parameters and the ϕ parameters.

For a specific example, suppose $\alpha_1 = 0.2, \zeta_1 = 1.5, \gamma_1 = 2.5$, and $\delta_1 = 4$ (so that the value of β_1 is irrelevant for doses 1, 2, 3, and 4). Then $\phi_1 = 0, \phi_2 = 0.1, \phi_3 = \phi_4 = 0.2$.

To complete the specification of the prior distribution, it is assumed that

$$\Delta \sim Beta\left(\frac{1}{3}, \frac{1}{3}\right), (\pi_0, \pi_1, \ldots, \pi_{30}) \sim \text{Dirichlet}\left(\frac{1}{3}, \frac{1}{3}, \ldots, \frac{1}{3}\right),$$

$$\alpha_1 \sim \text{Uniform}(-0.35, 0.35),$$

$$\alpha_2 \sim \text{Uniform}(-0.1334, 0.1334),$$

$$\beta_1 \sim \text{Uniform}(0,1), \text{ and}$$

$$\beta_2 \sim \text{Uniform}(0,1).$$

The limits for the α parameters are selected to be large but not completely absurd; e.g. $\alpha_1 = 0.35$ corresponds to a 5% benefit to a 20% mortality rate. Given P&VFD data, one can use a variable-at-a-time Metropolis-Hastings algorithm to sample from the posterior distribution of the unknown parameters $(\Delta, \pi_0, \pi_1, \ldots, \pi_{30}, \alpha_1, \zeta_1, \gamma_1, \delta_1, \beta_1, \alpha_2, \zeta_2, \gamma_2, \delta_2, \beta_2)$. These samples can be used to estimate the predictive probability of a successful Part 2, the probability that each doses maximizes the expected number of P&VFDs, and the probability that dose regimen 3 provides a larger value of expected P&VFDs than does dose regimen 2.

Response-Adaptive Randomisation Probabilities

Beginning with the first interim analysis, allocation probabilities for the selepressin arms are adjusted based on the posterior distribution (the allocation probability for placebo remains fixed at 1/3 throughout Part 1). The allocation probability for active arm j is proportional to the posterior probability that arm j is one of the arms that maximizes the expected number of P&VFD (where the mortality probabilities are included in the calculations). Note that the inverted-U models allow for multiple arms to have exactly the same expected number of P&VFD.

Posterior Predictive Probability Calculations

Computations of the predictive probability of a successful Part 2 are critical to the design. These computations proceed by drawing samples from the posterior distribution of the unknown parameters. We first discuss the predictive probability calculation for the simplified case in which there are no strata and the final analysis is a Wilcoxon test; this is the case that applies in the operating characteristics simulations presented in this report. For a given posterior sample, we use a normal approximation to the predictive distribution of the Wilcoxon statistic that will be obtained from the currently available data for placebo and the pooled active arms. The calculation is tedious but straightforward, based on writing the Wilcoxon statistic in terms of

$$\sum_{i=1}^{I} \sum_{j=1}^{J} sign(Y_{1i} - Y_{2j}),$$

where $Y_{11}, \ldots, Y_{1I}$ are the P&VFDs for sample 1 (i.e. the active arm) and $Y_{21}, \ldots, Y_{2J}$ are the P&VFDs for sample 2 (i.e. the placebo arm). For a given set of P&VFD probabilities for the two arms, one can calculate the expected value and variance of the Wilcoxon statistic, and use this to calculate the probability of a significant result (the variance depends on the number of ties, and we plug in the expected value of the tie component based on a Poisson approximation). The process is then repeated for more posterior samples, and the results are averaged to give the overall estimate of the predictive probability of a successful trial. The predictive probability calculation is performed for each active arm being the one that proceeds to Part 2. The calculation uses the same (pooled) data set for each arm, but each arm has a different posterior distribution of treatment effect and hence a different predictive distribution of future data.

The approximate formula for predictive probability of a successful Wilcoxon test is faster computationally than simulating many final data sets and calculating whether each one attains success, so it plays a key role in simulating the trial to estimate operating characteristics. When time permits, such as when the design is being executed, however, the direct Monte Carlo simulation should also be performed.

The extension to the case in which there are eight strata and in which the final analysis is a van Elteren test, is straightforward but adds another layer of complexity. For the purpose of this calculation, we introduce a Dirichlet-multinomial model for the stratum probabilities: the eight stratum probabilities begin with a Dirichlet prior distribution with parameters equal to 1/3 and then are updated with the stratum counts observed in the trial. This stratum probability model operates independently of the remainder of the statistical modeling. Suppose that we are interested in calculating the predictive probability of success assuming that half the remaining patients are allocated to the placebo and the other half are allocated to active arm 1. For a given posterior sample, we draw a sample from the Dirichlet posterior distribution of the stratum, and then draw multinomial samples for the stratum counts for the future patients allocated to placebo, and separately to the active arm. We then loop over the strata and either

- using the posterior sample, simulate numbers of deaths and P&VFDs for survivors for each arm and each stratum and then evaluate the Wilcoxon test statistic, its theoretical null hypothesis mean, and its theoretical null hypothesis variance (which depends on the numbers of ties in the data set), or
- based on the posterior sample and the current numbers of deaths and P&VFD counts in each arm and the current stratum, calculate the predictive mean and variance of the Wilcoxon statistic, its theoretical null hypothesis mean, and an approximation to the expected value of the theoretical null hypothesis variance. The current data for all the active arms are aggregated together.

Denoting the Wilcoxon test statistic for the sth stratum by T_s, the simulated final number of patients in stratum s by N_s, and the null hypothesis expected value and variance by $E(T_s|H_0)$ and $Var(T_s|H_0)$ respectively, the van Elteren test statistic is

$$T = \frac{\sum_{s=1}^{8}\{T_s - E(T_s|H_0)\}/(N_s+1)}{\{\sum_{s=1}^{8} Var(T_s|H_0)/(N_s+1)^2\}^{1/2}}$$

For the Monte Carlo estimate of predictive probability, evaluate this statistic for every simulated final data set and compute the fraction of final data sets for which this statistic exceeded the 97.5th Gaussian percentile. To use the approximate formula, denote the predictive mean of T_s by $E(T_s|D,i)$, the predictive variance by $Var(T_s|D,i)$, and the estimated null hypothesis variance by $E\{Var(T_s|H_0)|D,i\}$; D denotes the current data and i denotes that we are using the ith posterior sample. The approximate predictive probability for posterior sample i is then computed using Gaussian tail probabilities based on the expected value and variance of T given by

$$E(T|i) = \frac{\sum_{s=1}^{8}\{E(T_s|D,i) - E(T_s|H_0)\}/(N_s+1)}{\{\sum_{s=1}^{8} E\{Var(T_s|H_0)|D,i\}/(N_s+1)^2\}^{1/2}}$$

$$Var(T|i) = \frac{\sum_{s=1}^{8}\{Var(T_s|D,i)\}/\,(N_s+1)^2}{\sum_{s=1}^{8} E\{Var(T_s|H_0)|D,i\}/(N_s+1)^2}$$

Similar but simpler calculations apply for estimating the predictive probability that the final data set will have a mortality rate among the patients assigned to an active arm that is at least 2% higher than among the placebo patients.

Control of Type I Error

This design achieves control of Type I error through analytical means. While the trial can stop early for futility, a successful trial can only be achieved at a total of 1800 patients. At this time, a single test statistic is calculated, and it compares two populations that are defined before the trial begins, namely patients allocated to placebo compared to patients that are allocated to any active arm. In particular, no patients are excluded from the final analysis for any reason related to their outcomes (in contrast, if the final analysis compared placebo to the best performing of the active arms, that would inflate Type I error).

This argument demonstrates that Type I error is controlled even for the modification of the design in which early stopping for futility is disabled (See Appendix 6 Formal Proof of Type I Error Control for a formal proof of Type I error control). The potential for early stopping for futility, including the 25% predictive probability requirement at 800 patients, further reduces Type I error probability below the nominal value.

Appendix 6 Formal Proof of Type I Error Control

The following assumptions are made in the statistical discussions of type I error.

1. The primary analysis is based on a Wilcoxon-Mann-Whitney test (Van Elteren's Test) on the P&VFDs. The normal approximation to the sampling distribution of the test statistic is assumed for type I error discussions (as it would in a fixed trial).
2. The primary analysis combines all the active arms together for the final analysis. Under the null hypothesis, all arms (placebo and active) have the same mean and therefore the active arms can be combined in to a single arm.
3. The final analysis is based on 1800 patients.
4. The only "adaptive" aspect of the trial is the time in which randomisation switches from 2:1 (active to placebo) to (1:1).
    a. It is assumed that if the randomisation was 2:1 for the entire 1800 patients that type I error is controlled.
    b. It is assumed that if at a fixed point in time (deterministic) randomisation went from 2:1 to 1:1 that type I error is controlled.

We demonstrate the control of type I error by first considering a one-sample problem. We use the one-sample result to then extend to the two-sample problem. The proof focuses on the notion that when the data are positive in the first part of the trial it triggers a shift to a randomisation that increases the *effective* sample size of the trial. As demonstrated in Mehta and Pocock (2011)[3] this controls type I error when the data that triggers the shift are appropriately positive.

The hypothesis test is

$$H_o: \mu = 0$$

$$H_1: \mu > 0$$

Assume iid normally distributed random variables are observed with mean μ and known variance. At the interim time point a random variable, based on n_0 observations, $Z_0 = n_0\bar{X}_0$ is observed. After this time point two different random variables (with entirely different observations, X) could be observed: $Z_1 = n_1\bar{X}_1$ or $Z_2 = n_2\bar{X}_2$. Without loss of generality, under the null hypothesis, we assume that $Z_0 \sim$ N(0, 1) (with notation N(μ,σ^2) being a normal distribution with mean μ and variance σ^2).

The adaptive design specifies that if the data are unfavorable we will observe Z_1, and if the data are favorable we will observe Z_2. Therefore, for some value b,

1. If the data are unfavorable ($Z_0 < b$) we observe a second random variable $Z_1 \sim N(0, N)$, where N is the ratio of sample sizes between the second stage and the first stage. The trial is declared a success at the end of the second stage if

$$Z_0 + Z_1 > a\sqrt{1 + N}$$

where

$$a = \Phi^{-1}(1 - p).$$

If b=∞ then it is deterministic (select Z_1) and the probability of success is p (the type 1 error of a fixed design, under the null).

2. If the data are favorable ($Z_0 > b$) we observe the random variable $Z_2 \sim N(0, rN)$. The parameter r is a flexible parameter for increase (r>1) or decrease (r<1) in the sample size in the second part of the trial, so r>1 implies n2 > n1. The trial is declared a success at the end of the second stage if

$$Z_0 + Z_2 > a\sqrt{1 + rN}$$

The critical value has been set so that if b=–∞ (again deterministic to select Z_2) then the probability of success is p (type I error under a fixed design).

The value of b then determines the type I error of the adaptive design. The probability of a type I error of the adaptive design is then

$$\int_{-\infty}^{b} \phi(z)\, \Phi\left(\frac{z-a\sqrt{1+N}}{\sqrt{N}}\right) dz + \int_{b}^{\infty} \phi(z)\, \Phi\left(\frac{z-a\sqrt{1+rN}}{\sqrt{rN}}\right) dz \tag{1}$$

If r=1 there is no change in the trial and the above (1) is equal to p. In this trial $r > 1$. If the expression in (1) is nonincreasing in r then this demonstrates that the type I error is not inflated above p in this design. A sufficient condition is if $\frac{z-a\sqrt{1+rN}}{\sqrt{rN}}$ is decreasing in r. The derivative of this is

$$\frac{d}{dr}\left(\frac{z - a\sqrt{1+rN}}{\sqrt{rN}}\right) = -\frac{1}{2\sqrt{r^3N}}\left(z - \frac{a}{\sqrt{1+rN}}\right)$$

which is negative in the second integral ($z > b$) if

$$b > \frac{a}{\sqrt{1+rN}}. \tag{2}$$

Therefore, the type I error of the design is guaranteed if expression (2) holds. It is potentially possible to find a smaller b that guarantees a reduction in type I error, but that is not required here (see Mehta and Pocock (2011) for further discussion of this reduction). To interpret the constraint on *b*, note that the MLE of μ based on Z_0 is $\widehat{\mu_0} = \frac{Z_0}{n_0}$, and that the MLE of μ based on Z_0 and Z_2 is $\widehat{\mu_{02}} = \frac{Z_0+Z_2}{n_0+n_2}$. To elect to observe Z_2 we require

$$\widehat{\mu_0} = \frac{Z_0}{n_0} \geq \frac{b}{n_0} > \frac{a}{n_0\sqrt{1+rN}},$$

and to win the trial we require

$$\widehat{\mu_{02}} = \frac{Z_0+Z_2}{n_0+n_2} > \frac{a\sqrt{1+rN}}{n_0+n_2} = \frac{a\sqrt{1+rN}}{n_0(1+rN)} = \frac{a}{n_0\sqrt{1+rN}}.$$

In other words, if we are to elect to observe the larger sample size with Z_2, the observed data at the interim must achieve what would need to be observed at the conclusion of the trial in order to be a successful trial. This interpretation is convenient for discussing the Ferring decision rules.

Two-Sample Extension

The condition above is expressed as a single-sample case. We demonstrate the extension to the two-dimensional case. In the single-sample case the test-statistic based on unit-variance normal $X_1, \ldots, X_M$ is

$$\sum_{m=1}^{M} X_m = M\bar{X}_m,$$

which has variance M. For the two-sample case ($X_1, \ldots, X_M$ and $Y_1, \ldots, Y_N$) the analogous statistic is $\frac{MN}{M+N}(\bar{X}_M - \bar{Y}_N)$, which has variance $\frac{MN}{M+N}$ (functions like the sample size). The joint distribution of $\frac{M_k N_k}{M_k+N_k}\left(\bar{X}_{M_k} - \bar{Y}_{N_k}\right)$ for k=1,2,… where the sample sizes Mk and Nk are nondecreasing in k, is the distribution of Brownian motion with drift E(X_1) –E(Y_1) evaluated at the times $\frac{M_k N_k}{M_k+N_k}$: k=1,2,…, which is exactly the same as the one-sample case.

In the Ferring trial since for fixed $M+N$ the effective sample size $\frac{MN}{M+N}$ is maximized when $M=N$, an earlier shift to 1:1 randomisation is synonymous with an *increase* in sample size.

Decision to "Increase Sample Size"

In the Ferring trial the decision to shift to 1:1 and thus increase the sample size is not based on a conditional power or a point estimate, but rather is based on Bayesian predictive probability. The design will shift to 1:1 randomisation early if the predictive probability is greater than 90% (typically a 50% predictive probability is consistent with observed data at the interim larger than what would need to be seen at the final analysis). This can be demonstrated explicitly in the Gaussian case with prior distribution centered at no treatment effect. For the special case of a flat, improper prior and unit variances, we have that the predictive probability of success

$$\Pr\left\{\bar{X}_{M_0+M_2} - \bar{Y}_{N_0+N_2} > a\sqrt{\frac{1}{M_0+M_2} + \frac{1}{N_0+N_2}} \,|\bar{X}_{M_0}, \bar{Y}_{N_0}\right\}$$

is equal to

$$\Phi\left(\frac{\bar{X}_{M_0}-\bar{Y}_{N_0}-a\sqrt{\frac{1}{M_0+M_2}+\frac{1}{N_0+N_2}}}{\sqrt{\frac{1}{M_2}+\frac{1}{N_2}-\frac{1}{M_0+M_2}-\frac{1}{N_0+N_2}}}\right).$$

If the predictive probability is greater than 50% then the numerator is positive and the point estimate at the interim is larger than what is needed for success at the final analysis. If the prior is not improper, but is conjugate normal with mean zero, then the observed effect at the interim must be even larger for a 50% predictive probability of success. In the Ferring trial the prior is more complicated -- given the joint model over multiple active arms, but each of these arms has prior mean the same as the placebo.

Hence the condition of 90% predictive probability to shift to the 1:1 randomisation provides a conservative type I error for the final analysis.

All simulations have reinforced the conservative nature of the final analysis, accounting for the Wilcoxon final analysis.

Appendix 7 Randomisation Plan

Part 1: Burn-In:

During burn-in randomisation will be conducted using blocked randomisation. At each site separate lists will be conducted for each of the following 8 strata:

Strata	Mechanical Ventilation	Serum Creatinine	norepinephrine/ noradrenaline
1	Yes	<150 μmol/L	<30 μg/min
2	Yes	<150 μmol/L	≥30 μg/min
3	Yes	≥150 μmol/L	<30 μg/min
4	Yes	≥150 μmol/L	≥30 μg/min
5	No	<150 μmol/L	<30 μg/min
6	No	<150 μmol/L	≥30 μg/min
7	No	≥150 μmol/L	<30 μg/min
8	No	≥150 μmol/L	≥30 μg/min

Each blocked list will be generated using random block sizes of 9 and 18 with ratios of 3:2:2:2:0 for the placebo and active treatment arms 1, 2, 3, and 4, respectively.

Part 1: Adaptive Randomisation:

After burn-in randomisation will be conducted using a mixture of blocking and response adaptive randomisation (RAR) with stratification weighting.

A blocking system will be created for placebo and active arms within each of the 8 strata. Using random block sizes of 3 and 6, lists will be made with ratios of 1:2 for placebo to active. These lists will be created for each strata.

When an "active" slot is pulled a "coin flip" approach will be taken in order to select which active dose is selected. These probabilities will change monthly. These RAR probabilities will adjust for the 3 strata factors. The balancing approach for the RAR is described in Section "Balance Weighting."

Part 2:

In Part 2 of the trial there will be only one target active arm and placebo. During this part randomisation is done using random blocks of 2, 4, or 6 with equal randomisation (1:1) within each site, for each of the 8 strata.

The individual lists created at each stage of randomisation will be discontinued at the end of its stage, with the new lists within site being used.

Balance Weighting

The adaptive randomisation algorithm creates a vector of probabilities for the four active treatment arms. This is a "global" probability for the arms, meaning it is over all strata. This section describes the balancing of the adaptive randomisation across the different strata to maintain, as well as possible, the balance of the 8 strata within active treatment arms, while achieving the needed adaptive randomisation.

The 8 strata used to balance the randomisation are based on the two-way classification of mechanical ventilation (MV) (Yes/No), Serum Creatinine (Low/High), and norepinephrine/noradrenaline (NA) use (Low/High). We present the method for modifying the global adaptive randomisation probabilities to create different randomisation probabilities for each strata that will honor the goals of the response adaptive randomisation as well as the goal of balancing the strata in the treatment arms.

The outline of the approach at any interim point of the trial is:

1. The response adaptive randomisation probabilities are provided from the efficacy analysis. Label these π_1, π_2, π_3, and π_4, for the four active doses.
2. The odds-ratio of a stratum, for each arm, relative to all other arms, is calculated for the *previously* randomised patients.
3. Within each stratum the response adaptive randomisations are modified by the odds-ratios from the previous randomisations to create new odds for each arm that are strata specific.
4. The probabilities within each strata are normalized and used for randomisation.

The details of the balancing algorithm are presented below and an example data set is presented.

We label the 8 strata as s=1,…,8. For the previous randomisations, let N_{sa} be the number of stratum s randomised to arm a. The marginals of each stratum and/or arm are labeled with a + symbol.

The odds-ratio (modified by adding 0.5 to each cell for handling 0 counts) of the previous patients being randomised to arm a, within stratum s is labeled OR_{sa}:

$$OR_{sa} = \frac{\left(N_{sa} \Big/ \left(N_{+a} - N_{sa} \right) \right)}{\left(\left(N_{s+} - N_{sa} \right) \Big/ \left(N_{++} - N_{+a} - N_{s+} + N_{sa} \right) \right)}.$$

The global odds of randomising to each arm ($\pi_a/1-\pi_a$) for new patients is modified by the previous odds-ratio of randomisation to create the new randomisation probabilities by stratum for balancing future patients. The modified odds of randomising to arm *a*, within stratum *s* is

$$\left(\frac{\pi_a}{1-\pi_a} \right) \left(\frac{1}{OR_{sa}} \right).$$

These odds create unique probabilities for each arm, within each stratum.

$$\frac{\left(\frac{\pi_a}{1-\pi_a} \right) \left(\frac{1}{OR_{sa}} \right)}{1 + \left(\frac{\pi_a}{1-\pi_a} \right) \left(\frac{1}{OR_{sa}} \right)}$$

These are normalized (across a stratum to sum to 1) to form a probability distribution for arms within a stratum:

$$\Pr\left(\text{Arm a in Stratum s}\right) = \frac{\dfrac{\left(\frac{\pi_a}{1-\pi_a} \right) \left(\frac{1}{OR_{sa}} \right)}{1 + \left(\frac{\pi_a}{1-\pi_a} \right) \left(\frac{1}{OR_{sa}} \right)}}{\displaystyle\sum_{b=1}^{4} \dfrac{\left(\frac{\pi_b}{1-\pi_b} \right) \left(\frac{1}{OR_{sb}} \right)}{1 + \left(\frac{\pi_b}{1-\pi_b} \right) \left(\frac{1}{OR_{sb}} \right)}}$$

As an illustrative example, assume the high dose has not been opened (and the new randomisation probability π_4 is 0). Example of previous randomisations are presented in (Table 4):

Table 4: Example of randomisations to each arm within each stratum.

Strata	Arms			Total
	a=1	*a*=2	*a*=3	
1	5	5	5	15
2	9	1	5	15
3	2	2	2	6
4	1	0	0	1
5	15	18	20	53
6	2	8	8	18
7	7	8	9	24
8	3	4	1	8
Total	44	46	50	140

The odds-ratios of each arm, within each stratum, OR_{sa}, are presented in (Table 5):

Table 5: The odds-ratio of arm *a*, within each stratum, *s*, with the 0.5 factor added to each cell.

Strata	Arms		
	a=1	*a*=2	*a*=3
1	1.09	1.02	0.90
2	3.42	0.18	0.90
3	1.09	1.02	0.90
4	3.32	0.51	0.45
5	0.79	1.08	1.15
6	0.28	1.69	1.48
7	0.88	1.02	1.09
8	1.28	1.92	0.32

Assuming the global response adaptive randomisation probabilities are π_1=0.33, π_2=0.50, and π_3=0.17, the modified randomisation probabilities for each arm within each stratum are presented in (Table 6).

Table 6: The modified randomisation probabilities for each stratum

Strata	Arms		
	a=1	*a*=2	*a*=3
1	0.31	0.50	0.19
2	0.11	0.73	0.16
3	0.31	0.50	0.19
4	0.12	0.60	0.28
5	0.38	0.47	0.15
6	0.56	0.33	0.11
7	0.35	0.49	0.16
8	0.27	0.34	0.39

Appendix 8 Bias on Treatment Estimate for P&VFDs

The treatment estimates for the primary endpoint will be based on a comparison between all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm), and all patients on the placebo arm from both parts of the trial.

Additionally, the primary analysis will be repeated for the selected arm only, i.e. comparing all patients on the selected arm (from part 1) from both parts of the trial (pooled), to all patients on the placebo arm from both parts of the trial.

To address the issue of a potential bias on the treatment estimates a variety of different scenarios were simulated (Table 7). The results are based on 3000 simulated trials of scenarios where all arms are equally effective and where the mortality benefits and P&VFD benefits for survivors correspond. For each simulated trial, the treatment effect estimates are simple classical point estimates from the raw patient data, e.g. the mortality treatment effect is estimated by the raw mortality rate in the placebo group, minus the raw mortality rate in the active group.

Note that when investigating bias, it is not appropriate to restrict attention to simulated trials that are successful. Estimates from that approach will yield results that are larger than the simulated truths, and this is true for simple non-adaptive designs as well. In this trial the design is modified by removing all futility rules so that all simulated trials select an arm for Part 2 and enroll all 1800 patients. Some of these simulated trials reach 800 patients with the placebo arm outperforming all active arms; in these Part 2 is begun with the least badly performing active arm.

The table shows the true underlying benefit, the estimated benefits for the primary analysis (all selepressin arms from both parts of the trial compared to all patients on placebo from both parts of the trial), and the estimated benefits for the selected arm only (all patients on the selected arm from both parts of the trial compared to all patients on placebo from both parts of the trial).

The placebo arm was assumed to have a 25% mortality rate, a mean of 24 P&VFDs for survivors, and an overall mean of 18 P&VFDs (survivors and non-survivors).

If all active arms are included in the estimates (to correspond with the use of all active arms in the final van Elteren analysis as in the two middle columns), the estimates are unbiased and any differences from the truth are due to Monte Carlo variation. If only the selected arm is included in the estimates, there is some bias due to the fact that the selected arm must have performed relatively well in Part 1, the fact that Part 1 data are included in the estimates, and this bias is not completely neutralized by the introduction of a large sample of unbiased data in Part 2. The estimates of benefit in P&VFDs for survivors are nearly unbiased, while the relatively noisier mortality estimates have biases that can be on the same scale as the treatment effect. The largest bias is for the small mortality effect of 0.5%, where the design estimates an average effect of 0.77%, which is too small of a benefit to lead to a successful trial without a substantial effect on P&VFDs.

Table 7: Simulated treatment estimate bias for P&VFDs

True benefit		**Average estimated benefit (all selepressin arm)**		**Average estimated benefit (selected arm only)**	
Mortality	P&VFDs (survivors)	Mortality	P&VFDs (survivors)	Mortality	P&VFDs (survivors)
0%	0 days	-0.03%	0.01 days	0.21%	0.06 days
0.5%	0.5 days	0.56%	0.51 days	0.77%	0.56 days
1.0%	1.0 days	0.98%	0.98 days	1.17%	1.05 days
1.5%	1.5 days	1.45%	1.46 days	1.63%	1.54 days
2.0%	2.0 days	1.92%	1.95 days	2.05%	2.03 days
3.0%	3.0 days	2.97%	2.97 days	3.10%	3.03 days

In both scenarios, treatment estimates are either unbiased or the treatment bias is negligible, and hence treatment estimates will not be adjusted for treatment bias.

STATISTICAL ANALYSIS PLAN

A Double-blind, Randomised, Placebo-controlled Phase 2b/3 Adaptive Clinical Trial Investigating the Efficacy and Safety of Selepressin as Treatment for Patients with Vasopressor-dependent Septic Shock

SEPSIS-ACT

Selepressin Evaluation Programme for Sepsis-Induced Shock - Adaptive Clinical Trial

000133

Investigational Product: Selepressin; concentrate for solution for infusion
Placebo; sterile 0.9% sodium chloride solution

Indication: Vasopressor-dependent Septic Shock

Phase: 2b/3

Author: [redacted]

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Version: Final - Ver. 9.0

Change log

Version No.	Effective Date	Reason for the Change / Revision	Supersedes
1	Dec 17-2014	Original SAP	Not applicable
2	Dec 19-2014	Format change only. No content was changed	Version 1
3	Dec 19-2014	Format change only. No content was changed	Version 2
4	Mar 24-2015	Added endpoint: Episodes of hypotension Modified endpoint: Norepinephrine/ noradrenaline and other vasopressor doses Updated section on definition of P&VFDs and imputations for P&VFDs Various minor elaborations of endpoint definitions and minor changes to analyses	Version 3
5	May 27-2015	Added sensitivity analysis for primary endpoint. Appendix 4 notation updated and estimate for number of new organ dysfunctions and failures included Appendix 5 updated to reflect tenths of days. Appendix 6 updated to make proof more readable. Various editorial clarifications	Version 4

6	July 8 - 2016	Expanded definition of onset of shock to include ANY vasopressors Replaced two AE tables regarding adverse events based on changes in vital signs/laboratory values assessed as unanticipated in the setting of septic shock, as we do not collect sufficient data to produce these tables. Modified tables presented. The analysis of other vasopressors has been split into several components. Sensitivity analyses added to ICU-free days and ICU length of stay. Fluid balance/Urinary output analyses updated Terlipressin added to list of vasopressors for derivation of primary endpoint etc. Definition of primary endpoint imputation for patients lost to follow-up is extended to include any type of withdrawal before Day 30. List of critical adverse events updated (same as before, just re-grouped). No AE tables on AEs leading to withdrawal, as it is not an option to withdraw patient from trial due to AE. Added alternative analysis to “duration of” endpoints, in case normality cannot be assumed for the whole of the data. Small edit in formula in Appendix 5. Continuous version of baseline norepinephrine version is adjusted for weight (categorical version from strata is unadjusted) Added imputation rules for NE and other vasopressors (section 9.4.5) and IMP (section 8.1.1) Appendix 2 slightly updated with corrections. List of protocol deviations modified (section 5)	Version 5

7	Nov 9 - 2017	Additional analyses to MAP analyses. Analysis for duration of septic shock changed from ANCOVA to negative binomial (as blinded data suggests a better fit) with permutation test as backup in case model assumptions do not hold. Analyses for duration of ventilation and duration of RRT changed from ANCOVA to zero inflated negative binomial (as blinded data suggests a better fit) also with permutation test as backup in case model assumptions do not hold. Analysis for ICU length of stay and hospital length of stay changed from ANCOVA to permutation test (as blinded data suggested a non-parametric model). Covariates were dichotomized accordingly. Appendix 4 removed as no longer needed. Appendix 3 updated with variance correction for difference in PVFDs Analyses in 9.4.8, 9.4.9, and 9.4.10 will not be repeated for the selected arm only and data from part 2 only, as they are measured on a subset of patients, probably only in part 1. A “time” by “baseline value” interaction term has been added to the repeated ANCOVA analyses in 9.3.1.5, 9.3.3.1, 9.3.4.1, 9.3.4.2, and 9.4.5 as it is likely that the trajectory over time for each patient will depend on the baseline value. The supportive competing risk and Kaplan-Meier graphs for duration of RRT has been taken out as it makes no sense since RRT durations are short and repetitive. It is more meaningful to just look at 90 day mortality. For the remaining ‘duration’ endpoints withdrawal rates has been added to the competing risk and Kaplan-Meier graphs. As this is a superiority trial, the PP analysis will only be performed for the primary endpoint, and hence not performed for secondary as previously stated.	Version 6

		Only major protocol deviations will be listed. Adverse events graphs added to output. Fluid balance analyses updated. Analyses for norepinephrine/noradrenaline and other vasopressor doses updated. Forest plots added for incidence of RRT (section 9.3.1.8) and mortality (section 9.3.2.3).	
8	Nov 9 - 2017	Format change only. No content was changed	Version 7
9	Nov 20 - 2017	Format change only. No content was changed	Version 8

Signed agreement on Statistical Analysis Plan

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1 Introduction

This document describes the planned statistical analyses for selepressin (FE202158) 000133, and is based on protocol dated July 8th 2016 including amendments 1 and 2.

All analyses in the SAP were planned and pre-specified prior to trial termination due to futility.

1.1 Definitions/ Abbreviations

1.1.1 Definition of Terms

Terms	Definitions
Evaluable patient	Patient who has been treated with IMP and 30 days have passed since initiation of IMP infusion
Randomised	Patient randomised to trial treatment
Screened	Patient who enters the screening phase
Selepressin	FE 202158
Treated patient	Randomised and dosed
1-KM curve	1 minus Kaplan Meier curve

1.1.2 Abbreviations

Abbreviations	Meaning of abbreviations in document
AE	Adverse Event
ANCOVA	Analysis of covariance
AUC	Area Under the Curve
CVP	Central venous pressure
EVLW	Extra-vascular lung water
EQ-5D-5L	EuroQol – 5 Dimensions – 5 Levels
FAS	Full-Analysis Set
ICU	Intensive care unit
IMP	Investigational Medicinal Product
ITT	Intention-to-treat
LOCF	Last observation carried forward
MAP	Mean Arterial Pressure
MedDRA	Medical Dictionary for Regulatory Activities
NE	Norepinephrine
PK	Pharmacokinetic
PP	Per-Protocol
PPI	Pulmonary permeability index
PT	Preferred term
P&VFD	Vasopressor and mechanical ventilator free days
QALY	Quality adjusted life years
RAR	Response adaptive randomisation
RRT	Renal replacement therapy
ScvO2	Oxygen Saturation in Vena Cava Superior
SOFA	Sequential Organ Failure Assessment score

Abbreviations	**Meaning of abbreviations in document**
SOC	System Organ Class
WBC	White blood cells

2 Trial Objectives and Endpoints

2.1 Objectives

Primary Objective

- To demonstrate superiority of selepressin plus standard care versus placebo plus standard care in the number of vasopressor- and mechanical ventilator-free days (with penalty for mortality) in patients with vasopressor-dependent septic shock

Secondary Objectives

- To determine the efficacy of selepressin on:
  - Organ dysfunction
  - Morbidity and mortality
  - Fluid balance
  - Health-related quality of life
- To determine the safety profile of selepressin
- To determine the pharmacokinetics of selepressin
- To determine the health economics of selepressin
- To further evaluate a range of biomarkers in relation to the mode of action of selepressin

2.2 Endpoints

2.2.1 Primary Endpoint

- Vasopressor- and mechanical ventilator-free days (P&VFDs) up to day 30

2.2.2 Key Secondary Endpoints

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause, by the end of Day 90)
- Renal replacement therapy (RRT)-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)
- Intensive care unit (ICU)-free days up to Day 30

2.2.3 Secondary Endpoints

Organ dysfunction

- Vasopressor-free days up to Day 30
- Mechanical ventilator-free days up to Day 30
- Duration of septic shock up to Day 30

- Duration of mechanical ventilation up to Day 30
- Incidence of RRT up to Day 30 (counting patients who die as on RRT and excluding patients on RRT for chronic renal failure at time of randomisation)
- Duration of RRT up to Day 90 (excluding patients on RRT for chronic renal failure at time of randomisation)
- Daily overall and individual organ (cardiovascular, respiratory, renal, hepatic, coagulation) scores using the modified Sequential Organ Failure Assessment (SOFA) scores until ICU discharge
- Incidence of new organ dysfunctions and new organ failures (based on the SOFA score) up to Days 7 and 30

Morbidity/mortality

- ICU length of stay up to Day 30
- All-cause mortality (defined as the fraction of patients that have died, regardless of cause, by the end of Days 30 and 180)

Fluid balance

- Daily and cumulative fluid balance until ICU discharge (for a maximum of 7 days)
- Daily and cumulative urinary output until ICU discharge (for a maximum of 7 days)

Health-related quality of life

- Change in utility, based on EQ-5D-5L, up to Day 180

2.2.4 Safety Endpoints

- Incidence of adverse events (type, frequency, and intensity) with specific emphasis on:
  - Ischaemic events (e.g. myocardial, skin, cerebral, mesenteric, and limb ischaemia)
- Changes in vital signs and safety laboratory variables, including:
  - Number of clinically significant results assessed as unanticipated in the setting of septic shock
- Episodes of hypotension

2.2.5 Additional Endpoints

- Hospital-free days up to Day 90
- Hospital length of stay up to Day 90
- Patient residence at Day 30, Day 60, Day 90, and Day 180
- Mean arterial pressure (MAP), until ICU discharge (for a maximum of 7 days)

- Norepinephrine/noradrenaline and other vasopressor doses
- Pharmacokinetic response (in a subset of approximately 200 patients) - to be reported separately according to a pre-specified pharmacokinetic analysis plan
- Health economic evaluation – to be reported separately according to a pre-specified health economic analytical plan
- Creatinine Clearance
- PaO2/FiO2 ratio (in a subset of 100-350 patients)
- Extravascular Lung Water and Pulmonary Permeability Index (in a subset of 100-350 patients)
- Cardiac output (in a subset of 100-350 patients)
- Cytokines (in a subset of 100-350 patients)
- Angiopoietin 1 and 2 levels (in a subset of 100-350 patients)

2.2.6 Other Assessments

- Central Venous Pressure
- Central Venous Oxygen Saturation
- Arterial Blood Gases (PaO2, PaCO2, SaO2, pH, HCO3, base excess) and Lactate

3 Trial design

3.1 General Design Considerations

The overall adaptive design is a Phase 2b/3 trial, in which dose-ranging with response-adaptive randomization (RAR) (see Appendix 5 for details) is utilized in a first part (the Phase 2b part – Part 1), followed by a traditional 1:1 randomised comparison of selepressin to placebo in the second part (the Phase 3 part – Part 2). The final analysis uses patients from both parts of the trial. The entire trial, combining both parts, represents an adequate and well-controlled comparison of selepressin and placebo.

In Part 1 of the trial, up to four dosing regimens will be investigated.
Arm 1: Starting dose at 1.7 ng/kg/min, and a max. dose of 2.5 ng/kg/min
Arm 2: Starting dose at 2.5 ng/kg/min, and a max. dose of 3.75 ng/kg/min
Arm 3: Starting dose at 3.5 ng/kg/min, and a max. dose of 5.25 ng/kg/min
Arm 4: Starting dose at 5 ng/kg/min, and a max. dose of 7.5 ng/kg/min

Part 1 comprises a minimum of 300 evaluable patients and a maximum of 800 treated patients. During Part 1, patients will be randomised to placebo or selepressin (Arms 1 to 3). Arm 4 will only be opened between 200 evaluable- 600 treated patients and if there is at least a 50% probability that Arm 3 has a higher expected P&VFD than Arm 2 and if data from the lower dosing levels do not suggest any significant safety signals. To minimize the risk of imbalance between treatment arms, randomisation will be stratified based on trial site, the need for mechanical ventilation (Yes/No), norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and creatinine (< or ≥150 µmol/L) (See Appendix 7 for details).

Part 1 will begin with a 200-patients treated "burn-in" period during which fixed randomisation across the treatment arms will be used (one-third of the patients randomised to placebo and two-ninths of the patients to each of the selepressin arms [Arms 1 to 3]). The factors described above will be used to stratify the randomisation.

After completion of the burn-in period, Part 1 will utilize response-adaptive randomisation to preferentially place patients into the arms that appear to have the maximum benefit with respect to the primary endpoint. A fixed fraction (one third) of patients will be randomised to placebo throughout Part 1 to ensure contemporaneous control patients are enrolled throughout the trial.

If Part 1 culminates in the decision to run Part 2, Part 2 will be a 1:1 comparison of placebo to the best-performing active treatment arm. The best-performing active treatment arm will be identified at the end of Part 1. Part 2 will utilize a fixed 1:1 randomisation proportion, with stratified randomisation as described for Part 1. Part 2 can begin after any interim analysis after 300 evaluable -800 treated patients in Part 1 and the size of Part 2 will include enough patients to bring the total number of evaluable patients in Part 1 and Part 2 up to 1800, ensuring a minimum sample size of Part 2 of 1000 evaluable patients.

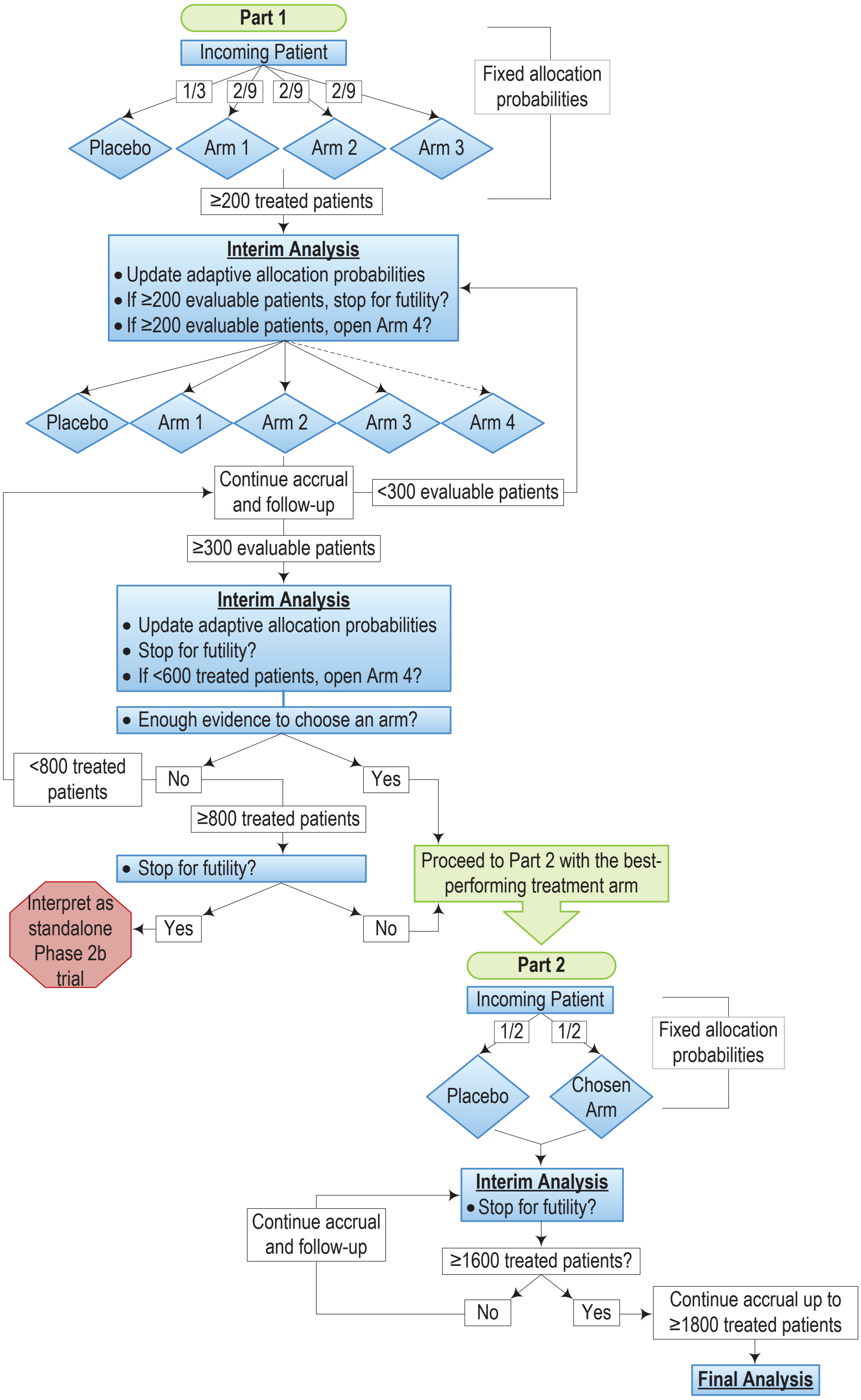


Figure 1: Design Flow Chart

3.2 Determination of Sample Size

At least 1800 evaluable patients combined for Part 1 and Part 2 are needed for the final analysis. The overall power of obtaining statistical significance based on combined evidence from Part 1 and Part 2 is 91% in situations where all 4 arms have a true underlying 1.5% lower mortality rate and a 1.5-day higher expected number of P&VFDs for survivors as compared to placebo (corresponding to an overall treatment effect of 1.5 P&VFDs). If the effect sizes are 2% on mortality and 2 days for P&VFDs in survivors for all 4 arms (corresponding to an overall treatment effect of 2 P&VFDs), then the overall power is 98%. In this latter case the probability of engaging into Part 2 is ~99%.

4 Patient Disposition

A summary table will present, for each part of the trial and overall, the number of patients in the population sets: ‘Screened’, ‘Intention to treat’, Full analysis set’, ‘Per protocol’, ‘Safety’, ‘Completed trial’, ‘Withdrawals’, and ‘IMP discontinuations’ with a breakdown of reasons/categories for trial withdrawals and IMP discontinuations.

The patient disposition table will be broken down by each of the stratification variables [the need for mechanical ventilation (Yes/No), norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and creatinine (< or ≥150 µmol/L)] and broken down chronologically displaying number of patients ‘completed’ and ‘withdrawn from trial’ at Day 30, Day 90 and Day 180.

The number of patients screened but not randomised/allocated to treatment will be presented with the reason(s) for screen failure in a data listing.

All major protocol violations (including misrandomisations), based on the Full analysis set, will be summarised for each part of the trial.

Furthermore 1-KM plots, based on the ITT, will be presented for the time to trial withdrawals/IMP discontinuations (whichever comes first) differentiated by reason of trial withdrawal/IMP discontinuation using cumulative incidence functions. Dropout rates between treatment groups will be evaluated by the log-rank test.

5 Protocol Deviations

Patient data in the full analysis set (FAS) will be excluded from the per-protocol (PP) analysis set if they meet any of the following criteria:

- Dosing errors expected to significantly impact efficacy
- More than 16 hours from onset of vasopressor treatment to start of IMP
- Failed to receive a continuous infusion of norepinephrine/noradrenaline base greater than 4.5 ug/min for at least one hour or received less than 4.5 ug/min of norepinephrine/noradrenaline base at start of IMP infusion
- Violation of exclusion criteria 2: Primary cause of hypotension not due to sepsis (e.g., major trauma including traumatic brain injury, hemorrhage, burns, or congestive heart failure/cardiogenic shock)
- Violation of exclusion criteria 6: Chronic mechanical ventilation for any reason OR severe chronic obstructive pulmonary disease (COPD) requiring either continuous daily oxygen use during the preceding 30 days or mechanical ventilation (for acute exacerbation of COPD) during the preceding 30 days
- Violation of exclusion criteria 9: Decision to limit full care taken before obtaining informed consent
- Violation of exclusion criteria 10: Use of vasopressin in the past 12 hours prior to start of IMP infusion or use of terlipressin within 7 days prior to start of IMP infusion
- Violation of exclusion criteria 12: Prior use of an investigational medicinal product within the last month OR planned or concurrent participation in a clinical trial for any investigational drug or investigational device

Furthermore, any other major protocol violations, such as serious unforeseen violations deemed to invalidate the data and affect the conclusions of the study will lead to exclusion of data from the PP analysis set.

Major protocol deviations will lead to exclusion of data from the PP analysis, while data will not be excluded because of minor protocol deviations. The list of major protocol deviations will be detailed and documented in the clean file document prior to database release.

All major protocol deviations will be listed in patient data listings.

6 Analysis sets

6.1 Intention-To-Treat Analysis Set

The intention-to-treat (ITT) analysis set comprises of all randomised (as planned) patients.

6.2 Full-Analysis Set

The FAS comprises data from all randomised (as planned) and dosed patients.

6.3 Per Protocol Analysis Set

Patients in the FAS will be excluded from the PP analysis set if they meet any major protocol violations defined in (Section 5). Data will be used up to the point of protocol violation.

6.4 Safety Analysis Set

The safety analysis set comprises all treated patients and are analysed according to the actual treatment received.

7 Trial population

7.1 Demographics and Other Baseline Characteristics

Categorical data will be summarised using numbers and percentages. The percentages are based on the total number of patients with a corresponding assessment. Continuous data will be presented, for example, using the number of patients (N), mean and standard deviation, median, interquartile range, minimum and maximum. All baseline characteristics will be listed.

Demographics and baseline characteristics of the study population will be summarised for the FAS.

7.1.1 Demographics

Descriptive statistics of baseline demographics variables will be summarized by treatment arm and total.

7.1.2 Vital Signs at Baseline

Baseline vital signs will be summarised by treatment arm and total.

7.1.3 SOFA Score, APACHE II Score and Septic Shock Characteristics

Baseline SOFA score (modified), APACHE II score and information on septic shock (infection proven/suspected, primary infection type and location will be summarised by treatment arm and total.

7.2 Medical History

Medical history recorded at screening visit will be summarised by treatment arm and total.

Furthermore, medical history will be presented in patient data listings.

7.3 Prior and Concomitant Medication

Prior and concomitant medication will be summarised by treatment arm and total.

Furthermore, concomitant medication will be presented in patient data listings.

8 Exposure and Treatment Compliance

8.1.1 Extent of Exposure

The total amount (adjusted by weight (µg/kg)) of selepressin administered and the number of days (reported to one decimal place) treated with selepressin will be summarised by (active) treatment arm and total (active treatment arms).

Furthermore, the mean cumulative amount administered and the mean infusion rate will be tabulated by treatment arm and presented graphically (also by treatment arm and total).

If a patient has missing infusion rate and the patient is still in the trial (not dead or withdrawn) it will be assumed that selepressin was not administered and a value of zero will be imputed, unless there is an interval in the timing log covering the exact time point (8 AM and 8 PM is the assumed time point for missing morning and evening collection time points). In that case LOCF will be used, but only within the time interval.

If a patients has missing cumulative selepressin volume and the patient is still in the trial (not dead or withdrawn), LOCF will be used assuming that selepressin was not administered and hence keeping the cumulative volume constant.

9 Efficacy

9.1 General Considerations

All statistical tests will be performed using a two-sided test at a 5% significance level.

If the trial is stopped prematurely due to e.g. futility, the data will be analysed as planned in this statistical analysis plan.

All efficacy endpoints will be analysed for the FAS analysis set, and as a sensitivity the analyses for the primary endpoint will be repeated for the PP population.

Categorical data will be summarised using counts and percentages, while continuous data will be presented using the number of patients (N), mean, standard deviation, median, interquartile range, minimum and maximum. All efficacy endpoints will be listed in patient data listings.

9.2 Primary Endpoint

- Vasopressor- and mechanical ventilator-free days (P&VFDs) up to day 30

This composite endpoint is defined as the number of days (reported to one decimal place (0.0 to 30.0)) from start of treatment with the investigational medicinal product (IMP) [selepressin or placebo] to 30.0 days thereafter during which the patient is: 1) alive; 2) free of treatment with intravenous vasopressors; and 3) free of any invasive mechanical ventilation (see definition below).

Patient Death

By definition, any patient that dies within this 30-day period will be assigned zero P&VFDs, even if there is a period during which the patient is alive and free of both vasopressor treatment and mechanical ventilation.

Definition of "Free of Vasopressors"

Free of vasopressors means less than 60 minutes during any contiguous 24-hour period (regardless of calendar day). If a patient requires periods of vasopressors longer than 60 minutes in total during any 24-hour period, the intervening intervals during which they are free of vasopressors will not be included in the period free of vasopressors in the determination of the number of P&VFDs. Thus, the period free of vasopressors begins at the end of the last use of vasopressors that was either: 1) longer than 60 minutes in duration; or 2) part of greater than 60 minutes of use within a contiguous 24-hour period.

Norepinephrine/noradrenaline, phenylephrine, dopamine, epinephrine/adrenaline, vasopressin, terlipressin, and IMP (i.e. selepressin and placebo) all constitute a vasopressor for the purpose of the primary analysis.

Vasopressor use due to anaesthesia or procedure-induced hypotension during and up to three hours after a surgery or procedure (including bedside) is exempt from this rule (i.e. such use of vasopressors would not affect the calculation of P&VFDs).

Definition of "Free of Mechanical Ventilation"

Mechanical ventilation is defined as use of endotracheal or tracheostomy tube assisted ventilation (>5 cm H_2O continuous positive airway pressure and >5 cm H_2O of pressure support from the ventilator in tracheostomy patients). End of mechanical ventilation is defined as: 1) extubation of intubated patients or 2) ≤5 cm H_2O continuous positive airway pressure and ≤5 cm H_2O of pressure support from the ventilator in tracheostomy patients. If non-invasive ventilation by mask or bag (>5 cm H_2O of pressure support) is deployed to avoid (re)intubation, it also counts as mechanical ventilation. However, all other uses of non-invasive ventilation such as chronic night-time use of positive airway pressure for chronic obstructive pulmonary disease (COPD) or sleep apnea does not count as mechanical ventilation (regardless of pressure). Free of mechanical ventilation means less than 60 minutes during any contiguous 24-hour period (regardless of calendar day). If a patient requires mechanical ventilation for periods longer than 60 minutes in total during any 24-hour period, the intervening intervals during which they are not receiving mechanical ventilation will not be included in the period free of mechanical ventilation in the determination of the number of P&VFDs. Thus, the period free of mechanical ventilation begins at the end of the last use of mechanical ventilation that was either: 1) longer than 60 minutes in duration; or 2) part of greater than 60 minutes of use within a contiguous 24-hour period.

The use of mechanical ventilation associated with anaesthesia or procedural sedation during and up to three hours after a surgery or procedure (including bedside) is exempt from this rule (i.e. such use of mechanical ventilation would not affect the calculation of P&VFDs).

It is important to note that the determination of freedom from vasopressors and freedom from mechanical ventilation are made separately; in other words, periods of vasopressor use and mechanical ventilation are not combined when determining whether 60 minutes of use has occurred within a 24-hour period.

Missing data during the time of hospitalization will be imputed using a worst case approach taking into account previous and subsequent starting and stopping times of vasopressor administration and mechanical ventilation (see Figure 2). If only the stop date but not time is given, the imputed time will be midnight of that date (example A), unless a subsequent starting time was recorded prior to midnight in which case the imputed time would be the start time of the subsequent record (example B). If neither stop date nor time is given, the imputed stop time will be the start date and time of the subsequent recording. Likewise, missing start dates and times would be imputed as worst case scenarios, i.e. is the patient found to be on mechanical ventilation with a date but no time for intubation, the imputed start time would be recorded as 00:01 of that day or the stop date of a preceding recording on that same date, whichever occurs last. If both start date and time is missing, the imputed start time would be the date and time of the preceding stop time recorded. In case of data being completely missing from a certain time point and onwards, the "last status carried forward" imputation (example C) will be applied. If a patient was last seen on either ventilator or vasopressors, it is assumed that the patient remained so, and is imputed to a value of 0 (zero) P&VFDs. If the patient was last seen off ventilator and vasopressors, it is also assumed that the patient remained so in the remaining 30-day period. If the patient was last seen (alive) on e.g. day

10 and at that point had been off both ventilator and vasopressors for three days, a value of 23 P&VFDs is imputed.

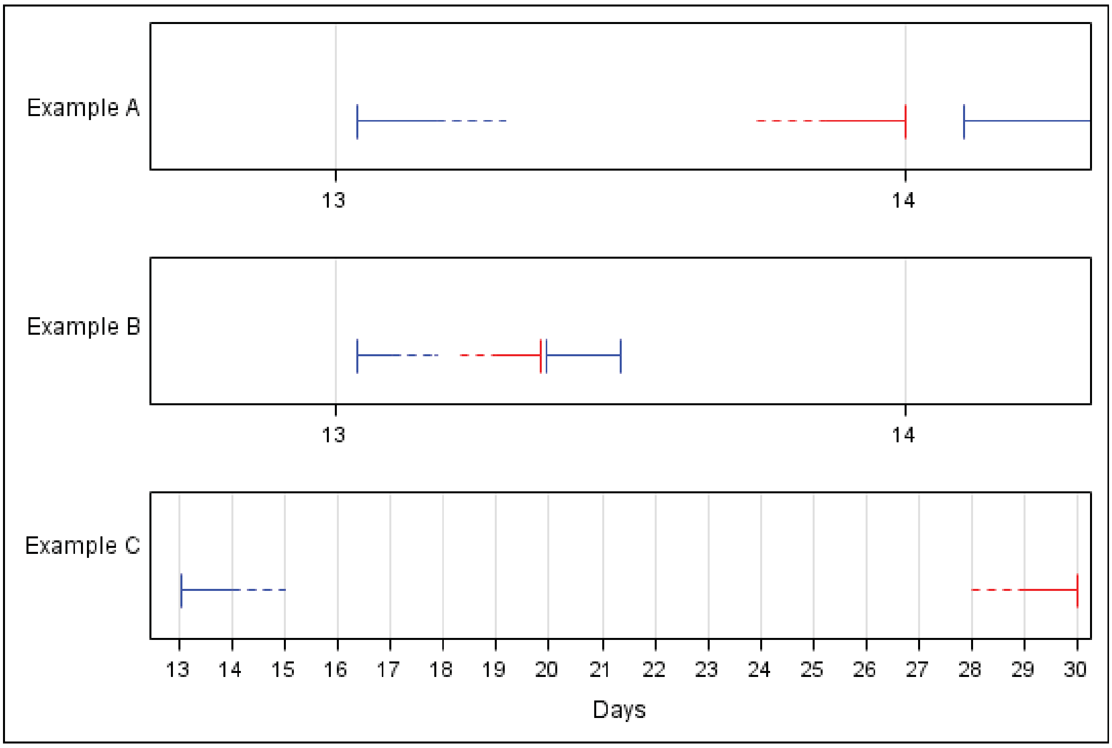


Figure 2: Missing Data Imputation of P&VFDs (Examples)

9.2.1 Primary Variable Analysis

The primary endpoint, P&VFDs, will be analyzed using a van Elteren test, stratified by need for ventilation (Yes/No), time from onset of shock (onset of any vasopressor) to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min).

The primary analysis will compare all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) to all patients on the placebo arm from both parts of the trial (see Appendix 7 for a discussion on treatment estimate bias).

The primary analysis will be a test of superiority using a two-sided 5% significance level test.

Treatment effects will be estimated assuming a negative binomial distribution (to allow for possible overdispersion in a Poisson distribution) for the quantity (30 minus P&VFDs) for survivors, and a binomial distribution to model the probability of surviving. Both models adjusted for need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) (see Appendix 3 for details). For completeness, the proportion of patients dying, and the P&VFDs for survivors will also be presented.

Furthermore, P&VFDs will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically by histograms and cumulative distributions functions.

The success (statistical/clinical significance) of the trial will be based upon the comparison of the analysis above (all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) compared to all patients on the placebo arm from both parts of the trial).

9.2.2 Sensitivity Analyses

The primary analysis will be repeated for the PP analysis set.

As the adaptations of the trial provide a conservative estimate of the p-value, sensitivity p-values will be provided using post-simulation bootstrap calculations (see Appendix 6 for details).

In order to check for consistency the primary endpoint treatment differences will, as a minimum, be estimated and presented by forest plots for the following subgroups

- region (US/Canada vs. Europe)
- age (<65, 65-74, 75-84, ≥85)
- gender
- race/ethnicity

Furthermore, the primary endpoint will be stratified by severity of the patients, with risk of dying as indicator of severity (Figure 3). Mortality (the risk of dying) will be predicted by a logistic regression model, with relevant baseline characteristics as covariates (e.g. the individual SOFA scores and age). The model used to generate the predicted risk (for all patients) will be based on patients in the placebo arm only, as the risk of dying should reflect the severity in the absence of selepressin. Stratified by the risk of dying (intervals of 20% if suitable, based on the mortality rates in the covariate categories in the model), the treatment effect of the primary endpoint will be presented graphically, in order to visually inspect whether the average treatment effect is distributed evenly across the severity of patients.

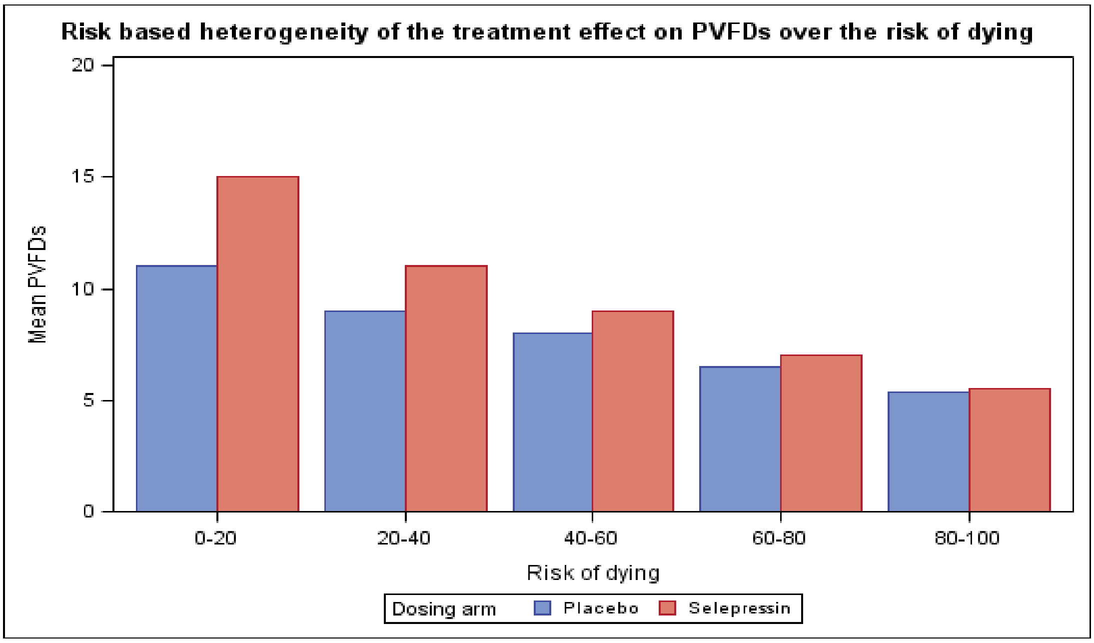


Figure 3: Risk Based Heterogeneity of the Treatment Effect on P&VFDs over the Risk of Dying (an Example)

The impact and robustness of the imputation of missing data will be checked by analysing data in the following ways

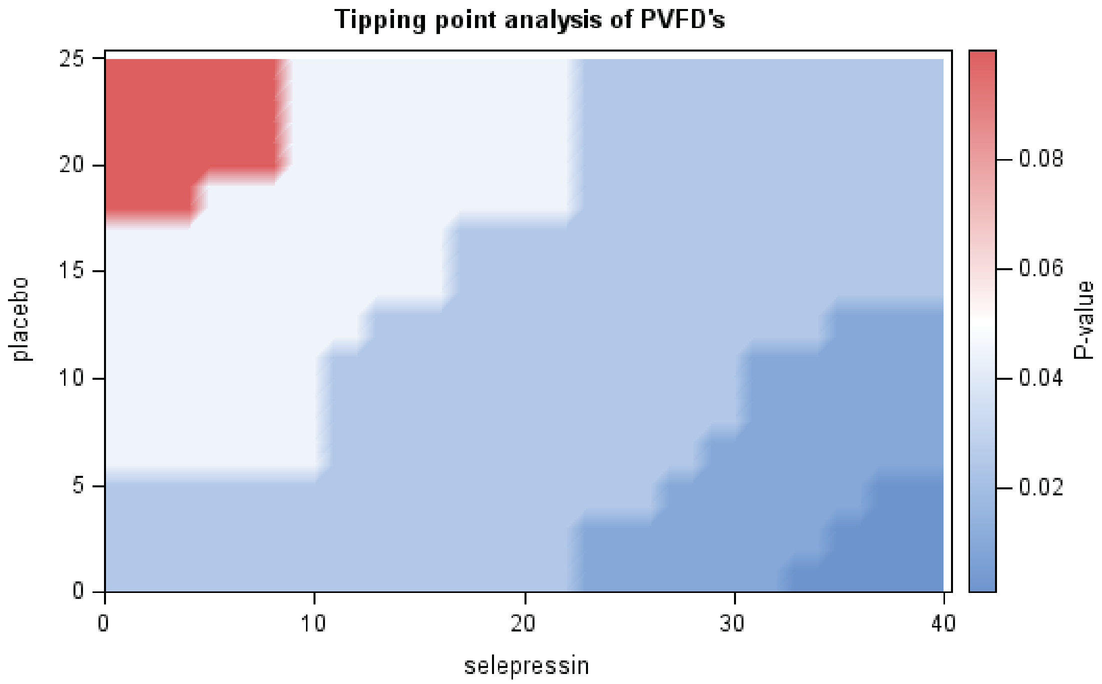
- excluding all patients with missing/imputed data
- imputing the 30-day P&VFD status for patients lost to follow up (or otherwise withdrawn from trial) using the observed ratio of P&VFDs at time of lost to follow up (or time of withdrawal), to the same proportion for a 30-day status

For this analysis the 30-day P&VFD status for patients lost to follow up (or otherwise withdrawn from trial) will be imputed so that the 30-day ratio of P&VFDs is equal to the ratio of P&VFDs at time of lost to follow up (or time of withdrawal). E.g. a patient being lost to follow up at Day 15 with 4 P&VFDs (a ratio of 4/15 P&VFDs per days observed) will be imputed to 8 P&VFDs at Day 30 (equivalent ratio 8/30 = 4/15). Patients having zero P&VFDs at time of lost to follow up will be imputed to a value of zero P&VFDs.

- tipping point analysis

The tipping point analysis will compare all possible combinations of 'best case' and 'worst case' scenarios between placebo and selepressin (Figure 4) for patients lost to follow up (or otherwise withdrawn from trial). Best case being an imputation assuming the remaining days off ventilator and vasopressors, and worst case being an imputation of 0 P&VFDs. Let N_p and N_s be the number of patients in the placebo and selepressin arms with missing data. The tipping point analysis will compare all combinations (from 0 to N_p) of X patients on placebo imputed best case and $N_p - X$

imputed worst case, to Y patients on selepressin imputed best case and N_s – Y imputed worst case. I.e all N_p +1 times N_s +1 combinations will be analysed for the primary endpoint. Since the 'best case' is not the same for all patients (depending on when they were last seen off both ventilator and vasopressors) there are multiple outcomes within each combination. For each combination, the average P-value of the multiple outcomes will be plotted in the tipping point analysis. Below is an example of a tipping point analysis of 25 placebo patients vs. 40 selepressin patients with imputed values. The x- and y-axis displays the number of patients with the 'best case' imputed. In the example below the red area displays the non-significant p-values, indicating that one would have to impute almost all placebo patients to a 'best case' and almost all selepressin to a 'worst case' in order to get non-significant p-values, and hence 'proving' the robustness of the imputation method.



The x- and y-axis displays the number of patients with the 'best case' imputed

Figure 4: Tipping Point Analysis of P&VFDs (an Example)

Also, to make sure that the use of vasopressor in each group is not simply being replaced by an increased use of inotropic agents (e.g. dobutamine, milrinone, levosimendan, and amrinone), there will be a sensitivity analysis of the primary endpoint in which the use of inotropic agents will count as vasopressor use.

9.2.3 Additional Analyses

The primary analysis will be repeated for:

- the selected arm only, i.e. comparing all patients on the selected arm (from part 1) from both parts of the trial (pooled), to all patients on the placebo arm from both parts of the trial.

- data from part 2 only, i.e. comparing the selected arm to placebo on data from part 2 only

9.3 Secondary Endpoints

For the purpose of a possible label inclusion, the Hochberg procedure (4) for adjustment on multiplicity will be implemented to selected key secondary endpoints. Only if the primary efficacy analysis leads to a statistically significant result at the (one-sided) 2.5% level, then the Hochberg procedure which is described below is applied to selected key secondary analyses. If the primary efficacy analysis does not result in statistical significance at the (one-sided) 2.5% level, then statistical significance (for the purpose of a possible label inclusion only) will not be declared for any of the key secondary analyses, regardless of their p-values.

The selected key secondary endpoints aimed at further demonstrating treatment effect are:

- All-cause mortality (defined as the fraction of patients that have died, regardless of cause, by the end of Day 90)
- Renal replacement therapy-free days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)
- ICU-free days up to Day 30

In this application of the Hochberg procedure there are three hypothesis tests of superiority for each of the selected secondary endpoints. The target alpha level is (one-sided) 2.5%. The Hochberg procedure is as follows:

- Order the p-values from the smallest to the largest value, $p(1) < p(2) < p(3)$, with corresponding null hypothesis $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$.
- Start with the highest p-value. If $p(3) < 2.5\%$ (one-sided), then stop and declare all three comparisons significant at the 2.5% (one-sided) level (i.e. reject $H_{(1)}$, $H_{(2)}$, and $H_{(3)}$). Otherwise, accept $H_{(3)}$ for the endpoint related to p(3), and go to p(2) the second highest p-value.
- If $p(2) < 2.5/2 = 1.25\%$ (one-sided), then stop and declare significance for $H_{(1)}$ and $H_{(2)}$. Otherwise, accept $H_{(2)}$, for the endpoint related to p(2), and go to p(1) the lowest p-value.
- If $p(1) < 2.5/3 = 0.833\%$ (one-sided), then stop and declare significance for $H_{(1)}$. Otherwise, accept $H_{(1)}$, for the endpoint related to p(1).

Regardless of the statistical significance declared according to the Hochberg procedure, all analysis will be included and presented in the statistical report.

As for the primary analysis, the primary comparison (which determines the success, i.e. statistical and clinical significance) for the secondary efficacy endpoints is between all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm) and all patients on the placebo arm from both parts of the trial.

As an additional analysis, all secondary efficacy analyses will, as for the primary, be repeated for:

- the selected arm only, i.e. comparing all patients on the selected arm (from part 1) from both parts of the trial (pooled), to all patients on the placebo arm from both parts of the trial.
- data from part 2 only, i.e. comparing the selected arm to placebo on data from part 2 only

All free-days endpoints will be reported to one decimal place.

9.3.1 Organ Dysfunction

9.3.1.1 Vasopressor-free Days up to Day 30

Vasopressor-free Days up to Day 30 will be defined and analyzed in a similar manner as the primary endpoint, with the van Elteren test stratified by need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min).

9.3.1.2 Mechanical Ventilator-free Days up to Day 30

Ventilator -free Days up to Day 30 will be defined and analyzed in a similar manner as the primary endpoint, with the van Elteren test stratified by need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min).

9.3.1.3 Duration of Septic Shock up to Day 30

Duration of septic shock is defined as the cumulative periods (>1 hour) from start of IMP until Day 30, on IMP or vasopressors. Vasopressor use due to anaesthesia / procedure-induced hypotension during - and up to three hours after - surgery / procedure (including bedside) is exempt from this rule

For patients withdrawn (in the survivors analysis) or dying (in the non-survivors analysis) while still in septic shock, the duration will be based on the data available up until the time of withdrawal or death.

Duration of septic shock will be analyzed separately for survivors, non-survivors (within the first 30 days) and overall, comparing treatment arms by a negative binomial model with time from onset of shock to start of treatment and norepinephrine/noradrenaline requirement at baseline (µg/kg/min) as covariates, and treatment and need for ventilation (Yes/No) as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented. As duration of septic shock is derived with two decimal points (with values in the range of 0.00, 0.01, ..., 30.00), duration of septic shock MAY be transformed (for model stability) to integer values (with values in the range of 0, 1, ..., 3000) by multiplying with 100. Treatment estimates will be back transformed

Some patients will get out of shock prior to Day 30 (and stay alive until Day 30), some will get out of shock and die later on (prior to Day 30), others will die or be withdrawn while still in shock (prior to Day 30), and the remaining few will not get out of shock prior to Day 30. This means that if mortality and withdrawal rates vary between treatment arms, the results of the analysis for the overall population will be influenced by the skewed mortality and withdrawal rates. Hence, for the overall population, the distribution of duration of shock (time to out of shock), will be presented graphically as competing risks between 'time to out of shock', 'withdrawn while in shock' and 'dying while in shock'. Further, Kaplan-Meier (sub)-graphs on 'time to death' and 'time to withdrawal' will be presented for those getting out of shock (for which some will die or be withdrawn later on, prior to Day 30). This is done in order to elucidate any skewness in mortality and withdrawal rates, influencing the results of the analysis.

Furthermore, duration of septic shock will be tabulated by treatment arm (including pooled active treatment arms).

In case the model assumptions does not hold, covariates will be dichotomised (in same manner as they have been dichotomised for other endpoint analyses) and duration of septic shock will be analysed using a stratified permutation test (Monte Carlo estimate of p-value). Treatment effects (and treatment difference) will be the raw (unadjusted/non-stratified) means. The confidence intervals for the individual treatment arms will be derived by bootstrapping 5000 samples (within treatment group) with same number of observations (within treatment group), and taking the 2.5 and 97.5% percentiles from the distribution of the 5000 means from the bootstrapped samples. The confidence interval for the treatment difference will be derived via significance testing (based on the stratified permutation test), i.e. the confidence interval will be constructed by including the 95% confidence region for all those values for which the significance test of the hypothesis that the true value is the given value is not rejected at a 5% significance level (e.g. if we test is treatment difference=1 and p-value >0.05 then we include 1 in the confidence interval, and so on). The seed for both the Monte Carlo derived p-value and the bootstrapped confidence intervals will (appropriately) be seed=133.

9.3.1.4 Duration of Mechanical Ventilation up to Day 30

Duration of mechanical ventilation is defined as the cumulative periods (>1 hour) from start of IMP until Day 30, on mechanical ventilation. Mechanical ventilation during - and up to three hours after - surgery / procedure is exempt from this rule.

For patients withdrawn or dying while still on mechanical ventilation, the duration will be based on the data available up until the time of withdrawal or death.

Duration of mechanical ventilation will be analyzed separately for survivors, non-survivors (within the first 30 days) and overall using a zero inflated negative binomial model with time from onset of shock to start of treatment, and norepinephrine/noradrenaline requirement at baseline (µg/kg/min) as covariates, and treatment and need for ventilation as factors. As duration of ventilation is derived with two decimal points (with values in the range of 0.00, 0.01, ..., 90.00), duration of ventilation will be transformed (for model stability) to integer values (with values in the range of 0, 1, ..., 9000) by multiplying with 100. Treatment estimates will be back transformed.

In case the model assumptions for duration of ventilation does not hold, the permutation test specified in Section 9.3.1.3 will be used including an analysis of the expected proportion of patients with 0 duration, and estimates of the duration for those with a positive duration.

9.3.1.5 Daily Overall and Individual Organ (Cardiovascular, Respiratory, Renal, Hepatic, Coagulation) Scores using the Modified Sequential Organ Failure Assessment (SOFA) Scores Until ICU Discharge

Last observation carried forward (LOCF) will be used for missing SOFA scores on Days 2-7. No LOCF for Day 1 (as previous value is baseline). Patients dying will be imputed with a worst possible outcome, i.e. a value of 4 for each individual SOFA score.

Daily overall (modified) and individual SOFA scores will be compared between treatment arms up until Day 7 using a repeated measures ANCOVA model with baseline SOFA score as covariate, treatment, time and treatment by time interaction as factors, baseline SOFA score by time interaction, and patient as the experimental unit. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented.

Furthermore, daily overall and individual SOFA scores will be tabulated by treatment arm (including pooled active treatment arms).

9.3.1.6 Incidence of New Organ Dysfunctions and New Organ Failures (Based on the SOFA score) up to Days 7 and 30

Incidence of new organ failures is defined as a change in any of the individual SOFA scores from (0,1,2) at baseline to (3,4) post baseline up until the end of the period (Day 7 or 30) (if the SOFA scores goes from (0,1,2) to (3,4) and back to (0,1,2) again within the period, that will still count as a new organ failure). If a patient dies within the period, he is considered to fail on all organs, and the number of new organ failures will be all organs except those already failed at baseline. Patients withdrawn within the period will be evaluated based on the data available at time of withdrawal.

Incidence of new organ dysfunction is defined as an increase ≥ 1 from baseline to post baseline up until the end of the period (e.g. going from 1 to 2) in any of the individual SOFA scores. Patients with an individual SOFA score of 4 at baseline can per default not have a new organ dysfunction. If a patient dies within the period, he is considered to have dysfunction on all organs, and the number of new organ dysfunctions will be all organs except those already having a score of 4 at baseline. Patients withdrawn within the period will be evaluated based on the data available at time of withdrawal.

As the SOFA score is only collected for patients still in the ICU, it is assumed that as soon as the patient leaves the ICU, the patient will not experience any new organ dysfunctions or organ failures. Unless of course the patient dies.

Incidence of at least one new organ failure will be analyzed for any new organ failure (across all organ systems) and by individual organ systems, and compared between treatment arms using a logistic regression model with age, modified SOFA score and norepinephrine/noradrenaline requirement at baseline (μg/kg/min) as covariates and gender and treatment arm as factors, presenting odds ratios with 95% confidence intervals.

Incidence of at least one new organ dysfunction will be analyzed for any new organ dysfunction (across all organ systems) and by individual organ systems, and will be analyzed as above for new organ failures.

The number of new organ failures and the number of new organ dysfunctions will be compared between treatment arms using a negative binomial model with age, modified SOFA score and norepinephrine/noradrenaline requirement at baseline (μg/kg/min) as covariates, and gender and treatment as factors. The estimated treatment difference (to placebo) with a 95% confidence interval will be presented. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method (see Appendix 4 for details).

Furthermore, incidence of any new organ failure and any new organ dysfunction, and the number of new organ failures and new organ dysfunctions will be tabulated by treatment arm (including pooled active treatment arms).

9.3.1.7 Renal Replacement Therapy (RRT)-free Days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)

RRT-free Days is defined as for the primary endpoint with free of treatment with any form of renal replacement therapy defined as continuous renal replacement therapy, intermittent hemodialysis or peritoneal dialysis.

RRT-free Days will be analyzed excluding patients on RRT for chronic renal failure at time of randomisation.

RRT-free Days up to Day 30 will be analyzed in a similar manner as the primary endpoint, with the van Elteren test stratified by need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), norepinephrine/noradrenaline requirement at baseline (< or ≥30 μg/min) and baseline creatinine (< or ≥150 μmol/L).

9.3.1.8 Incidence of RRT up to Day 30 (counting patients who die as on RRT and excluding patients on RRT for chronic renal failure at time of randomisation)

RRT is defined as any form of renal replacement therapy defined as continuous renal replacement therapy, intermittent hemodialysis or peritoneal dialysis. In order to ensure that any reduction in incidence of RRT is not caused by an increase in mortality, all patients dying within the 30-day period will be counted as on RRT. For patients withdrawn prior to Day 30, incidence of RRT will be based on the data available up until the time of withdrawal.

Incidence of RRT will be analysed by a logistic regression model with time from onset of shock to start of treatment, baseline creatinine and norepinephrine/noradrenaline requirement at baseline (µg/kg/min) as covariates, and treatment and need for ventilation as factors. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method (see Appendix 4 for details). Patients already on RRT for chronic renal failure at time of inclusion will be excluded from the analysis of incidence of RRT.

Non-inferiority will be claimed if the upper limit of the two-sided 95% CI of the adjusted difference in proportions is less than 20% of the estimated incidence of RRT in the placebo group. Superiority can be claimed (5) if the upper limit is less than 0.

I.e., let $\widehat{p_S}$ and $\widehat{p_P}$ be the estimated incidences of RRT in the combined selepressin groups and the placebo group respectively. Non-inferiority will then be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0.2 * \widehat{p_P}$$

and superiority will be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0$$

A forest plot including the non-inferiority tests for all analyses will be presented.

Furthermore, incidence of RRT will be tabulated by treatment arm (including pooled active treatment arms).

A subgroup analysis will be performed on patients without acute RRT at baseline.

9.3.1.9 Duration of RRT up to Day 90 (excluding patients on RRT for chronic renal failure at time of randomisation)

Duration of RRT is defined as the cumulative periods with RRT (continuous renal replacement therapy, intermittent hemodialysis or peritoneal dialysis) and will be analyzed excluding patients on RRT for chronic renal failure at time of randomisation.

For patients withdrawn or dying while still on RRT, the duration will be based on the data available up until the time of withdrawal or death.

Duration of RRT will be analyzed as for duration of ventilation (also by transforming to integers) in Section 9.3.1.4 with time from onset of shock to start of treatment, baseline creatinine and norepinephrine/noradrenaline requirement at baseline (µg/kg/min) as covariates, and treatment and need for ventilation as factors. The supportive competing risk and Kaplan-Meier graphs described in Section 9.3.1.4 will not be performed as it makes no sense since RRT durations are short and repetitive. It is more meaningful to just look at 90 day mortality and withdrawal rates to take eventual skewed mortality and withdrawal rates into account.

9.3.2 Morbidity/Mortality

9.3.2.1 Intensive Care Unit (ICU)-free Days up to Day 30

ICU -free Days up to Day 30 will be defined and analyzed in a similar manner as the primary endpoint, with the van Elteren test stratified by need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and baseline creatinine (< or ≥150 µmol/L).

A sensitivity analysis will be performed where the definition of time spent in the ICU will include first admission to emergency department, as some patients will be enrolled and have first IMP treatment in the emergency department.

9.3.2.2 ICU Length of Stay up to Day 30

ICU length of stay is defined as the cumulative periods spent in ICU from start of IMP to 30 days after.

For patients withdrawn or dying while still in ICU up to Day 30, the duration will be based on the data available up until the time of withdrawal or death.

ICU length of stay will be analyzed between treatment groups using the permutation test specified in Section 9.3.1.3 stratified by the combination of time from onset of shock to start of treatment (< or ≥6 hours), baseline creatinine (< or ≥150 µmol/L), norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and need for ventilation (Yes/No).

A sensitivity analysis will be performed where the definition of time spent in the ICU will include first admission to emergency department, as some patients will be enrolled and have first IMP treatment in the emergency department.

9.3.2.3 All-cause Mortality (Defined as the Fraction of Patients That Have Died, Regardless of Cause, by the end of Day 30, Day 90, and Day 180)

Mortality will be analysed by a logistic regression model with the individual SOFA scores and age as covariates and treatment arm as factor. The 95% confidence interval for the difference in proportions between treatment groups will be constructed using the delta method as for incidence of RRT (section 9.3.1.8). There will be no imputations for mortality.

Non-inferiority will be claimed if the upper limit of the two-sided 95% CI of the adjusted difference in proportions is less than 30% of the estimated incidence of mortality in the placebo group. Superiority can be claimed (5) if the upper limit is less than 0.

I.e., let $\widehat{p_S}$ and $\widehat{p_P}$ be the estimated incidences of mortality in the combined selepressin groups and the placebo group respectively. Non-inferiority will then be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0.3 * \widehat{p_P}$$

and superiority will be claimed if

$$\widehat{p_S} - \widehat{p_P} + 1.96 * \sqrt{var(\widehat{p_S} - \widehat{p_P})} < 0$$

Assuming observed mortality rates of 20-25% in the placebo group, a non-inferiority limit of 30% corresponds to a maximum observed mortality rate of 2-3% in the combined selepressin groups in order for selepressin to be non-inferior to placebo. A forest plot including the non-inferiority tests for all analyses will be presented.

Furthermore, mortality will be tabulated by treatment arm (including pooled active treatment arms), and the time to death presented graphically by a Kaplan-Meier plot.

9.3.3 Health-Related Quality of Life

9.3.3.1 Change in utility, based on EQ-5D-5L, up to Day 180

EQ-5D-5L will be analyzed by the index value, the overall QALY (Quality-Adjusted Life Years) at Day 30 and 180 (see Appendix 1 for details), and the VAS score.

The QALY scores will NOT be adjusted to e.g. a half yearly time scale at Day 180.

As the QALY is not defined for patients with all remaining values missing, and hence also not defined for those dead, the analyses will automatically only be analyzed for those surviving up until Day 30 and 180 respectively.

For patients with missing baseline index value, the QALY score will also be set to missing. For robustness, a sensitivity analyses will be performed, imputing the missing baseline scores with the overall mean of the baseline health index. Baseline is the timing prior to acute admission.

The QALY at Day 30 and 180 will be compared between treatment arms using an ANCOVA model with baseline health index as covariate, and treatment as factor. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

The index value and VAS scores will be analysed separately for survivors and non-survivors at Day 180 (since all non-survivors will have non-random missing values, and hence would artificially inflate the mean estimates if survivors and non-survivors were analysed together) and will be compared between treatment arms using a repeated measures ANCOVA model with baseline health index/VAS score as covariate, treatment, time and treatment by time interaction as factors, baseline index/VAS score by time interaction, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented for Day 30, 60, 90 and 180. There will be no imputations for missing values.

Furthermore, the QALY, index value and VAS scores will be tabulated treatment arm (including pooled active treatment arms), and the index value and VAS scores will be presented graphically.

9.3.4 Fluid Balance

9.3.4.1 Daily and Cumulative Fluid Balance Until ICU Discharge (for a Maximum of 7 Days)

Fluid overload is defined as fluid balance volume (L) as a percentage of baseline weight. E.g. if a patient weighs 90 kg at baseline and has a fluid balance of 9 liters, fluid overload is then 100% * 9L / 90kg = 10%.

Fluid balance (as a rate of time) and cumulative fluid balance (total volume) will be presented both unadjusted and adjusted for weight.

Baseline fluid balance (as a rate of time) and baseline fluid balance volume (mL) will be based on the time from onset of sepsis induced hypotension to start of IMP.

All analyses will be presented for 'all patients' and for 'patients in ICU throughout Day 0-7'.

Fluid balance, cumulative fluid balance, fluid overload and cumulative fluid overload will all be compared between treatment arms using a repeated measures ANCOVA model with baseline (baseline fluid balance volume (mL) or baseline fluid overload) as covariate, treatment, time and treatment by time interaction as factors, baseline score by time interaction, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

Fluid overload and balance will be based on a sectioning of the intervals between the actual sampling time points on baseline, Day 1, 2, etc., in order to be able to make 24 hour intervals from start of IMP.. For Days 1, 2, etc. sampling time points will be every 24 hours from start of IMP. Patients withdrawn or dead will be set to missing.

Endpoints (absolute values and change from baseline) will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

Furthermore, in order to assess all patients at Day 3 and 7 (regardless of mortality), the composite endpoint 'critical edema-free survival' will be derived at both Day 3 and 7.
A patient will be categorised as being critically edema-free and alive at Day 3 (or 7 respectively) if:

- fluid overload < 10% at Day 3, or
- patient is discharged from ICU and emergency department prior to Day 3 with a fluid overload < 10% at day of discharge (or previous day if discharged before fluid balance measurement), and patient is alive at Day 3.

The patient will be categorised as not being critically edema free or dead if:

- fluid overload ≥ 10% at Day 3, or

- patient is discharged from ICU and emergency department prior to Day 3 with a fluid overload ≥ 10% at day of discharge (or previous day if discharged before fluid balance measurement), or
- patient is dead on Day 3 (prior to fluid balance measurement)

Patients withdrawn at the given time point will be set to missing (less than 20 patients total in the trial are expected to be withdrawn prior to Day 7). The endpoints will be analysed using a logistic regression model with baseline fluid overload as a covariate and treatment arm as a factor, presenting odds ratios with 95% confidence intervals.

9.3.4.2 Daily and Cumulative Urinary Output Until ICU Discharge (for a Maximum of 7 Days)

Urinary output and cumulative urinary output (absolute values) will all be will be compared between treatment arms using a repeated measures ANCOVA model with baseline urinary output volume (mL) as covariate, treatment, time and treatment by time interaction as factors, baseline score by time interaction, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

Urinary output and cumulative urinary output will be presented both unadjusted and adjusted for weight.

All analyses will be presented for ‘all patients’ and for ‘patients in ICU throughout Day 0-7’.

Urinary output will be derived in the same manner as fluid balance.

Endpoints (absolute values and change from baseline) will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

9.4 Other Endpoints/Assessments

9.4.1 Hospital-free Days up to Day 90

Hospital stay is defined (from the eCRF) as “Other acute care hospital” or “Still in trial hospital”

Hospital-free Days up to Day 90 will be defined and analyzed in a similar manner as the primary endpoint, with the van Elteren test stratified by need for ventilation (Yes/No), time from onset of shock to start of treatment (< or ≥6 hours), and norepinephrine/noradrenaline requirement at baseline (< or ≥30 μg/min) and baseline creatinine (< or ≥150 μmol/L).

9.4.2 Hospital Length of Stay up to Day 90

Hospital length of stay is defined as the cumulative periods spent in hospital (“Other acute care hospital” or “Still in trial hospital”) from start of IMP to 90 days after.

For patients withdrawn or dying while still in hospital up to Day 90, the duration will be based on the data available up until the time of withdrawal or death.

Hospital length of stay will be analyzed between treatment groups using the permutation test specified in Section 9.3.1.3 stratified by the combination of time from onset of shock to start of treatment (< or ≥6 hours), baseline creatinine (< or ≥150 µmol/L), norepinephrine/noradrenaline requirement at baseline (< or ≥30 µg/min) and need for ventilation (Yes/No).

9.4.3 Patient Residence at Day 30, Day 60, Day 90, and Day 180

Patient location at Day 30, 60, 90 and 180 will be summarized by treatment arm (including pooled active treatment arms). Baseline is the timing prior to acute admission.

Shift tables will be presented at each time point to assess whether patients have returned to their location at enrollment. There will be no imputations of missing values.

9.4.4 Mean Arterial Pressure, Until ICU Discharge (for a Maximum of 7 Days)

Mean arterial pressure will be tabulated by treatment arm (including pooled active treatment arms) and presented graphically (at pre-specified time points, i.e. not when NE/NA infusion changes, and only until ICU discharge/day 7) for both MAP alone, and the difference from MAP to target MAP (which is 65, unless the investigator judges it to be otherwise). There will be no imputations of missing values.

MAP will be displayed both for MAP measurements taken while patients are in and out of septic shock, i.e. while patients are on and off IMP/vasopressors (as defined for the primary endpoint).

Difference to target MAP will only be displayed for MAP measurements taken while patients are in septic shock.

9.4.5 Norepinephrine/Noradrenaline and Other Vasopressor Doses

The cumulative dose of norepinephrine/noradrenaline administered (adjusted for baseline weight) will be derived as an area under the curve (AUC) of the actual norepinephrine doses and actual time points (although analysed at planned time points). Linear interpolation will be used to derive the AUC (taking into account that only patients enrolled before noon will have a measurement at 36 hours after IMP start). Patients dead or withdrawn will be set to missing. The cumulative doses (µg/kg) will be compared between treatment arms using a repeated measures ANCOVA model with baseline dose of norepinephrine/noradrenaline (µg/kg/min) as covariate, treatment, time and treatment by time interaction as factors, baseline dose by time interaction, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

If a patient has missing values and the patient is still in the trial (not dead or withdrawn) it will be assumed that the specific vasopressor was not given and a value of zero will be imputed, unless there is an interval in the timing log covering the exact time point (8 AM and 8 PM is the assumed time point for missing morning and evening collection time points). In that case LOCF will be used, but only within the time interval.

The mean dose and cumulative dose administered will be tabulated by treatment arm (including pooled active treatment arms), and presented graphically.

To assess the time needed to wean the patients off norepinephrine, the percentage change from baseline in norepinephrine dose (µg/kg/min) will be compared between treatment arms using a repeated measures ANCOVA model with baseline dose of norepinephrine/noradrenaline (µg/kg/min) as covariate, treatment, time and treatment by time interaction as factors, baseline dose by time interaction, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented at 1, 3, 6, 12 and 24 hours after start of IMP treatment.

The same analyses (adjusted for baseline dose of norepinephrine/noradrenaline (µg/kg/min)) will be performed for the following endpoints:

- Catecholamines (defined as the sum of doses of norepinephrine/noradrenaline, epinephrine/adrenaline, dopamine, and phenylephrine) (ug/kg)
- Catecholamines excluding norepinephrine/noradrenaline (ug/kg)
- Vasopressin (U/kg)

For the sum of catecholamine doses we define 100 µg dopamine, 1 µg epinephrine, and 2.2 µg phenylephrine all equivalent to 1 µg norepinephrine.

Also, the number of patients receiving terlipressin will be summarised.

9.4.6 Pulmonary Function (PaO2/FiO2) (in a subset of patients)

Baseline for PaO2/FiO2 will be the values obtained at the last assessment prior to the first dose of IMP. There will be no imputations of missing values.

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point by treatment arm (including pooled active treatment arms).

9.4.7 Arterial Blood Gases and Acid/Base Status (PaO2, PaCO2, pH, SaO2, Bicarbonate, Base Excess), Lactate and Oxygen Saturation in Vena Cava Superior (ScvO2)

Baseline for arterial blood gases, lactate and ScvO2 will be the values obtained at the last assessment prior to the first dose of IMP. There will be no imputations of missing values.

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point by treatment arm (including pooled active treatment arms).

9.4.8 Cytokines, ANG-1 and ANG-2 (in a Subset of 100-350 Patients)

Baseline values will be the values obtained at the last assessment prior to the first dose of IMP. There will be no imputations of missing values.

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point by treatment arm (including pooled active treatment arms).

These analyses will not be repeated for the selected arm only and data from part 2 only.

9.4.9 EVLW and PPI (in a Subset of 100-350 Patients)

Baseline for EVLW and PPI will be the values obtained at the last assessment prior to the first dose of IMP. There will be no imputations of missing values.

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point by treatment arm (including pooled active treatment arms).

These analyses will not be repeated for the selected arm only and data from part 2 only.

9.4.10 Cardiac Output (in a Subset of 100-350 Patients)

Baseline for cardiac output values will be the values obtained at the last assessment prior to the first dose of IMP. There will be no imputations of missing values.

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point by treatment arm (including pooled active treatment arms).

These analyses will not be repeated for the selected arm only and data from part 2 only.

9.4.11 Creatinine Clearance

Creatinine clearance is determined by estimated glomular filtration rate (using creatinine, age, and gender as per Cockcroft-Gault).

Cockcroft-Gault equation: creatinine clearance (mL/min) = ((140 - age in years) x weight (kg)) / creatinine (µmol/L) for women. For men, multiply result by 1.2.

Creatinine clearance will be analyzed up until day 3 as for fluid balance in Section 9.3.4.1, with baseline creatinine clearance as a covariate, treatment, time and treatment by time interaction as factors, and patient as the experimental unit.

10 Safety

10.1 General Considerations

Safety parameters will be evaluated for the safety analysis data set.

All safety summaries will be tabulated by treatment arm (including pooled active treatment arms).

10.2 Adverse Events

Adverse events (AEs) are classified according to the Medical Dictionary for Regulatory Activities (MedDRA). The version of MedDRA will be documented in the clinical report.

Written narratives will be issued for all serious AEs (including deaths) and AEs leading to withdrawal.

A pre-treatment adverse event is any untoward medical occurrence arising or observed between informed consent and administration of the IMP.

A treatment emergent adverse event is any adverse event occurring after the administration of the IMP and within the time of residual drug effect, or a pre-treatment adverse event or pre-existing medical condition that worsens in intensity after start of IMP and within the time of residual drug effect.

The time of residual drug effect is the estimated period of time after the end of the administration of the IMP, where the effect of the product is still considered to be present based on pharmacokinetic, pharmacodynamic or other substance characteristics. A generally accepted time for residual drug effect is 5 half-lives. The terminal half-life of the IMP is expected to be not more than 1.8 hours, and treatment-emergent AEs are defined as AEs occurring after the start of study drug infusion to within 12 hours after study drug infusion is stopped.

A post-treatment adverse event is any adverse event occurring after the residual drug effect period. Missing values will be treated as missing, except for causality, intensity, seriousness, and outcome of adverse events. A "worst case" approach will be used: if causality is missing, the adverse event will be regarded as related to the IMP; if the intensity of an adverse event is missing, the adverse event will be regarded as severe; if seriousness is missing the adverse event will be regarded as serious; if start date is missing or incomplete, worst case will be assumed and the AE will be regarded as treatment emergent (only if the incomplete start date is not compromised). If start date is completely missing, start date will be set as same day as start of treatment. If start date is incomplete, the date closest to start of treatment will be assumed, without compromising the incomplete data available on the start date; if outcome is missing and no date of outcome is present the outcome is regarded as 'not recovered'.

10.2.1 Overview of Adverse Events

AE overview summary tables will be prepared for treatment-emergent AEs and all AEs (treatment-emergent and non treatment-emergent) during the treatment period, including the number of patients reporting an AE, the percentage of patients (%) with an AE, and the number of events (E) reported, for the following categories:

- Adverse events
- Deaths
- Serious adverse events
- Adverse events leading to discontinuation of IMP
- Severe and life threatening adverse events
- Adverse drug reactions

10.2.2 Incidence of Adverse Events

Adverse events will be summarised in a Table by SOC and PT for MedDRA. The Table will display the total number of patients reporting an AE, the percentage of patients (%) with an AE, and the number of events (E) reported. AEs will be presented by system organ class (SOC) sorted alphabetically and preferred term (PT) sorted in decreasing frequency of occurrence.

For both treatment-emergent AEs and all AEs (treatment-emergent and non treatment-emergent) during the treatment period, summary tables will be prepared for:

- All adverse events
- Adverse events with an incidence ≥ 5% of patients in any treatment arm
- Non-serious adverse events with an incidence ≥ 5% of patients in any treatment arm
- Critical adverse events (see Appendix 2 for details)
- Adverse events by causality (related/unrelated)
- Adverse events leading to death
- Adverse events by intensity
- Serious adverse events
- Adverse events leading to discontinuation of IMP (related/unrelated)

Supporting data listings will be provided for:

- All adverse events sorted by centre and patient no.
- All adverse events sorted by MedDRA Preferred Term
- Serious adverse events
- Adverse events leading to death
- Adverse events leading to discontinuation of IMP (related/unrelated)
- Post treatment-emergent adverse events.

Furthermore, for both treatment-emergent AEs and all AEs (treatment-emergent and non treatment-emergent) during the treatment period, graphs showing the most frequent adverse events and the difference and 95% confidence interval between placebo and selepressin pooled will be presented for:

- All adverse events
- Adverse events with an incidence ≥ 5% of patients in any treatment arm
- Critical adverse events (see Appendix 2 for details)
- Adverse events leading to death
- Serious adverse events

10.3 Safety Laboratory Variables

Baseline for all laboratory analyses will be the values obtained at the last assessment prior to (or at) the first dose of the investigational medicinal product (IMP). End of treatment period will include the last post-baseline observation during the trial up until Day 30.

Laboratory variables will be grouped under "Haematology", "Clinical Chemistry" or "Coagulation".

10.3.1 Summary Statistics

Mean change and mean percentage (%) change from baseline at end of treatment period will be presented for each laboratory variable. In addition, descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each laboratory variable.

Furthermore, summary tables will be presented, displaying by time and laboratory parameter, the number of patients with a clinically significant result assessed as unanticipated.

Also, a summary table will be prepared for selected laboratory variables that display the number and percentage of patients in each treatment arm with X% increments (increase or decrease) from baseline at pre-specified timepoints and end of treatment period. The following categories for summary tables are defined:

- ≤ -3*X%: Values with more than 3*X% decrease from baseline
- > -3*X% - -2*X%: Values with 2-3*X% decrease from baseline
- > -2*X% - -1*X%: Values with 1-2*X% decrease from baseline
- > -1*X% - 0*X%: Values with 0-1*X% decrease from baseline
- < 0*X%- < 1*X%: Values with 0-1*X% increase from baseline

- 1*X%- < 2*X%: Values with 1-2*X% increase from baseline
- 2*X%- < 3*X%: Values with 2-3*X% increase from baseline
- ≥ 3*X%: Values with more than 3*X% increase from baseline

The following key laboratory variables will be summarised:

- Hemoglobin: 20% change
- WBC: 50% change
- Platelets: 50% change
- Creatinine: 20% change
- Sodium: 5% change
- Lactate: 50% change

10.3.2 Data Listings

Data listings will be prepared by centre, treatment arm, patient and time-point (including baseline) displaying all laboratory values for all patients.

10.4 Vital Signs (including CVP)

Baseline for all vital signs analyses will be the values obtained at the last assessment prior to the first dose of IMP.

10.4.1 Summary Statistics

Descriptive statistics, i.e., the number of patients with data, mean (standard deviation), median, interquartile range, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each vital signs variable.

Furthermore, summary tables will be presented, displaying by time and vital signs parameter, the number of patients with a clinically significant result assessed as unanticipated.

Also, a summary table will be prepared for each vital signs variable that display the number and percentage of patients in each treatment arm with X% increments (increase or decrease) from baseline. The following categories for summary tables are defined:

- ≤ -3*X%: Values with more than 3*X% decrease from baseline
- > -3*X% - -2*X%: Values with 2-3*X% decrease from baseline
- > -2*X% - -1*X%: Values with 1-2*X% decrease from baseline
- > -1*X% - 0*X%: Values with 0-1*X% decrease from baseline
- < 0*X%- < 1*X%: Values with 0-1*X% increase from baseline

- 1*X%- < 2*X%: Values with 1-2*X% increase from baseline
- 2*X%- < 3*X%: Values with 2-3*X% increase from baseline
- ≥ 3*X%: Values with more than 3*X% increase from baseline

The following % changes will be summarised:

- Heart rate: 50% change
- Blood pressure: 25% change

10.4.2 Data Listings

Data listings will be prepared by centre, treatment arm, patients and time-point (including baseline) displaying all vital signs values for all patients with an indication of abnormal values.

10.5 Episodes of Hypotension

Descriptive statistics of number of patients with episodes of hypotension and the total length of periods with hypotension will be summarized by treatment arm.

The total length of periods with hypotension will be summarized for both all patients, and patients having one or more episodes of hypotension.

11 Interim analyses

There will be no interim analyses with the potential to stop the trial early for treatment efficacy. However, once the “burn-in” period in Part 1 (first 200 treated patients) is completed, regular interim analyses will be conducted to improve the efficiency of dose selection and to allow early termination of the part or the trial for futility or for successful dose selection. The following steps will be considered at each interim analysis:

- When 200 patients are treated, the allocation probabilities for the active treatment arms are changed using response-adaptive randomisation (with placebo still 1/3). For the two-thirds of patients assigned to the active arms, the probability that a given active arm is assigned to a patient is proportional to the probability that that arm is the arm with the largest expected number of P&VFD
- Potentially stopping the trial for futility during Part 1. This occurs if no active arm has better than a 5% predictive probability of a significant result in Part 2 if it were to start immediately. This decision can occur at any interim during Part 1 after 200 evaluable patients.
- Potentially ending Part 1 and selecting an active treatment arm to continue to Part 2. This decision can occur at any interim analysis between 300 evaluable and 800 treated patients, and it occurs if some arm has a predictive probability of a successful trial of at least 90% before 800 treated patients, and the threshold drops to 25% for the final Part 1 interim analysis at 800 treated patients. The selected arm is the arm with the largest posterior predictive probability of trial success. This will generally be the best-performing active arm, but if multiple arms are performing equally well, it will be the arm with the lowest dosing level. If Part 1 ends after N patients, then Part 2 will consist of up to 1800 - N evaluable patients.
- If the trial is not stopped for futility or proceeding to Part 2 and active treatment Arm 4 has not yet been approved for assignment of patients, the decision can be made to open up Arm 4. Arm 4 is only opened between 200 evaluable and 600 treated patients and if there is at least a 50% probability that Arm 3 has a higher expected P&VFD than Arm 2 and if data from the lower dosing levels do not suggest any significant safety signals.
- If Part 1 reaches its maximum of 800 treated patients and no arm has a predictive probability of Part 2 success of more than 25%, the trial stops with an inconclusive result and will be interpreted as a standalone Phase 2b trial.

During Part 2, interim analyses will be conducted regularly (until 1600 patients have been treated) to allow early termination of the trial for futility. This occurs if the predictive probability of an overall significant result is less than 5%. In addition, if the predictive probability of observing a more than 2% higher mortality in the active arms compared to placebo is greater than 90% then the trial will stop for futility.

12 Deviations from protocol analysis

See change log.

13 References

1 The EuroQol Group (1990). EuroQol-a new facility for the measurement of health-related quality of life. *Health Policy* 16(3): 199-208

2 EuroQoL Home page: http://www.euroqol.org/home.html

3 Mehta, C.R. and Pocock, S.J. (2011) Adaptive increase in sample size when interim results are promising: A practical guide with examples. *Statistics in Medicine*, 30, 3267-3284.

4 Hochberg (1988). A sharper Bonferroni procedure for multiple tests of significance. *Biometrika* 75(4):800-802

5 EMEA. (2000). Points to consider on switching between superiority and non-inferiority. CPMP/EWP/482/99.

14 Tables, Listings and Figures

The document with tables, figures and listings (TLF) shells will be presented in a separate document.

Appendix 1 EQ-5D-5L Quality Adjusted Life Year (QALY)

We calculate Quality Adjusted Life Year (QALY) (1), (2) at Day 30 and 180 in three steps:

(1) A unique EQ-5D-5L health state is defined by combining 1 level from each of the 5 dimensions of EQ-5D-5L. Each health state is referred to in terms of a 5 digit code. For example, state 12345 indicates no problems with mobility, slight problems with washing or dressing, moderate problems with doing usual activities, severe pain or discomfort and extreme anxiety or depression.

(2) Convert each EQ-5D-5L health state into a single EQ-5D-5L index value. The index values are country specific and we will use the value sets for US and apply these values to all patients in this trial.

(3) QALY for a patient is then defined to be the area under the curve (AUC) for a Time (with unit of Year) versus index values. AUC will be calculated by the linear trapezoidal method. See below for a schematic presentation where the y-axis is the index value with y_0, y_1 and y_2, etc. represent the index values at baseline, Day 30, 60, 90 and 180. The x-axis is Time (in Years) and the t_0 is the start of treatment period, i.e. baseline, and t_1 and t_2 are time of the actual Day 30 and 60, respectively, and so on.

If the index value at baseline is missing then we set QALYs at Day 30 and 180 to missing. No LOCF imputation will be used. However, linear interpolation will be used between data points with missing data in between (e.g. t_0 to t_2, if t_1 is missing).

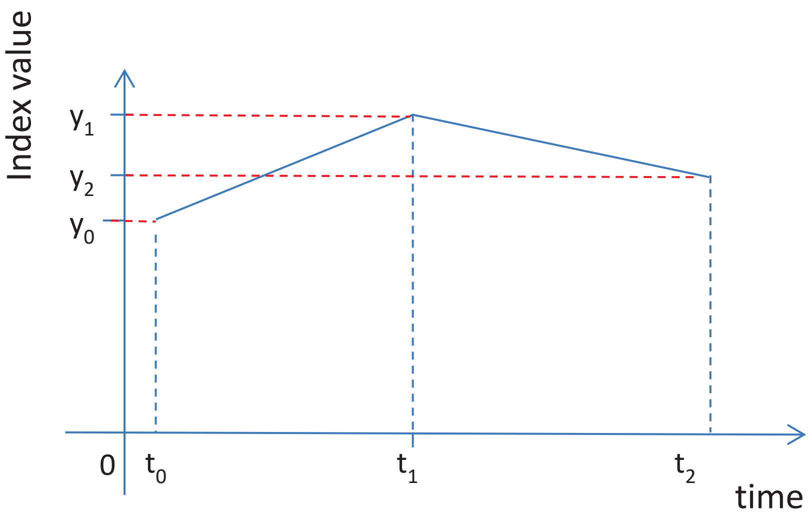


Figure 5: EQ-5D-5L QALY Calculation

Appendix 2 Critical Adverse Events and Classification of Adverse Events Based on Changes in Safety Laboratory Variables

Table 1: Critical Adverse Events

Critical adverse event	MedDRA terms or SMQ used for search
Potential hypersensitivity reactions	Anaphylactic reaction (SMQ) (Narrow Scope) Angioedema (SMQ) (Narrow Scope)
Myocardial infarction / ischemia	Ischaemic heart disease (SMQ) (Broad scope)
Episodes of atrial fibrilation and other cardiac arrythmias	Cardiac Arrhythmias (SMQ) (Broad scope)
Cerebrovascular accident	Haemorrhagic central nervous system vascular conditions (SMQ) (Broad Scope) Ischaemic central nervous system vascular conditions (SMQ) (Broad Scope) Conditions associated with central nervous system haemorrhages and cerebrovascular accidents (SMQ) (Broad scope)
Digital ischemia	Peripheral ischaemia Pallor Peripheral coldness
Renal failure	Acute Renal failure (SMQ)
Mesenteric ischemia	Ischaemic colitis (SMQ) (Narrow scope)
Hepato-biliary adverse events	Drug related hepatic disorders - severe events only (SMQ) (Broad Scope)

Appendix 3 Estimation of Treatment Effects for P&VFDs

Let Y be the number of P&VFDs for both survivors and non-survivors, let p be the probability of surviving, and say that for survivors (30-Y) has a negative binomial distribution (with for Y=0 consuming all values ≥30).

$$P(Y=y)=\begin{cases}(1-p)+p*\sum_{k\geq 30}\frac{\Gamma(d^{-1}+\mathrm{k})}{\Gamma(d^{-1})k!}\left(\frac{\mu*d}{1+\mu*d}\right)^{k}\left(\frac{1}{1+\mu*d}\right)^{d^{-1}} & for\ y=0\\ p*\frac{\Gamma(d^{-1}+30-\mathrm{y})}{\Gamma(d^{-1})(30-y)!}\left(\frac{\mu*d}{1+\mu*d}\right)^{(30-y)}\left(\frac{1}{1+\mu*d}\right)^{d^{-1}} & for\ y>0\end{cases}$$

In the trial it is expected we have a mean of around 5-8 days on vasopressors and mechanical ventilation for those surviving.

Therefore, the probability of getting zero P&VFDs for those surviving

$\sum_{k\geq 30}\frac{\Gamma(d^{-1}+\mathrm{k})}{\Gamma(d^{-1})k!}\left(\frac{\mu*d}{1+\mu*d}\right)^{k}\left(\frac{1}{1+\mu*d}\right)^{d^{-1}}$ is negligible (and can be omitted from the model),

For d=1/16

μ	5	6	7	8	9	10	11
Var =μ+ μ^2d	6.56	8.25	10.06	12	14.06	16.25	18.56
P(K≥30)	$1.4*10^{-9}$	$4.2*10^{-8}$	$6*10^{-7}$	$5*10^{-6}$	$2.8*10^{-5}$	$1.2*10^{-4}$	$4*10^{-4}$

μ	12	13	14	15
Var =μ+ μ^2d	21	23.56	26.25	26.09
P(K≥30)	$1.1*10^{-3}$	$2.5*10^{-3}$	$5.4*10^{-3}$	$1*10^{-2}$

For $Y_1,...,Y_N$ we then get two likelihood functions,

$$L_1=\prod_{y_i=0}(1-p)$$

and,

$$L_2=\prod_{y_i>0}p*\frac{\Gamma(d^{-1}+30-\mathrm{y})}{\Gamma(d^{-1})(30-y)!}\left(\frac{\mu*d}{1+\mu*d}\right)^{(30-y)}\left(\frac{1}{1+\mu*d}\right)^{d^{-1}}$$

Assuming that p is modeled as $\frac{e^{\beta_1}}{1+e^{\beta_1}}$, and μ as e^{β_2}, the two log-likelihoods then become,

$$l_1=\sum_{y_i=0}\ln\left(\frac{1}{1+e^{\beta_1}}\right)=\sum_{y_i=0}-\ln(1+e^{\beta_1})$$

and,

$$l_2 = \sum_{y_i>0} \beta_1 - \ln(1 + e^{\beta_1}) + \ln\big(\Gamma(d^{-1} + 30 - y_i)\big) - \ln\big(\Gamma(d^{-1})\big) - \ln\big((30 - y_i)!\big) + (30 - y_i)\big(\beta_2 + \ln\big(1 + e^{\beta_2}\big)\big) - d^{-1} * \ln\big(1 + e^{\beta_2}\big)$$

Maximizing the full log-likelihood is thus maximizing $l_1 + l_2$.

And as can be clearly seen, the maximization of l_1 and l_2 with regards to β_1 does not depend on β_2 and vice-versa. Hence, β_1 and β_2 can be estimated separately from two independent models, i.e. a logistic regression for the probability of surviving, and a poisson regression (or negative binomial to allow for overdispersion) for the distribution of days on vasopressors and mechanical ventilation for those surviving.

In practice, and with the model adjusted for A, B and C, this means that the probability of surviving will be estimated from a logistic regression adjusted for A, B and C and treatment group. From each treatment group we can do an LSMEANS and get an ‘overall’ β_1 along with the standard error of β_1.

For a given treatment group the mean probability of surviving is given as:

$$\frac{e^{\beta_1}}{1 + e^{\beta_1}}$$

The distribution of days on vasopressors and mechanical ventilation (30- PVFD’s) for those surviving will be estimated from a negative binomial regression (poisson with a potential overdispersion), also adjusted for A, B and C and treatment group. From each treatment group we can do an LSMEANS and get an ‘overall’ β_2 along with the standard error of β_2.

For a given treatment group the mean PVFD’s for survivors is given as:

$$(30 - e^{\beta_2})$$

The mean PVFD’s for all subjects (survivors and non-survivors) is then given as:

$$\frac{e^{\beta_1}}{1 + e^{\beta_1}} * (30 - e^{\beta_2})$$

Let

$$f(\beta_1, \beta_2) = \frac{e^{\beta_1}}{1 + e^{\beta_1}} * (30 - e^{\beta_2})$$

Using the delta method we can calculate the variance of f(β_1, β_2):

$$f'(\beta_1,\beta_2) = \left((30 - e^{\beta_2}) * \frac{e^{\beta_1}}{(1+e^{\beta_1})^2}, \frac{e^{\beta_1}}{(1+e^{\beta_1})} * (-e^{\beta_2}) \right)$$

Hence,

$$var[f(\beta_1,\beta_2)] = f'(\beta_1,\beta_2) \begin{pmatrix} \sigma^2_{\beta_1} & 0 \\ 0 & \sigma^2_{\beta_2} \end{pmatrix} f'(\beta_1,\beta_2)^t$$

$$= \left(\sigma^2_{\beta_1} * (30 - e^{\beta_2}) * \frac{e^{\beta_1}}{(1+e^{\beta_1})^2} \quad \sigma^2_{\beta_2} * \frac{e^{\beta_1}}{(1+e^{\beta_1})} * (-e^{\beta_2}) \right) \begin{pmatrix} (30 - e^{\beta_2}) * \frac{e^{\beta_1}}{(1+e^{\beta_1})^2} \\ \frac{e^{\beta_1}}{(1+e^{\beta_1})} * (-e^{\beta_2}) \end{pmatrix}$$

$$= \sigma^2_{\beta_1} * (30 - e^{\beta_2})^2 * \left(\frac{e^{\beta_1}}{(1+e^{\beta_1})^2} \right)^2 + \sigma^2_{\beta_2} * \left(\frac{e^{\beta_1}}{(1+e^{\beta_1})} \right)^2 * e^{2\beta_2}$$

The 95% CI for the mean PVFD's then become

$$\frac{e^{\widehat{\beta_1}}}{1+e^{\widehat{\beta_1}}} * (30 - e^{\widehat{\beta_2}}) \pm 1.96 * \sqrt{var[f(\widehat{\beta_1},\widehat{\beta_2})]}$$

Let s indicate selepressin and p indicate placebo, the estimated treatment difference then becomes,

$$d = \frac{e^{\widehat{\beta_{1s}}}}{1+e^{\widehat{\beta_{1s}}}} * (30 - e^{\widehat{\beta_{2s}}}) - \frac{e^{\widehat{\beta_{1p}}}}{1+e^{\widehat{\beta_{1p}}}} * (30 - e^{\widehat{\beta_{2p}}})$$

With 95% CI,

$$\frac{e^{\widehat{\beta_{1s}}}}{1+e^{\widehat{\beta_{1s}}}} * (30 - e^{\widehat{\beta_{2s}}}) - \frac{e^{\widehat{\beta_{1p}}}}{1+e^{\widehat{\beta_{1p}}}} * (30 - e^{\widehat{\beta_{2p}}}) \pm 1.96 * \sqrt{var[d]}$$

Where $var[d]$ is derived again using the delta method as,

$$var[d(\beta_{1s},\beta_{1p},\beta_{2s},\beta_{2p})] = d' \begin{pmatrix} \sigma^2_{\beta_{1s}} & \sigma^2_{\beta_{1s}\beta_{1p}} & 0 & 0 \\ \sigma^2_{\beta_{1s}\beta_{1p}} & \sigma^2_{\beta_{1p}} & 0 & 0 \\ 0 & 0 & \sigma^2_{\beta_{2s}} & \sigma^2_{\beta_{2s}\beta_{2p}} \\ 0 & 0 & \sigma^2_{\beta_{2s}\beta_{2p}} & \sigma^2_{\beta_{2s}} \end{pmatrix} d'^t$$

With,

$$d'(\beta_{1s}, \beta_{1p}, \beta_{2s}, \beta_{2p})^t = \begin{pmatrix} \left(30 - e^{\beta_{2s}}\right) * \dfrac{e^{\beta_{1s}}}{(1 + e^{\beta_{1s}})^2} \\ -\left(30 - e^{\beta_{2p}}\right) * \dfrac{e^{\beta_{1p}}}{(1 + e^{\beta_{1p}})^2} \\ -\dfrac{e^{\beta_{1s}}}{(1 + e^{\beta_{1s}})} * e^{\beta_{2s}} \\ \dfrac{e^{\beta_{1p}}}{\left(1 + e^{\beta_{1p}}\right)} * e^{\beta_{2p}} \end{pmatrix}$$

In the above reasoning, it is assumed that P&VFDs can only take on integer values between 0 and 30. In practice, P&VFDs will be analysed with one decimal (with values in the range of 0, 0.1, ..., 29.9, 30.0) and hence the distribution of days on vasopressors and mechanical ventilation for those surviving will be estimated from a negative binomial regression model scaled up to 300 (transforming the P&VFDs from 0.0, 0.1, ..., 29.9, 30.0 to 0, 1, ..., 299, 300), and later scaled back to one decimal.

Let X = 10*Y (scaling Y from 0 to 300) and δ_1 and δ_2 the corresponding estimated parameters from the logistic regression and negative binomial regression models based on the modeling of X.

Transforming back to Y, the 95% CI for the mean P&VFDs (E(Y)) then become

$$\frac{e^{\widehat{\delta_1}}}{1 + e^{\widehat{\delta_1}}} * \left(30 - \frac{e^{\widehat{\delta_2}}}{10}\right) \pm 1.96 * \sqrt{\frac{var[f(\widehat{\delta_1}, \widehat{\delta_2})]}{100}}$$

Again with s indicating selepressin and p indicating placebo, the estimated treatment difference then becomes,

$$\frac{e^{\widehat{\delta_{1s}}}}{1+e^{\widehat{\delta_{1s}}}} * \left(30 - \frac{e^{\widehat{\delta_{2s}}}}{10}\right) - \frac{e^{\widehat{\delta_{1p}}}}{1+e^{\widehat{\delta_{1p}}}} * (30 - \frac{e^{\widehat{\delta_{2p}}}}{10})$$

With 95% CI,

$$\frac{e^{\widehat{\delta_{1s}}}}{1 + e^{\widehat{\delta_{1s}}}} * \left(30 - \frac{e^{\widehat{\delta_{2s}}}}{10}\right) - \frac{e^{\widehat{\delta_{1p}}}}{1 + e^{\widehat{\delta_{1p}}}} * \left(30 - \frac{e^{\widehat{\delta_{2p}}}}{10}\right) \pm 1.96 * \sqrt{\frac{var\left[f\left(\widehat{\delta_{1s}}, \widehat{\delta_{2s}}, \widehat{\delta_{1p}}, \widehat{\delta_{2p}}\right)\right]}{100}}$$

Appendix 4 Estimation of Treatment Effects for Difference of Proportions in Incidence of RRT, and Difference in Number of New Organ Failures and Number of New Organ Dysfunctions

Estimation of Treatment Effects for Difference of Proportions in Incidence of RRT

Let Y be the incidence of RRT (and/or death).

Y can then be modelled using a logistic regression, adjusted for various factors and covariates.

In practice, and with the model adjusted for A, B and C, this means that the probability of RRT incidence will be estimated from a logistic regression (PROC GENMOD preferred over PROC LOGISTIC as estimates can be subtracted by ODS output) adjusted for A, B and C and treatment group. From each treatment group we can do an LSMEANS and get an 'overall'(adjusted) β along with the standard error of β and the covariance between the estimates from each treatment group. I.e. we get β_s, β_p, σ_{β_s},σ_{β_p}, and $\sigma^2_{\beta_s\beta_p}$.

For a given treatment group the mean probability of RRT incidence is given as:

$$\frac{e^{\beta}}{1+e^{\beta}}$$

The difference in proportions of RRT incidence is then given as:

$$\frac{e^{\beta_s}}{1+e^{\beta_s}} - \frac{e^{\beta_p}}{1+e^{\beta_p}}$$

Using the delta method we can calculate the variance of f(β_s, β_p):

$$f'(\beta_s,\beta_p) = \left(\frac{e^{\beta_s}}{(1+e^{\beta_s})^2}, -\frac{e^{\beta_p}}{\left(1+e^{\beta_p}\right)^2}\right)$$

Hence,

$$var[f(\beta_s,\beta_p)] = f'(\beta_s,\beta_p)\begin{pmatrix}\sigma^2_{\beta_s} & \sigma^2_{\beta_s\beta_p}\\ \sigma^2_{\beta_s\beta_p} & \sigma^2_{\beta_p}\end{pmatrix} f'(\beta_s,\beta_p)^t$$

$$= \left(\frac{\sigma^2_{\beta_s} * e^{\beta_s}}{(1+e^{\beta_s})^2} - \frac{\sigma^2_{\beta_s\beta_p} * e^{\beta_p}}{\left(1+e^{\beta_p}\right)^2}, \frac{-\sigma^2_{\beta_p} * e^{\beta_p}}{\left(1+e^{\beta_p}\right)^2} + \frac{\sigma^2_{\beta_s\beta_p} * e^{\beta_s}}{(1+e^{\beta_s})^2}\right)\begin{pmatrix}\frac{e^{\beta_s}}{(1+e^{\beta_s})^2}\\ \frac{-e^{\beta_p}}{(1+e^{\beta_p})^2}\end{pmatrix}$$

$$= \sigma^2_{\beta_s} * \left(\frac{e^{\beta_s}}{(1+e^{\beta_s})^2}\right)^2 + \sigma^2_{\beta_p} * \left(\frac{e^{\beta_p}}{\left(1+e^{\beta_p}\right)^2}\right)^2 - 2 * \sigma^2_{\beta_s\beta_p} * \frac{e^{\beta_s}}{(1+e^{\beta_s})^2} * \frac{e^{\beta_p}}{\left(1+e^{\beta_p}\right)^2}$$

The 95% CI for the difference in proportions of RRT incidence then become

$$\frac{e^{\widehat{\beta_s}}}{1+e^{\widehat{\beta_s}}} - \frac{e^{\widehat{\beta_p}}}{1+e^{\widehat{\beta_p}}} \pm 1.96 * \sqrt{var\left[f\left(\widehat{\beta_s}, \widehat{\beta_p}\right)\right]}$$

Estimation of Treatment Effects for Difference in Number of New Organ Failures and Number of New Organ Dysfunctions

Let Y be the number of new organ dysfunctions (or organ failures).

Y can then be modelled using a negative binomial model (poisson model with possible overdispersion), adjusted for various factors and covariates.

In practice, and with the model adjusted for A, B and C, this means that the number of new organ failures will be estimated from a negative binomial model (PROC GENMOD) adjusted for A, B and C and treatment group. From each treatment group we can do an LSMEANS and get an 'overall'(adjusted) β along with the standard error of β and the covariance between the estimates from each treatment group. I.e. we get β_s, β_p, σ_{β_s},σ_{β_p}, and $\sigma^2_{\beta_s\beta_p}$.

For a given treatment group the mean number of new organ dysfunctions is given as:

$$e^{\beta}$$

The difference in the number of new organ dysfunctions is then given as:

$$e^{\beta_s} - e^{\beta_p}$$

Using the delta method we can calculate the variance of f(β_s, β_p):

$$f'\left(\beta_s, \beta_p\right) = \left(e^{\beta_s}, -e^{\beta_p}\right)$$

Hence,

$$var\left[f\left(\beta_s, \beta_p\right)\right] = f'\left(\beta_s, \beta_p\right)\begin{pmatrix} \sigma^2_{\beta_s} & \sigma^2_{\beta_s\beta_p} \\ \sigma^2_{\beta_s\beta_p} & \sigma^2_{\beta_p} \end{pmatrix} f'\left(\beta_s, \beta_p\right)^t$$

$$= \left(\sigma^2_{\beta_s} * e^{\beta_s} - \sigma^2_{\beta_s\beta_p} * e^{\beta_p}, \quad \sigma^2_{\beta_s\beta_p} * e^{\beta_s} - \sigma^2_{\beta_p} * e^{\beta_p}\right)\begin{pmatrix} e^{\beta_s} \\ -e^{\beta_p} \end{pmatrix}$$

$$= \sigma^2_{\beta_s} * e^{2\beta_s} + \sigma^2_{\beta_p} * e^{2\beta_p} - 2 * \sigma^2_{\beta_s\beta_p} * e^{\beta_s} * e^{\beta_p}$$

The 95% CI for the difference in the number of new organ dysfunctions then become

$$e^{\widehat{\beta_s}} - e^{\widehat{\beta_p}} \pm 1.96 * \sqrt{var\left[f\left(\widehat{\beta_s}, \widehat{\beta_p}\right)\right]}$$

Appendix 5 Statistical Model for Adaptive Design Decisions

An Example Trial

This section presents the results of an example trial in order to illustrate how the design behaves. The selected scenario includes defining the probability distributions that describe how patients given placebo behave, and also defining the effects of all four selepressin dosing regimens. In the example trial we work with P&VFD data recorded to the nearest day instead of the nearest tenth of a day.

Figure 6 shows the data available at the first interim analysis, and the results of the statistical analyses performed using those data that are then used to make decisions. The leftmost of the three plots shows the raw data for each of the five arms, with mortality data in the legend and P&VFD data for survivors in the barplots. Red represents the placebo arm, and the shades of blue and black represent the active arms, with darker colors indicating larger maximum doses. The curves added to the plot are naïve density estimates that are not related to the statistical models used in the trial, scaled so that they have the same maximum. Active arm 2 has observed the highest mortality rate, while active arms 1 and 3 have seen tentative improvements compared to placebo. Active arm 3 has had the more surviving patients with small numbers of P&VFDs than the other arms. Active arm 4 is not yet allowed to accept patients. The middle plot displays estimates of mean P&VFDs (with fatalities scored as zero P&VFDs), together with 95% uncertainty intervals. The placebo arm is estimated to be the least effective, and uncertainties are smallest for the intermediate doses. The rightmost plot displays predictive probabilities and updated allocation probabilities. The circles show the allocation probabilities for the next 30 days: the probability for placebo remains at 1/3 for the duration of Part 1, and active arm 3 is assigned the lowest probability for any active arm. The rightmost plot also features squares showing the predictive probability that this trial would be successful if Part 1 were to terminate and Part 2 were to begin with each of the four arms as the chosen arm (It is too early to choose an arm: this can only happen when final data have been observed for at least 300 patients). If all four active arms had predictive probabilities less than 0.05 the trial would terminate for futility. Also, if the predictive probability were at least 90% that the final data set would show an observed increase in mortality of greater than 2%, regardless of which of the four active arms were selected, the trial would stop for futility for that reason, but these predictive probabilities are not shown in the figures.

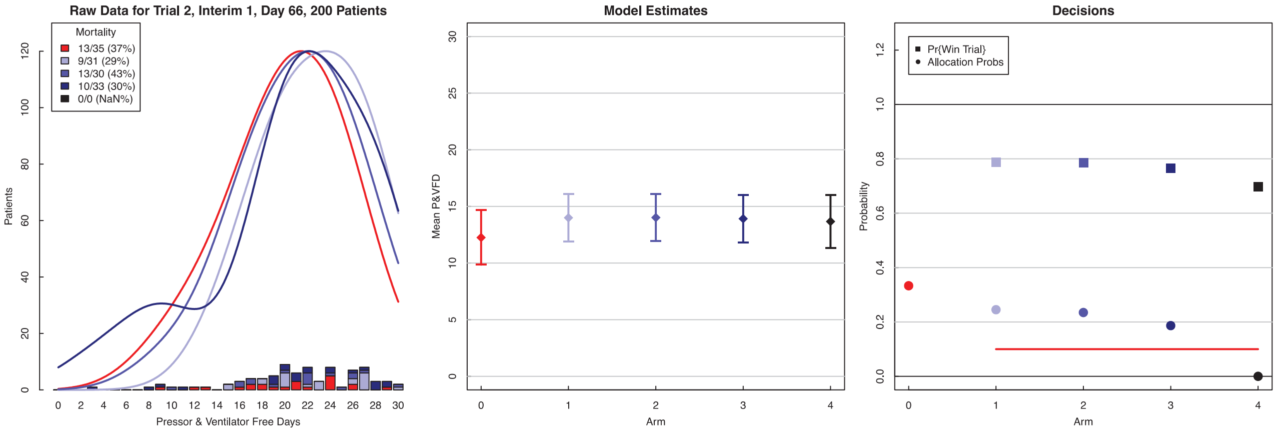


Figure 6: Data and interim analysis results at time of first interim analysis in an example trial

Moving ahead to the second interim analysis shown in Figure 7, when we have observed final data for 199 patients. Mortality has remained high for active arm 2. For survivors, the frequency of small numbers of P&VFDs is lowest for the placebo arm, but all three active arms have seen more patients with very large numbers of P&VFDs than has placebo. According to the rightmost plot, predictive probabilities for trial success are around 40% for all active arms. For the next 30 days, arm 3 will receive the highest allocation probability among the active arms, and since (not shown) the probability is at least 50% that arm 3's expected P&VFDs is higher than for arm 2, arm 4 becomes eligible for patient allocation to explore whether the apparent increasing trend in efficacy continues.

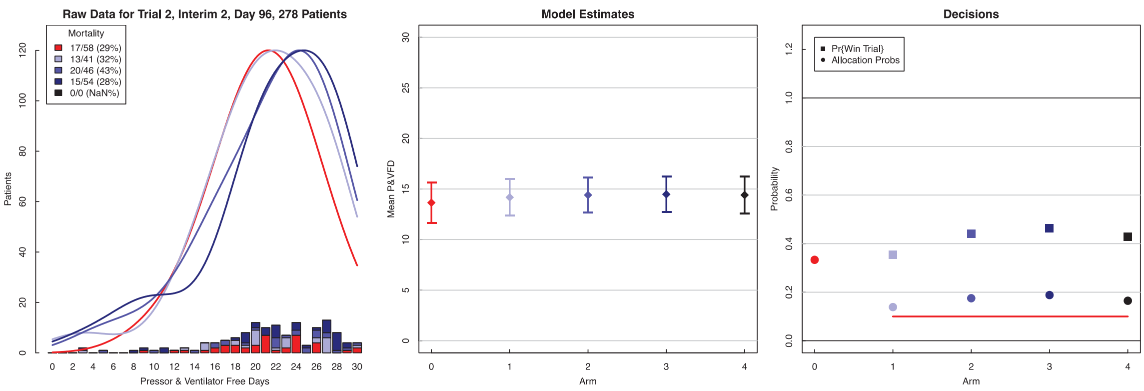


Figure 7: Data and analysis results for second interim analysis in example trial.

We skip ahead to the fourth interim analysis, shown in Figure 8, which is the first interim analysis after final data are available for at least 300 patients, so this is the first opportunity to choose a dose and move on to Part 2. The first data from arm 4 have come in, and arms 1, 3, and 4 are all achieving mortality rates no worse than placebo. The placebo arm continues to have the smallest probability of large numbers of P&VFDs among survivors. Since the predictive probability of trial success is largest for arm 3 and its value exceeds 90% (shown by the light green line), the trial elects to terminate Part 1 and proceed to Part 2 with arm 3. The allocation probabilities change to 50% each for placebo and active arm 3. Unless the trial stops earlier for futility, Part 2 will consist of 1430 patients since 370 are in the trial currently. The decision to select arm 3 occurs with only 11 patients allocated to arm 4, and with arm 4 looking promising thus far, but arm 3 has a high enough probability of a successful trial that the trial advances to Part 2.

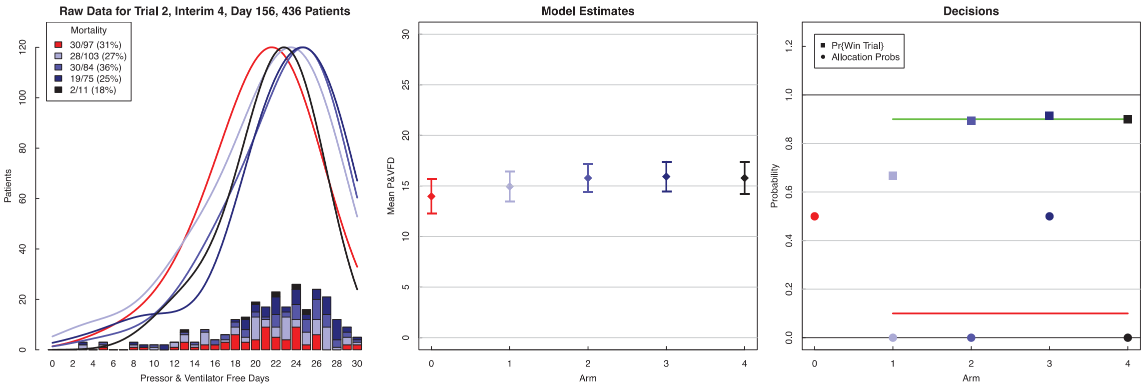


Figure 8: Data and analysis results for fourth interim analysis in example trial

During this interim analysis, Part 1 terminates and the trial proceeds to Part 2 with active arm #3.

The first interim analysis of Part 2 takes place after 63 more patients are allocated to arm 3 or placebo. Final data continue to come in for the other active arms as well. Since data are relatively favourable for the placebo arm in this month, the predictive probability of a successful trial at 1800 patients drops to about 0.80, which is much larger than the 5% futility boundary for Part 2 of the trial.

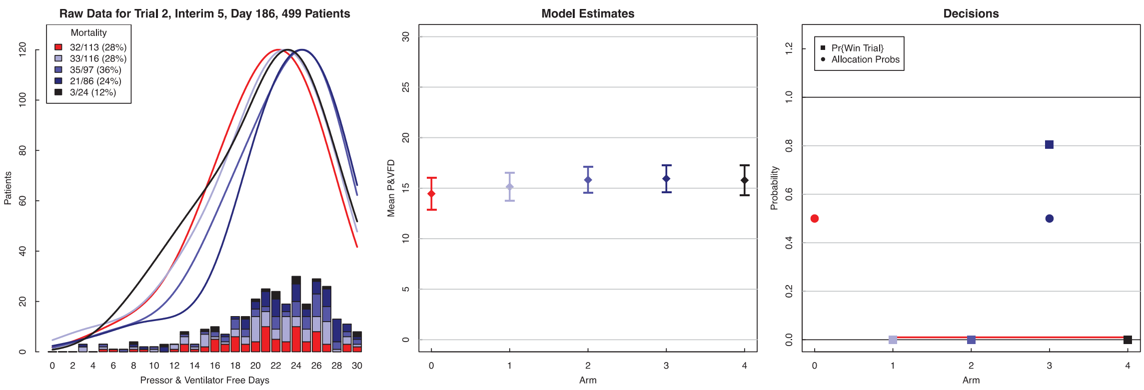


Figure 9: First interim analysis during Part 2

Interim analyses continue every 30 days (frequency chosen for this example), with the only available decision being whether or not to stop for futility. The predictive probability of trial success never approaches the 5% value that would trigger a futility stop. The final data at the end of the trial are shown in Figure 9. Observed mortality was 2% lower for active arm 3 than for placebo, and the final analysis will be based on a smaller difference than this due to the inclusion of the data from other active arms. The simulation assumed true mortalities and expected P&VFD distributions for survivors are shown in the middle plot: the doses increase in effectiveness at both preventing mortality and increasing P&VFDs for survivors, with arms 3 and 4 each reducing mortality by 1.5% and increasing expected P&VFDs by 1.5 days for survivors. The rightmost plot shows the final p-value for the Wilcoxon test: the p-value for the comparison between patients given placebo and patients given any active arm is lower than 0.025, so this is a successful trial.

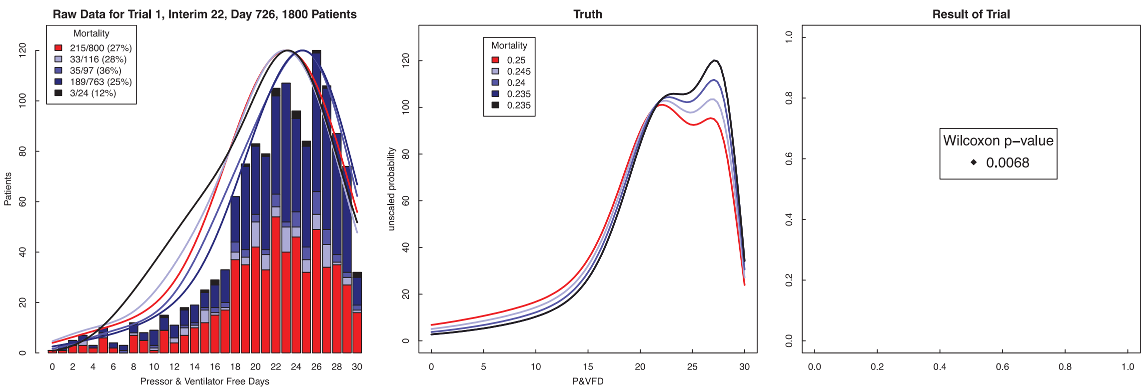


Figure 10: Results of final analysis. The trial is successful, with a p-value of 0.0068.

Statistical Model for Adaptive Design Decisions

A patient's outcome can be either death or a number of vasopressor and mechanical ventilator-free tenths of days between 0 and 300. Label d_i as the dose regimen assigned to patient i, and denote by s_i the stratum to which the patient belongs ($s_i \in \{0,1,\ldots,7\}$). First, define the distribution of these potential outcomes for patients treated with placebo ($d_i = 0$). If the i'th patient dies, write $X_i = 1$, otherwise $X_i = 0$. So,

$$\Pr\{X_i = 1 |\, d_i = 0, s_i = 0\} = \Delta.$$

Intuitive parameterization of the P&VFD model

To motivate the strategy for modeling the distribution of the number of P&VFD given placebo and survival, we first define a version of the model that is more intuitive but less computationally convenient and is asymmetric with respect to the strata. The version actually recommended is defined below in the section "*Symmetric, computational parameterization of the P&VFD model* ". The distribution can be modeled nonparametrically:

$$\Pr\{Y_i = k | d_i = 0, s_i = 0, X_i = 0\} = \pi_k, \text{ with } \sum_{j=0}^{300} \pi_j = 1.$$

Note that patients who die are modelled separately from patients who survive, but nevertheless accumulate zero P&VFD, although these patients are handled together in the final analysis. We model the treatment effect for a given dose d with two parts: the effect on mortality ϕ_d and the effect on P&VFD given survival, θ_d. The differences between the strata are modeled similarly to the effects of the different doses, with a stratum effect on mortality denoted by ψ_s and a stratum effect on P&VFD given survival denoted by ω_s. By definition $\psi_0 = \omega_0 = 0$. We model the effect on mortality, ϕ_d, on the log-odds scale:

$$\Pr\{X_i = 1 | d_i = d, s_i = s\} = \frac{\Delta}{\{\Delta + (1 - \Delta)\exp(\phi_d + \psi_s)\}},$$

where we have defined the effect so that larger values of ϕ_d or ψ_s are beneficial (decrease mortality). We model the effects of the dose arm and the stratum on the number of P&VFDs for survivors using an exponential family whose sufficient statistic is the number of P&VFD:

$$\Pr\{Y_i = k | d_i = d, s_i = s, X_i = 0\} = \frac{\pi_k e^{k(\theta_d + \omega_s)}}{\sum_{j=0}^{300} \pi_j e^{j(\theta_d + \omega_s)}}.$$

In particular, given a dose arm, a sample of P&VFD, and a probability vector π, the maximum likelihood estimators of θ_d and ω_s set the expected values of P&VFD equal to their sample means.

In the final analysis, however, patients who died are treated in the same way as patients who survived but who had 0 P&VFD.

For the simpler statistical model with no consideration of stratum effects, the ψ_s and ω_s are omitted.

Symmetric, computational parameterization of the P&VFD model

The version of the statistical model we will actually use is as follows. There are still parameters $\pi_0, \ldots, \pi_{300}$, but they do not correspond directly to any stratum and do not have a clear interpretation of their own. Instead of assuming that $\psi_0 = \omega_0 = 0$, we use another choice of identifiability assumptions: $\sum_{s=1}^{8} \psi_s = 0$ and $\sum_{s=1}^{8} \omega_s = 0$. Now we have

$$\Pr\{Y_i = k | d_i = d, s_i = s, X_i = 0\} = \frac{\pi_k e^{k(\theta_d + \omega_s)}}{\sum_{j=0}^{300} \pi_j e^{j(\theta_d + \omega_s)}}$$

for all d and s, where we have defined the placebo parameter $\theta_0 = 0$. This parameterization facilitates the definition of prior distributions for the ψ_s and ω_s, and ensures that the results of the analysis do not depend on which stratum is identified with $s = 0$, etc. We assume hierarchical models on the stratum parameters, with $\psi_s \sim N(0, \tau_\psi^2)$ and $\omega_s \sim N(0, \tau_\omega^2)$ (conditionally on observing the identifiability assumptions), and with $\tau_\psi \sim$Uniform(0,1) and $\tau_\omega \sim$Uniform(0,1).

Inverted-U dose-response models for the dose effects ϕ and θ.

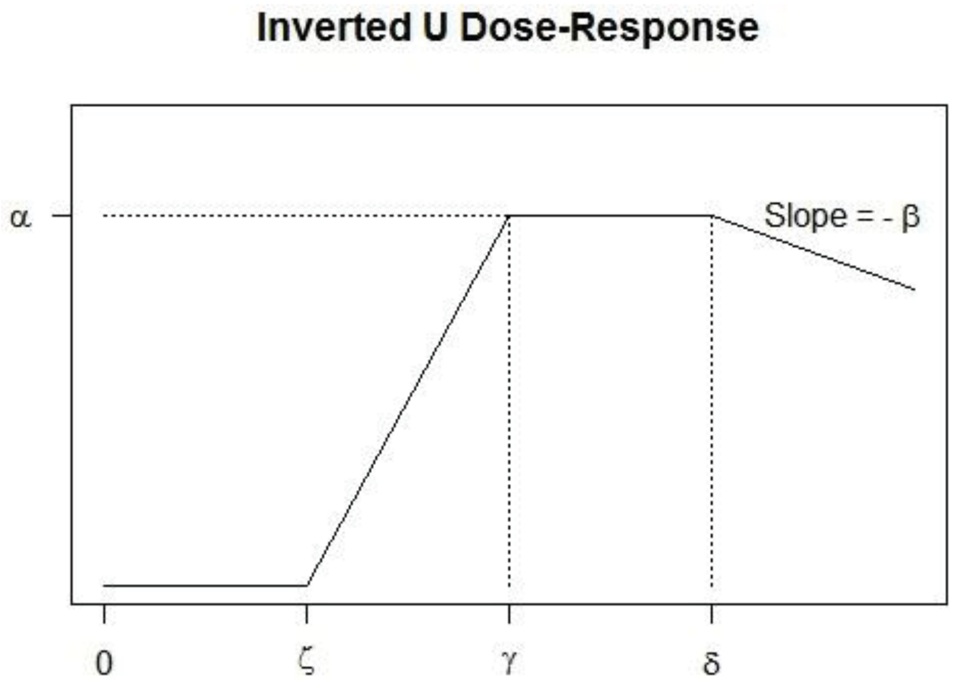


Figure 11: Inverted-U dose-response model
The x-axis represents dose regimen (which is interpreted as maximum dose in the selepressin trial), and the y-axis represents the treatment effect. The model parameters ζ, γ, δ define theoretical special doses (e.g. γ is the smallest dose that gives maximal effect), while α denotes the maximum achievable treatment effect.

An inverted-U dose-response model is used for the effects on both mortality and P&VFD. This is a flexible family that allows for the possibilities that small doses have no effect at all (if those doses are smaller than ζ), and that large doses can start to become less effective than smaller doses (if those doses are larger than δ). Most importantly for this trial, the model allows all doses to be equally effective, since, for a given patient, any assigned maximum dose could be titrated to a similar delivered treatment. In the parameterization, shown in Figure 10, γ is the smallest dose that delivers maximal effect, δ is the largest dose that delivers maximal effect, ζ is the largest dose that delivers no effect, β is the rate at which performance degrades beyond dose δ, and α is the size of the largest effect ($\alpha \leq 0$ is also allowed and there is a prior distribution for α which is symmetric about zero. To maintain an inverted U shape, β is constrained to be positive). Care must be taken to avoid important identifiability problems: in particular, it is insisted that the interval (γ, δ) contains at least one dose for which data can potentially be obtained, otherwise the largest treatment effect is not uniquely defined. However, γ can be smaller than any active dose, in which case the data contain no information about ζ, and δ can be larger than any active dose, in which case the data contain no information about β, but neither of these nonidentifiabilities are serious problems since they do not impact the ability to predict future data.

In the case where the candidate doses are 1, 2, 3, and 4, a prior distribution is assumed in which

$$\delta - \gamma \sim \text{Uniform}(1{,}5),$$

$$\gamma|\delta \sim \text{Uniform}(0, \delta - 1), \text{ and}$$

$$\zeta|\gamma \sim \text{Uniform}(0, \gamma).$$

It is assumed that $\phi_d = IU(d|\alpha_1, \zeta_1, \gamma_1, \delta_1, \beta_1)$ and $\theta_d = IU(d|\alpha_2, \zeta_2, \gamma_2, \delta_2, \beta_2)$ for $d = 1{,}2{,}3{,}4$, where the inverted-U function is defined as

$$IU(d|\alpha, \zeta, \gamma, \delta, \beta) = \alpha\{(1 - \left[1 - \frac{(d-\zeta)_+}{(\gamma-\zeta)}\right]_+\Big)_+ - \beta(d - \delta)_+\}, \text{ where } x_+ = \max(x, 0).$$

There is no assumed relationship between the θ parameters and the ϕ parameters.

For a specific example, suppose $\alpha_1 = 0.2, \zeta_1 = 1.5, \gamma_1 = 2.5,$ and $\delta_1 = 4$ (so that the value of β_1 is irrelevant for doses 1, 2, 3, and 4). Then $\phi_1 = 0, \phi_2 = 0.1, \phi_3 = \phi_4 = 0.2$.

To complete the specification of the prior distribution, it is assumed that

$$\Delta \sim Beta\left(\frac{1}{3}, \frac{1}{3}\right), (\pi_0, \pi_1, \ldots, \pi_{300}) \sim \text{Dirichlet}\left(\frac{1}{30}, \frac{1}{30}, \ldots, \frac{1}{30}\right),$$

$$\alpha_1 \sim \text{Uniform}(-0.35{,}0.35),$$

$$\alpha_2 \sim \text{Uniform}(-0.01334{,}0.01334),$$

$$\beta_1 \sim \text{Uniform}(0{,}10), \text{ and}$$

$$\beta_2 \sim \text{Uniform}(0{,}1).$$

The limits for the α parameters are selected to be large but not completely absurd; e.g. $\alpha_1 = 0.35$ corresponds to a 5% benefit to a 20% mortality rate. Given P&VFD data, one can use a variable-at-a-time Metropolis-Hastings algorithm to sample from the posterior distribution of the unknown parameters $(\Delta, \pi_0, \pi_1, \ldots, \pi_{300}, \alpha_1, \zeta_1, \gamma_1, \delta_1, \beta_1, \alpha_2, \zeta_2, \gamma_2, \delta_2, \beta_2)$. These samples can be used to estimate the predictive probability of a successful Part 2, the probability that each doses maximizes the expected number of P&VFDs, and the probability that dose regimen 3 provides a larger value of expected P&VFDs than does dose regimen 2.

The scale of α_2 depends on how P&VFDs are measured. Here they are recorded as integer numbers of tenths. If integer days are used instead, the prior range should be expanded by a factor of ten. The Dirichlet prior exponents we use also depend on how P&VFDs are measured: with integer days, we use exponents of 1/3 instead of 1/30. In either case the prior distribution corresponds to approximately ten equivalent observations.

Response-Adaptive Randomisation Probabilities

Beginning with the first interim analysis, allocation probabilities for the selepressin arms are adjusted based on the posterior distribution (the allocation probability for placebo remains fixed at 1/3 throughout Part 1). The allocation probability for active arm j is proportional to the posterior probability that arm j is one of the arms that maximizes the expected number of P&VFD (where the mortality probabilities are included in the calculations). Note that the inverted-U models allow for multiple arms to have exactly the same expected number of P&VFD.

Posterior Predictive Probability Calculations

Computations of the predictive probability of a successful Part 2 are critical to the design. These computations proceed by drawing samples from the posterior distribution of the unknown parameters. We first discuss the predictive probability calculation for the simplified case in which there are no strata and the final analysis is a Wilcoxon test; this is the case that applies in the operating characteristics simulations presented in this report. For a given posterior sample, we use a normal approximation to the predictive distribution of the Wilcoxon statistic that will be obtained from the currently available data for placebo and the pooled active arms. The calculation is tedious but straightforward, based on writing the Wilcoxon statistic in terms of

$$\sum_{i=1}^{I}\sum_{j=1}^{J} sign(Y_{1i} - Y_{2j}),$$

where $Y_{11}, \dots, Y_{1I}$ are the P&VFDs for sample 1 (i.e. the active arm) and $Y_{21}, \dots, Y_{2J}$ are the P&VFDs for sample 2 (i.e. the placebo arm). For a given set of P&VFD probabilities for the two arms, one can calculate the expected value and variance of the Wilcoxon statistic, and use this to calculate the probability of a significant result (the variance depends on the number of ties, and we plug in the expected value of the tie component based on a Poisson approximation). The process is then repeated for more posterior samples, and the results are averaged to give the overall estimate of the predictive probability of a successful trial. The predictive probability calculation is performed for each active arm being the one that proceeds to Part 2. The calculation uses the same (pooled) data set for each arm, but each arm has a different posterior distribution of treatment effect and hence a different predictive distribution of future data.

The approximate formula for predictive probability of a successful Wilcoxon test is faster computationally than simulating many final data sets and calculating whether each one attains success, so it plays a key role in simulating the trial to estimate operating characteristics. When time permits, such as when the design is being executed, however, the direct Monte Carlo simulation should also be performed.

The extension to the case in which there are eight strata and in which the final analysis is a van Elteren test, is straightforward but adds another layer of complexity. For the purpose of this calculation, we introduce a Dirichlet-multinomial model for the stratum probabilities: the eight stratum probabilities begin with a Dirichlet prior distribution with parameters equal to 1/3 and then are updated with the stratum counts observed in the trial. This stratum probability model operates independently of the remainder of the statistical modeling. Suppose that we are interested in calculating the predictive probability of success assuming that half the remaining patients are allocated to the placebo and the other half are allocated to active arm 1. For a given posterior sample, we draw a sample from the Dirichlet posterior distribution of the stratum, and then draw multinomial samples for the stratum counts for the future patients allocated to placebo, and separately to the active arm. We then loop over the strata and either

- using the posterior sample, simulate numbers of deaths and P&VFDs for survivors for each arm and each stratum and then evaluate the Wilcoxon test statistic, its theoretical null hypothesis mean, and its theoretical null hypothesis variance (which depends on the numbers of ties in the data set), or
- based on the posterior sample and the current numbers of deaths and P&VFD counts in each arm and the current stratum, calculate the predictive mean and variance of the Wilcoxon statistic, its theoretical null hypothesis mean, and an approximation to the expected value of the theoretical null hypothesis variance. The current data for all the active arms are aggregated together.

Denoting the Wilcoxon test statistic for the sth stratum by T_s, the simulated final number of patients in stratum s by N_s, and the null hypothesis expected value and variance by $E(T_s|H_0)$ and $Var(T_s|H_0)$ respectively, the van Elteren test statistic is

$$T = \frac{\sum_{s=1}^{8}\{T_s - E(T_s|H_0)\}/(N_s+1)}{\{\sum_{s=1}^{8} Var(T_s|H_0)/(N_s+1)^2\}^{1/2}}$$

For the Monte Carlo estimate of predictive probability, evaluate this statistic for every simulated final data set and compute the fraction of final data sets for which this statistic exceeded the 97.5th Gaussian percentile. To use the approximate formula, denote the predictive mean of T_s by $E(T_s|D,i)$, the predictive variance by $Var(T_s|D,i)$, and the estimated null hypothesis variance by $E\{Var(T_s|H_0)|D,i)\}$; D denotes the current data and i denotes that we are using the ith posterior sample. The approximate predictive probability for posterior sample i is then computed using Gaussian tail probabilities based on the expected value and variance of T given by

$$E(T|i) = \frac{\sum_{s=1}^{8}\{E(T_s|D,i) - E(T_s|H_0)\}/(N_s+1)}{\{\sum_{s=1}^{8} E\{Var(T_s|H_0)|D,i)\}/(N_s+1)^2\}^{1/2}}$$

$$Var(T|i) = \frac{\sum_{s=1}^{8}\{Var(T_s|D,i)\}/\,(N_s+1)^2}{\sum_{s=1}^{8} E\{Var(T_s|H_0)|D,i)\}/(N_s+1)^2}$$

Similar but simpler calculations apply for estimating the predictive probability that the final data set will have a mortality rate among the patients assigned to an active arm that is at least 2% higher than among the placebo patients.

Control of Type I Error

This design achieves control of Type I error through analytical means. While the trial can stop early for futility, a successful trial can only be achieved at a total of 1800 patients. At this time, a single test statistic is calculated, and it compares two populations that are defined before the trial begins, namely patients allocated to placebo compared to patients that are allocated to any active arm. In particular, no patients are excluded from the final analysis for any reason related to their outcomes (in contrast, if the final analysis compared placebo to the best performing of the active arms, that would inflate Type I error).

This argument demonstrates that Type I error is controlled even for the modification of the design in which early stopping for futility is disabled (See Appendix 6 for a formal proof of Type I error control). The potential for early stopping for futility, including the 25% predictive probability requirement at 800 patients, further reduces Type I error probability below the nominal value.

Appendix 6 Formal Proof of Type I Error Control

The following assumptions are made in the statistical discussions of type I error.

1. The primary analysis is based on a Wilcoxon-Mann-Whitney test (Van Elteren's Test) on the P&VFDs. The normal approximation to the sampling distribution of the test statistic is assumed for type I error discussions (as it would in a fixed trial).
2. The primary analysis combines all the active arms together for the final analysis. Under the null hypothesis, all arms (placebo and active) have the same mean and therefore the active arms can be combined in to a single arm.
3. The final analysis is based on 1800 patients.
4. The only "adaptive" aspect of the trial is the time in which randomisation switches from 2:1 (active to placebo) to (1:1).
   a. It is assumed that if the randomisation was 2:1 for the entire 1800 patients that type I error is controlled.
   b. It is assumed that if at a fixed point in time (deterministic) randomisation went from 2:1 to 1:1 that type I error is controlled.

We demonstrate the control of type I error by first considering a one-sample problem. We use the one-sample result to then extend to the two-sample problem. The proof focuses on the notion that when the data are positive in the first part of the trial it triggers a shift to a randomisation that increases the *effective* sample size of the trial. As demonstrated in Mehta and Pocock (2011) (3) this controls type I error when the data that triggers the shift are appropriately positive.

The hypothesis test is

$$H_o: \mu = 0$$

$$H_1: \mu > 0$$

Assume iid normally distributed random variables are observed with mean μ and known variance of 1. At the interim time point a random variable, based on n_0 observations, $Z_0 = n_0\bar{X}_0$ is observed. After this time point two different random variables (with entirely different observations, X) could be observed: $Z_1 = n_1\bar{X}_1$ or $Z_2 = n_2\bar{X}_2$. We use the notation $N(\mu,\sigma^2)$ for a normal distribution with mean μ and variance σ.^{2}

So, under the null hypotheses,

$$Z_o \sim N(0, n_0)$$
$$Z_1 \sim N(0, n_1)$$

and,

$$Z_2 \sim N(0, n_2).$$

The adaptive design specifies that if the data are unfavorable we will observe Z_1, and if the data are favorable we will observe Z_2. Therefore, for some value *b*,

1. If the data are unfavorable ($Z_0 < b$) we observe a second random variable $Z_1 \sim N(0, n_1)$. The trial is declared a success at the end of the second stage if

$$Z_0 + Z_1 > a\sqrt{n_0 + n_1}$$

where

$$a = \Phi^{-1}(1 - p).$$

If $b=\infty$ then it is deterministic (select Z_1) and the probability of success is p (the type 1 error of a fixed design, under the null).

2. If the data are favorable ($Z_0 > b$) we observe the random variable $Z_2 \sim N(0, n_2)$. We assume that the sample size for Z_2 is $n_2 = rn_1$. The parameter *r* is a flexible parameter for increase ($r>1$) or decrease ($r<1$) in the sample size in the second part of the trial, so r>1 implies $n_2 > n_1$. The trial is declared a success at the end of the second stage if

$$Z_0 + Z_2 > a\sqrt{n_0 + rn_1}$$

The critical value has been set so that if $b=-\infty$ (again deterministic to select Z_2) then the probability of success is p (type I error under a fixed design).

The value of *b* then determines the type I error of the adaptive design. The probability of a type I error of the adaptive design, for *f* the pdf of Z_0, is then

$$\int_{-\infty}^{b} f(z)\,\Phi\left(\frac{z-a\sqrt{n_0+n_1}}{\sqrt{n_1}}\right)dz + \int_{b}^{\infty} f(z)\,\Phi\left(\frac{z-a\sqrt{n_0+rn_1}}{\sqrt{rn_1}}\right)dz \qquad (1)$$

If $r=1$ there is no change in the trial and the above (1) is equal to *p*. In this trial $r > 1$. If the expression in (1) is nonincreasing in *r* then this demonstrates that the type I error is not inflated above *p* in this design. A sufficient condition is if $\frac{z-a\sqrt{n_0+rn_1}}{\sqrt{rn_1}}$ is decreasing in *r*. The derivative of this is

$$\frac{d}{dr}\left(\frac{z-a\sqrt{n_0+rn_1}}{\sqrt{rn_1}}\right) = -\frac{n_1}{2(rn_1)^{\frac{3}{2}}}\left(z - \frac{an_0}{\sqrt{n_0+rn_1}}\right)$$

which is negative in the second integral ($z > b$) if

$$b > \frac{an_0}{\sqrt{n_0+rn_1}}. \qquad (2)$$

Therefore, the type I error of the design is guaranteed if expression (2) holds. It is potentially possible to find a smaller *b* that guarantees a reduction in type I error, but that is not required here (see Mehta and Pocock (2011) for further discussion of this reduction). To interpret the constraint on *b*, note that the MLE of μ based on Z_0 is $\widehat{\mu_0} = \frac{Z_0}{n_0}$, and that the MLE of μ based on Z_0 and Z_2 is $\widehat{\mu_{0+2}} = \frac{Z_0+Z_2}{n_0+n_2}$. To elect to observe Z_2 we require

$$\widehat{\mu_0} = \frac{Z_0}{n_0} \geq \frac{b}{n_0} > \frac{a}{\sqrt{n_0+rn_1}},$$

and to show significance at the end of the trial we require

$$\widehat{\mu_{0+2}} = \frac{Z_0+Z_2}{n_0+n_2} > \frac{a\sqrt{n_0+rn_1}}{n_0+rn_1} = \frac{a}{\sqrt{n_0+rn_1}}.$$

In other words, if we are to elect to observe the larger sample size with Z_2, the observed data at the interim must achieve what would need to be observed at the conclusion of the trial in order to be a successful trial (meeting the definition of 50% conditional power). This interpretation is convenient for discussing the Ferring decision rules.

Two-Sample Extension

The condition above is expressed as a single-sample case. We demonstrate the extension to the two-dimensional case. In the single-sample case the test-statistic based on unit-variance normal $X_1, \ldots, X_M$ is

$$\sum_{m=1}^{M} X_m = M\bar{X}_m,$$

which has variance M. For the two-sample case ($X_1, \ldots, X_M$ and $Y_1, \ldots, Y_N$) the analogous statistic is $\frac{MN}{M+N}(\bar{X}_M - \bar{Y}_N)$, which has variance $\frac{MN}{M+N}$ (functions like the sample size). The joint distribution of $\frac{M_k N_k}{M_k+N_k}(\bar{X}_{M_k} - \bar{Y}_{N_k})$ for k=1,2,… where the sample sizes M_k and N_k are nondecreasing in k, is the distribution of Brownian motion with drift E(X_1) –E(Y_1) evaluated at the times $\frac{M_k N_k}{M_k+N_k}$: k=1,2,…, which is exactly the same as the one-sample case.

In the Ferring trial since for fixed *M*+*N* the effective sample size $\frac{MN}{M+N}$ is maximized when *M*=*N*, an earlier shift to 1:1 randomisation is synonymous with an *increase* in sample size.

Decision to "Increase Sample Size"

In the Ferring trial the decision to shift to 1:1 and thus increase the sample size is not based on a conditional power or a point estimate, but rather is based on Bayesian predictive probability. The design will shift to 1:1 randomisation early if the predictive probability is greater than 90% (typically a 50% predictive probability is consistent with observed data at the interim larger than what would need to be seen at the final analysis). This can be demonstrated explicitly in the

Gaussian case with prior distribution centered at no treatment effect. For the special case of a flat, improper prior and unit variances, we have that the predictive probability of success

$$\Pr\left\{\bar{X}_{M_0+M_2} - \bar{Y}_{N_0+N_2} > a\sqrt{\frac{1}{M_0+M_2} + \frac{1}{N_0+N_2}} \,|\bar{X}_{M_0}, \bar{Y}_{N_0}\right\}$$

is equal to

$$\Phi\left(\frac{\bar{X}_{M_0} - \bar{Y}_{N_0} - a\sqrt{\frac{1}{M_0+M_2} + \frac{1}{N_0+N_2}}}{\sqrt{\frac{1}{M_2} + \frac{1}{N_2} - \frac{1}{M_0+M_2} - \frac{1}{N_0+N_2}}}\right).$$

If the predictive probability is greater than 50% then the numerator is positive and the point estimate at the interim is larger than what is needed for success at the final analysis. If the prior is not improper, but is conjugate normal with mean zero, then the observed effect at the interim must be even larger for a 50% predictive probability of success. In the Ferring trial the prior is more complicated -- given the joint model over multiple active arms, but each of these arms has prior mean the same as the placebo.

Hence the condition of 90% predictive probability to shift to the 1:1 randomisation provides a conservative type I error for the final analysis.

All simulations have reinforced the conservative nature of the final analysis, accounting for the Wilcoxon final analysis.

Post-Simulation P-value

Next to the p-value based on the asymptotic normality of the Van Elteren test, post-simulation bootstrap p-values will be provided as sensitivity analyses.
To this end, the primary outcome for each patient in the trial, regardless of the treatment arm (restricted to the evaluable patients) will be placed in a vector and used to simulate complete trials and bootstrapped test-statistics.

The following procedure will be used:

1. The adaptive trial will be simulated exactly as designed in the protocol
2. The following assumptions will be used for the simulated trial:
    a. The day of enrollment during the actual trial, for each patient that was evaluable will be noted. These days will be used deterministically for the simulated trials.
    b. Additionally the strata in which that actual patient belonged will be recorded and used deterministically in the trial.
3. The randomization rules will follow the adaptive algorithm and design.
4. All, patients, from any arm, will be simulated with replacement from all patients in the actual trial with the same stratification membership.
5. The final test-statistic (Van Elteren's) will be calculated for each simulated trial.

6. This process will be repeated 100,000 times. The probability of a test statistic more extreme than the observed test-statistic is the empirical estimate of the p-value

A second simulation will run identically, but there will be no futility stopping in the simulated trial.

Appendix 7 Randomisation Plan

Part 1: Burn-In:

During burn-in randomisation will be conducted using blocked randomisation. At each site separate lists will be conducted for each of the following 8 strata:

Strata	Mechanical Ventilation	Creatinine	norepinephrine/ noradrenaline
1	Yes	<150 µmol/L	<30 µg/min
2	Yes	<150 µmol/L	≥30 µg/min
3	Yes	≥150 µmol/L	<30 µg/min
4	Yes	≥150 µmol/L	≥30 µg/min
5	No	<150 µmol/L	<30 µg/min
6	No	<150 µmol/L	≥30 µg/min
7	No	≥150 µmol/L	<30 µg/min
8	No	≥150 µmol/L	≥30 µg/min

Each blocked list will be generated using random block sizes of 9 and 18 with ratios of 3:2:2:2:0 for the placebo and active treatment arms 1, 2, 3, and 4, respectively.

Part 1: Adaptive Randomisation:

After burn-in randomisation will be conducted using a mixture of blocking and response adaptive randomisation (RAR) with stratification weighting.

A blocking system will be created for placebo and active arms within each of the 8 strata. Using random block sizes of 3 and 6, lists will be made with ratios of 1:2 for placebo to active. These lists will be created for each strata.

When an “active” slot is pulled a “coin flip” approach will be taken in order to select which active dose is selected. These probabilities will change monthly. These RAR probabilities will adjust for the 3 strata factors. The balancing approach for the RAR is described in Section “Balance Weighting.”

Part 2:

In Part 2 of the trial there will be only one target active arm and placebo. During this part randomisation is done using random blocks of 2, 4, or 6 with equal randomisation (1:1) within each site, for each of the 8 strata.

The individual lists created at each stage of randomisation will be discontinued at the end of its stage, with the new lists within site being used.

Balance Weighting

The adaptive randomisation algorithm creates a vector of probabilities for the four active treatment arms. This is a "global" probability for the arms, meaning it is over all strata. This section describes the balancing of the adaptive randomisation across the different strata to maintain, as well as possible, the balance of the 8 strata within active treatment arms, while achieving the needed adaptive randomisation.

The 8 strata used to balance the randomisation are based on the two-way classification of mechanical ventilation (MV) (Yes/No), Creatinine (Low/High), and norepinephrine/noradrenaline (NA) use (Low/High). We present the method for modifying the global adaptive randomisation probabilities to create different randomisation probabilities for each strata that will honor the goals of the response adaptive randomisation as well as the goal of balancing the strata in the treatment arms.

The outline of the approach at any interim point of the trial is:

1. The response adaptive randomisation probabilities are provided from the efficacy analysis. Label these π_1, π_2, π_3, and π_4, for the four active doses.
2. The odds-ratio of a stratum, for each arm, relative to all other arms, is calculated for the *previously* randomised patients.
3. Within each stratum the response adaptive randomisations are modified by the odds-ratios from the previous randomisations to create new odds for each arm that are strata specific.
4. The probabilities within each strata are normalized and used for randomisation.

The details of the balancing algorithm are presented below and an example data set is presented.

We label the 8 strata as s=1,…,8. For the previous randomisations, let N_{sa} be the number of stratum s randomised to arm a. The marginals of each stratum and/or arm are labeled with a + symbol.

The odds-ratio (modified by adding 0.5 to each cell for handling 0 counts) of the previous patients being randomised to arm *a*, within stratum *s* is labeled OR_{sa}:

$$OR_{sa} = \frac{\left(N_{sa} \middle/ \left(N_{+a} - N_{sa}\right) \right)}{\left(\left(N_{s+} - N_{sa}\right) \middle/ \left(N_{++} - N_{+a} - N_{s+} + N_{sa}\right) \right)}.$$

The global odds of randomising to each arm ($\pi_a/1-\pi_a$) for new patients is modified by the previous odds-ratio of randomisation to create the new randomisation probabilities by stratum for balancing future patients. The modified odds of randomising to arm *a*, within stratum *s* is

$$\left(\frac{\pi_a}{1-\pi_a}\right)\left(\frac{1}{OR_{sa}}\right).$$

These odds create unique probabilities for each arm, within each stratum.

$$\frac{\left(\frac{\pi_a}{1-\pi_a}\right)\left(\frac{1}{OR_{sa}}\right)}{1+\left(\frac{\pi_a}{1-\pi_a}\right)\left(\frac{1}{OR_{sa}}\right)}$$

These are normalized (across a stratum to sum to 1) to form a probability distribution for arms within a stratum:

$$\Pr(\text{Arm a in Stratum s}) = \frac{\dfrac{\left(\frac{\pi_a}{1-\pi_a}\right)\left(\frac{1}{OR_{sa}}\right)}{1+\left(\frac{\pi_a}{1-\pi_a}\right)\left(\frac{1}{OR_{sa}}\right)}}{\displaystyle\sum_{b=1}^{4} \frac{\left(\frac{\pi_b}{1-\pi_b}\right)\left(\frac{1}{OR_{sb}}\right)}{1+\left(\frac{\pi_b}{1-\pi_b}\right)\left(\frac{1}{OR_{sb}}\right)}}$$

As an illustrative example, assume the high dose has not been opened (and the new randomisation probability π_4 is 0). Example of previous randomisations are presented in Table 2:

Table 2: Example of randomisations to each arm within each stratum.

Strata	Arms			Total
	a=1	a=2	a=3	
1	5	5	5	15
2	9	1	5	15
3	2	2	2	6
4	1	0	0	1
5	15	18	20	53
6	2	8	8	18
7	7	8	9	24
8	3	4	1	8
Total	44	46	50	140

The odds-ratios of each arm, within each stratum, OR_{sa}, are presented in Table 3:

Table 3: The odds-ratio of arm *a*, within each stratum, *s*, with the 0.5 factor added to each cell

Strata	Arms		
	a=1	a=2	a=3
1	1.09	1.02	0.90
2	3.42	0.18	0.90
3	1.09	1.02	0.90
4	3.32	0.51	0.45
5	0.79	1.08	1.15
6	0.28	1.69	1.48
7	0.88	1.02	1.09
8	1.28	1.92	0.32

Assuming the global response adaptive randomisation probabilities are π_1=0.33, π_2=0.50, and π_3=0.17, the modified randomisation probabilities for each arm within each stratum are presented in Table 4.

Table 4: The modified randomisation probabilities for each stratum

Strata	Arms		
	a=1	a=2	a=3
1	0.31	0.50	0.19
2	0.11	0.73	0.16
3	0.31	0.50	0.19
4	0.12	0.60	0.28
5	0.38	0.47	0.15
6	0.56	0.33	0.11
7	0.35	0.49	0.16
8	0.27	0.34	0.39

Appendix 8 Bias on Treatment Estimate for P&VFDs

The treatment estimates for the primary endpoint will be based on a comparison between all patients on all selepressin arms from both parts of the trial (pooled together and treated as a single arm), and all patients on the placebo arm from both parts of the trial.

Additionally, the primary analysis will be repeated for the selected arm only, i.e. comparing all patients on the selected arm (from part 1) from both parts of the trial (pooled), to all patients on the placebo arm from both parts of the trial.
To address the issue of a potential bias on the treatment estimates a variety of different scenarios were simulated (Table 5). The results are based on 3000 simulated trials of scenarios where all arms are equally effective and where the mortality benefits and P&VFD benefits for survivors correspond. For each simulated trial, the treatment effect estimates are simple classical point estimates from the raw patient data, e.g. the mortality treatment effect is estimated by the raw mortality rate in the placebo group, minus the raw mortality rate in the active group.

Note that when investigating bias, it is not appropriate to restrict attention to simulated trials that are successful. Estimates from that approach will yield results that are larger than the simulated truths, and this is true for simple non-adaptive designs as well. In this trial the design is modified by removing all futility rules so that all simulated trials select an arm for Part 2 and enroll all 1800 patients. Some of these simulated trials reach 800 patients with the placebo arm outperforming all active arms; in these Part 2 is begun with the least badly performing active arm.

The table shows the true underlying benefit, the estimated benefits for the primary analysis (all selepressin arms from both parts of the trial compared to all patients on placebo from both parts of the trial), and the estimated benefits for the selected arm only (all patients on the selected arm from both parts of the trial compared to all patients on placebo from both parts of the trial).

The placebo arm was assumed to have a 25% mortality rate, a mean of 24 P&VFDs for survivors, and an overall mean of 18 P&VFDs (survivors and non-survivors).

If all active arms are included in the estimates (to correspond with the use of all active arms in the final van Elteren analysis as in the two middle columns), the estimates are unbiased and any differences from the truth are due to Monte Carlo variation. If only the selected arm is included in the estimates, there is some bias due to the fact that the selected arm must have performed relatively well in Part 1, the fact that Part 1 data are included in the estimates, and this bias is not completely neutralized by the introduction of a large sample of unbiased data in Part 2. The estimates of benefit in P&VFDs for survivors are nearly unbiased, while the relatively noisier mortality estimates have biases that can be on the same scale as the treatment effect. The largest bias is for the small mortality effect of 0.5%, where the design estimates an average effect of 0.77%, which is too small of a benefit to lead to a successful trial without a substantial effect on P&VFDs.

Table 5: Simulated treatment estimate bias for P&VFDs

True benefit		**Average estimated benefit (all selepressin arm)**		**Average estimated benefit (selected arm only)**	
Mortality	P&VFDs (survivors)	Mortality	P&VFDs (survivors)	Mortality	P&VFDs (survivors)
0%	0 days	-0.03%	0.01 days	0.21%	0.06 days
0.5%	0.5 days	0.56%	0.51 days	0.77%	0.56 days
1.0%	1.0 days	0.98%	0.98 days	1.17%	1.05 days
1.5%	1.5 days	1.45%	1.46 days	1.63%	1.54 days
2.0%	2.0 days	1.92%	1.95 days	2.05%	2.03 days
3.0%	3.0 days	2.97%	2.97 days	3.10%	3.03 days

In both scenarios, treatment estimates are either unbiased or the treatment bias is negligible, and hence treatment estimates will not be adjusted for treatment bias.

STATISTICAL ANALYSIS PLAN

ADDENDUM

A Double-blind, Randomised, Placebo-controlled Phase 2b/3 Adaptive Clinical Trial Investigating the Efficacy and Safety of Selepressin as Treatment for Patients with Vasopressor-dependent Septic Shock

SEPSIS-ACT

Selepressin Evaluation Programme for Sepsis-Induced Shock - Adaptive Clinical Trial

000133

Investigational Product:	Selepressin; concentrate for solution for infusion Placebo; sterile 0.9% sodium chloride solution
Indication:	Vasopressor-dependent Septic Shock
Phase:	2b/3
Author:	Allan Blemings
Date of issue:	November 20th - 2017
Version:	Final – Ver. 1.0

Change log

Version No.	Effective Date	Reason for the Change / Revision	Supersedes
1	Nov 20-2017	Original SAP addendum	Not applicable

Signed agreement on Statistical Analysis Plan Addendum

The original analysis plan addendum was reviewed by,

- [redacted] Global Biometrics, Ferring Pharmaceuticals A/S
- [redacted] Global Clinical Operations, CDS, Medical Writing & Selepressin, , Ferring Pharmaceuticals A/S
- [redacted] Medical Writing, Ferring Pharmaceuticals A/S
- [redacted] Selepressin, Ferring Pharmaceuticals A/S
- [redacted] Selepressin, Ferring Pharmaceuticals A/S

And reviewed and approved (signed electronically) by,

- [redacted] Biometrics, Ferring Pharmaceuticals A/S

TABLE OF CONTENTS

1 Introduction

This document describes the statistical analyses for selepressin (FE202158) 000133 planned after the trial was stopped for futility, but before the blind was broken. It is an addendum to version 9.0 of the statistical analysis plan.

1.1 Abbreviations

Abbreviations	**Meaning of abbreviations in document**
IMP	Investigational medicinal product
P&VFDs	Vasopressor- and mechanical ventilator-free days

2 Post-futility / Pre-unblinding Planned Analyses

2.1 Primary Endpoint

To explore whether there was a 'learning curve' effect of administering the drug, the primary analysis will be conducted without the first 3 patients at each site.

Due to trial logistics (e.g. preparation time for pharmacy etc.) or safety concerns, some patients may receive norepinephrine instead of the investigational medicinal product (IMP) during parts of the treatment period where they have a vasopressor need. To try to mimic the 'near perfect' conditions, where IMP is used as the primary vasopressor, the primary analysis will be conducted on a subset of the per-protocol analysis set in which patients who did not have norepinephrine weaned before IMP (unless due to death), or patients in which IMP was not restarted within 6 hours upon restart of norepinephrine, will be excluded.

To evaluate the new septic shock criteria (1), the primary endpoint will be evaluated for patients with lactate <2 mmol/L at baseline (sepsis) and patients with lactate ≥2 mmol/L (septic shock), respectively.

The VASST trial identified a potential survival benefit using vasopressin as compared to norepinephrine in patients with less severe septic shock defined as a baseline norepinephrine dose of less than 15 µg/min. Similarly, to assess whether there was a potential benefit of selepressin in vasopressor- and mechanical ventilator-free days (P&VFDs) in patients with less severe septic shock, the treatment effect of the primary endpoint will be presented graphically, in order to visually inspect whether the average treatment effect is distributed evenly across the baseline norepinephrine levels (≤15 µg/min, >15 µg/min).

To further assess if baseline norepinephrine impacts the treatment effect, the same analysis will be repeated for baseline norepinephrine levels (≤0.1µg/kg/min,]0.1; 0.20 µg/kg/min],]0.2; 0.30 µg/kg/min],]0.3; 0.45µg/kg/min],]0.45; 0.60µg/kg/min], >0.60µg/kg/min).

To assess if there is a treatment duration effect, patients will be stratified by cumulative duration of IMP infusion (<1 day, [1; 3 days[, [3; 7 days[, ≥7 days) and the treatment effect of the primary endpoint will be presented graphically, in order to visually inspect whether the average treatment effect is distributed evenly across the duration of treatment with IMP.

2.2 Organ Dysfunction

2.2.1 Mechanical Ventilator-free Days up to Day 30

To assess if there is an effect of baseline lung function on the potential treatment effect of selepressin on lung function, the treatment effect of mechanical ventilator-free days will be presented graphically, in order to visually inspect whether the average treatment effect is distributed evenly across baseline PaO_2/F_iO_2 levels (<100mmHg, [100; 200 mmHg[, [200; 300 mmHg[,≥300 mmHg).

2.2.2 Duration of Mechanical Ventilation up to Day 30

The graphical presentation in section 2.2.1 will be repeated for duration of mechanical ventilation.

2.2.3 Renal Replacement Therapy (RRT)-free Days up to Day 30 (excluding patients on RRT for chronic renal failure at time of randomisation)

RRT-free days will also be evaluated using the new septic shock definition (1) as for the primary endpoint.

2.3 Morbidity/Mortality

2.3.1 Intensive Care Unit (ICU)-free Days up to Day 30

ICU-free days will also be evaluated using the new septic shock definition as for the primary endpoint.

2.3.2 All-cause Mortality (Defined as the Fraction of Patients That Have Died, Regardless of Cause, by the end of Day 30, Day 90, and Day 180)

Mortality will also be evaluated using both the new septic shock definition, and the baseline norepinephrine cut-offs as for the primary endpoint.

2.4 Daily and Cumulative Urinary Output Until ICU Discharge (for a Maximum of 7 Days)

Urinary output and cumulative urinary output analyses will be repeated for the two components that make up total output volume; spontaneous urine output and output fluids collected from drainages, suction devices and respiratory fluid loss estimation (if transpiration was performed).

2.5 Pulmonary Function (PaO2/FiO2) (in a subset of patients)

PaO_2/F_iO_2 up to and incl. Day 7 will be evaluated as an area under the curve (AUC).
Linear interpolation will be used to derive the AUC (taking into account that not all patients will have measurements taken between 24 hours post baseline and Day 2). Patients dead or withdrawn will be set to missing. The cumulative PaO_2/F_iO_2 will be compared between treatment arms using a repeated measures ANCOVA model with baseline PaO_2/F_iO_2 as covariate, treatment, time and treatment by time interaction as factors, baseline PaO_2/F_iO_2 by time interaction, and patient as the experimental unit. Estimated treatment differences (to placebo) along with a 95% confidence interval will be presented.

2.6 Arterial Blood Gases and Acid/Base Status (PaO2, PaCO2, pH, SaO2, Bicarbonate, Base Excess), Lactate and Oxygen Saturation in Vena Cava Superior (ScvO2)

Lactate up to and incl. Day 7 will be evaluated as for PaO_2/F_iO_2 in section 2.5 above.

2.7 Time to Out of Shock

A Kaplan-Meier analysis will be performed for time to out of shock, defined as the first time where the patient has no vasopressor for at least 24 hours. Patients who die or are withdrawn while still on vasopressors and before first 24 hour vasopressor-free episode will be censored. The same applies for patients prematurely discontinued from IMP.

2.8 Time to Out of Mechanical Ventilation

Time to out of mechanical ventilation will be defined and analyzed similarly as for time to out of shock in section 2.7 above.

2.9 Proportion of Patients maintaining Target MAP while on IMP

The proportion of patients maintaining target MAP while on IMP will be compared between treatment groups using a logistic regression model.

2.10 Episodes of Hypotension

The proportion of patients having one or more episodes of hypotension will be compared between treatment groups using a logistic regression model.

The cumulative duration of periods of hypotension will (ideally) be compared between treatment groups using a zero inflated negative binomial model. In case model assumptions do not hold, a permutation test will be used.

3 References

1 Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016. 315(8):801-810.