SEDANAMEDICAL

A Randomised Active-controlled Study to Compare Efficacy and Safety of Inhaled Isoflurane Delivered by the AnaConDa-S (Anaesthetic Conserving Device) to Intravenous Midazolam for Sedation in Mechanically Ventilated Paediatric Patients 3 to 17 (Less than 18) Years Old

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National Coordinating Investigators:	
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	
Sponsor's Medical Representative:	Peter Sackey, MD, PhD, Chief Medical Officer Sedana Medical AB Vendevägen 89 SE-182 32, Danderyd, Sweden

PROTOCOL APPROVAL PAGE

National Coordinating Investigators

[redacted]

Sponsor's Medical Representative

……

Peter Sackey, MD, PhD, Chief Medical Officer
Sedana Medical AB
Danderyd, Sweden

Date (DD/MMM/YYYY)

INVESTIGATOR AGREEMENT PAGE

I confirm agreement to conduct the study in compliance with the protocol, Good Clinical Practice (GCP), Declaration of Helsinki and applicable regulations.

I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described clinical study.

I agree to ensure that all associates, colleagues and clinical staff assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

_____ _____

Principal Investigator's signature Date (DD/MMM/YYYY)

_____

Principal Investigator's name

_____

Site number

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Appendix A COMFORT Behavior Scale

Appendix B Sophia Observation Withdrawal Symptoms Scale and Delirium

1. PROTOCOL SYNOPSIS

Study code	SED002
Study title	A Randomised Active-controlled Study to Compare Efficacy and Safety of Inhaled Isoflurane Delivered by the AnaConDa-S (Anaesthetic Conserving Device) to Intravenous Midazolam for Sedation in Mechanically Ventilated Paediatric Patients 3 to 17 (Less than 18) Years Old
Coordinating investigators	[redacted]
Clinical phase	Therapeutic confirmatory (phase III)
Study sites	Approximately 20 European sites (Intensive Care Units)
Study period	Estimated first patient first visit (FPFV): Q4 2020 Estimated last patient last visit (LPLV): Q1 2023

PRIMARY STUDY OBJECTIVE AND ENDPOINT

Primary objective

To compare the percentage of time adequate sedation depth is maintained within the individually prescribed target range in absence of rescue sedation as assessed according to the COMFORT-B scale, in isoflurane vs midazolam treated paediatric patients for an expected minimum of 12 hours.

Primary endpoint

Percentage of time of adequately maintained sedation within the COMFORT-B interval (light, moderate or deep sedation) prescribed at randomisation, monitored every 2 hours for a minimum of 12 hours (up to 48 hours ± 6 hours).

The primary endpoint assessment will start 2 hours after initiating study sedative treatment (or in the case of ongoing sedation, 2 hours after terminating ongoing sedatives). The primary endpoint assessment will be collected either until the study treatment is replaced with the standard treatment (at 48± 6 hours from study treatment initiation) or when the wake-up for extubation is started, whichever comes first.

SECONDARY OBJECTIVES AND ENDPOINTS

Secondary objectives, efficacy

- Compare the use of opiates, and the development of tolerance to the sedative regimen as measured by the change in dose of study drug, opiates and other analgesics, over time in isoflurane- vs midazolam-treated patients.
- Compare the need for rescue sedatives and other sedatives in isoflurane- vs midazolam-treated patients.

Secondary objectives, safety

- Compare time from sedation termination to extubation in isoflurane- vs midazolam-treated patients.
- Compare the proportion of time with spontaneous breathing in isoflurane- vs midazolam-treated patients.

- Evaluate haemodynamic effect as indicated by inotropic/vasopressor agent administration in patients sedated with isoflurane compared with midazolam.
- Evaluate the frequency of withdrawal symptoms in isoflurane- vs midazolam-treated patients.
- Evaluate the frequency of delirium in isoflurane- vs midazolam-treated patients.
- Evaluate the frequency of neurological symptoms or psychomotor dysfunction during and up to 48 hours after discontinuation of isoflurane and midazolam treatment, and the association with duration of treatment, and total exposure (MAC hours and midazolam doses) over time.
- Compare the 30 days/hospital mortality in isoflurane- vs midazolam-treated patients.
- Compare ventilator-free days up to 30 days in isoflurane- vs midazolam-treated patients.
- Compare the time in ICU/hospital up to 30 days in isoflurane- vs midazolam-treated patients.
- Compare ICU-free days up to 30 days in isoflurane- vs midazolam-treated patients.
- Compare the safety profile in terms of experienced adverse events, safety laboratory values, blood gases, vital signs, body temperature and urinary output in isoflurane- vs midazolam-treated patients.

Secondary endpoints, efficacy

- Dose of opiates, study drugs and other analgesics required, from first blinded COMFORT-B assessment (at +2 hours) to end of study treatment period, given per 24 hours.
- Mean dose of study drugs, opiates and other analgesics required, during the last 4 hours of study treatment, as compared to the first 4 hours of study treatment after first blinded COMFORT-B assessment.
- Mean dose of rescue propofol (mg/kg/24 hours) and mean dose of rescue ketamine/es-ketamine (converted to ketamine-equivalents mg/kg/24 hours), and mean dose of α2-adrenergic agonists (mg/kg/24 hours) to maintain the COMFORT-B score in the individually prescribed range, in isoflurane- vs midazolam-treated children (time window: from 2 hours after initiating study sedative treatment to end of sedative treatment).
- Number of doses of rescue sedation (propofol, ketamine, es-ketamine) given per 24 hours from first blinded COMFORT-B assessment (at +2 hours) to end of study treatment period.

Secondary endpoints, safety

- Time from end of study drug administration to extubation if study drug is terminated for extubation.
- Proportion of observations with spontaneous breathing efforts during study treatment.
- Need for additional inotropic/vasopressor agent and change in VIS score during study treatment period compared to baseline.
- Presence of withdrawal symptoms as assessed using the SOS-PD scale in patients exposed to more than a total of 96 hours sedation (including pre-study sedation period) until the end of the 48-hour post study treatment monitoring or ICU discharge, whichever comes first.
- Presence of delirium as assessed using the SOS-PD scale in patients admitted to the ICU for at least 48 hours (including period prior to study enrolment) until the end of the 48-hour post study treatment monitoring or ICU discharge, whichever comes first.

- Proportion of patients experiencing psychomotor dysfunction or neurological symptoms during sedation and/or in the 48 hours after discontinuation of isoflurane or midazolam treatment, in relation to duration of exposure to isoflurane or midazolam, and to cumulative midazolam mg/kg or isoflurane exposure (MAC hours).
- 30 days/hospital mortality.
- Ventilator-free days at 30 days from start of study treatment period.
- Time in intensive care unit/hospital at day 30 from start of study treatment period.
- Days alive and not in the ICU at day 30 from start of study treatment period.
- Proportion of patients with common as well as sedation-related adverse events, and frequencies of these adverse events from start of study treatment to end of 48-hour post study treatment monitoring.
- Frequency and intensity of adverse events from start of study treatment to day 30.
- Changes in vital signs, blood gases, body temperature and urinary output from baseline to end of study treatment.
- Changes in clinical chemistry and haematology parameters from baseline up to the 48-hour post-study treatment monitoring.

EXPLORATORY OBJECTIVES AND ENDPOINTS

Exploratory objectives

- Determine the isoflurane dosage, end-tidal concentrations and infusion rates, and the midazolam dosage, required for adequate sedation in mechanically ventilated paediatric patients.
- Evaluate frequency and type of AnaConDa-S device deficiencies when used for sedating patients with isoflurane.

Exploratory endpoints

- The mean and median dose (MAC value and end-tidal concentration) of isoflurane and mean and median dose of midazolam required for achieving the target level of sedation, over time, by age group.
- Number of study drug bolus doses given per 24 hours during midazolam and isoflurane sedation of mechanically ventilated patients.
- Ventilator parameters (ventilation mode, tidal volume, minute volume, fraction of inspired oxygen (FiO_2), end tidal carbon dioxide [$EtCO_2$], total breathing rate, positive end-expiratory pressure [PEEP], set inspiratory pressure [Pinsp], level of pressure support [PS] above PEEP).
- Frequency and type of AnaConDa-S device deficiencies during isoflurane sedation.

STUDY DESIGN AND METHODOLOGY

This is a therapeutic confirmatory (phase III), multicentre, open label with a blinded assessor, randomised, age-stratified, active controlled study vs. standard treatment. Patients will be assigned to a sedative treatment through randomisation in a 2:1 ratio to either inhaled isoflurane via the AnaConDa-S, or standard treatment (IV midazolam). The study treatment duration is expected to be a minimum of 12 hours and up to 48±6 hours with a follow-up period of 30±2 days.

Screening: Paediatric patients (3-17 years old) will be recruited at the study sites and may be referred from other departments at the same hospital or from another hospital. Potential candidates will be patients of age 3-7, 8-11 or 12-17 years that require mechanical ventilation for at least 12 hours. Patients eligible for the study will involve two categories; either they are

planned to undergo mechanical ventilation in the ICU after surgery, or they are admitted to ICU in an unplanned situation and require mechanical ventilation. The study will be stratified by this category of reason for ICU admission as well as age group and country.

Patients will be switched from ongoing sedative treatment to the randomised study treatment. Ongoing sedative treatment must not have exceeded 72 hours at the time of randomisation. When possible, informed consent for postoperative patients (and assent when appropriate) should be obtained prior to surgery. If the patient is unconscious at the time of inclusion, information about the study will be given and assent obtained as soon as their condition allows. Informed consent will be obtained from the legal guardian(s) whether the patient is conscious or unconscious at the time of inclusion in accordance with local regulation. Following the informed consent procedure, patients will be formally screened and enrolled into the study if all criteria are met. Shortly prior to randomisation, the Investigator will prescribe the "target sedation depth" as either light, moderate or deep, defined according to the COMFORT-B scores.

Treatment: Study treatment will be titrated to achieve and maintain the target sedation depth. The sedation depth will be assessed through blinded COMFORT-B assessments every 2 hours throughout the study treatment period and will, in the statistical analysis, be adjusted for potential confounding factors such as the need of rescue sedation, α2-adrenergic agonists, additional sedation agents administered due to therapeutic or diagnostic procedures and use of analgesia (incl. opiates). Therefore, administration of rescue and procedural sedation agents as well as analgesia will be recorded throughout the study treatment period including doses administered. One change of the target sedation depth (light, moderate or deep) will be allowed for the welfare of the patient during the study treatment period. This optional single change of prescribed target sedation depth is not for shorter medical procedures, when the sedation/analgesia is permitted to the clinically indicated level.

Vital signs and ventilator parameters will be collected every 2 hours, blood gases, body temperature and urinary output will be collected every 8 hours. A safety laboratory assessment will be performed once after 24 hours. The SOPHIA Observation Withdrawal Symptoms Scale – Paediatric Delirium (SOS-PD) will be evaluated in patients which have been admitted to the ICU for 48 hours or more (SOS-PD delirium module) and in patients which have been exposed to a total of 96 hours of sedation or more, including the pre-study sedation period (SOS-PD withdrawal module). From these time points, the SOS-PD delirium and withdrawal modules will be evaluated every 8 hours respectively during the remaining study treatment period until the end of 48-hour post-study treatment period or until the patient is discharged from ICU, whichever comes first.

Follow-up: After the study treatment period of up to 48±6 hours, the treating physician may continue sedation of the patient according to local practice. The patient will be followed closely for 48 hours after end of study treatment, including a follow-up safety laboratory assessment, or until the patient is discharged, if this is earlier than 48 hours after end of study treatment. After this initial 48-hour post-study treatment monitoring the patient will be followed up on a weekly basis up to 30±2 days after the end of study treatment. Adverse events will be recorded weekly, either by examining the patient in the hospital or through contact with the patient/legal guardian(s) or caregiver if patient is discharged. On day 30, the Investigator will record, based on review of the patient's medical records, or by contacting the patient, legal guardian(s) or caregiver, the number of ventilator-free days, time in ICU/hospital and mortality.

In the case of identified neurological symptoms, such as psychomotor events (known event in young patients exposed for longer periods of time to isoflurane) each event will be monitored at least daily in the first week after end of study treatment or until resolution. Other ongoing AEs will be monitored until resolution or referral to a physician for follow-up.

PATIENT POPULATION

No more than 100 patients will be randomised in order to reach 90 evaluable patients.

ELIGIBILITY CRITERIA

Inclusion criteria

1. Paediatric patients at least 3 years to 17 (less than 18) years at the time of randomisation, admitted to an ICU/with planned ICU admission. Patients who will turn 18 years during the study (including study follow-up period) will not be included.
2. Expected mechanical (invasive) ventilation and sedation for at least 12 hours.
3. Informed consent obtained from the patient, patient's legal guardian(s) as required by local regulations. Where applicable, assent obtained from the patient to participate in the clinical study.
4. **Germany only:** Women of childbearing potential who are sexually active: The patient does already use a highly effective contraception method or agrees to use a highly effective contraception method from signing the informed consent form (screening) until the operation/study treatment. According to Clinical Trials Facilitation Group (CTFG) recommendations, methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include:
    - combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal)
    - progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable)
    - intrauterine device
    - intrauterine hormone-releasing system (IUS)
    - bilateral tubal occlusion
    - vasectomised partner
    - sexual abstinence or absence of sexual relations with men

Exclusion criteria

1. Ongoing seizures requiring acute treatment.
2. Continuous sedation for more than 72 hours at time of randomisation.
3. Less than 24 hours post cardiopulmonary resuscitation.
4. Uncompensated circulatory shock.
5. Known hypersensitivity to isoflurane or to other halogenated anaesthetics (such as halothane), benzodiazepines, non-investigational medicinal product(s) (analgesics, additional rescue sedatives that may be required during the study) or to any of their formulation ingredients.
6. Known or suspected genetic susceptibility to malignant hyperthermia.
7. Patients with acute asthma or obstructive lung disease symptoms requiring treatment at inclusion.
8. Patient with tidal volumes below 30 mL or above 800 mL.
9. Inability to perform reliable COMFORT-B assessment in the opinion of the Investigator e.g. due to (not limited to):

Severe traumatic brain injury, intracranial pathology (tumour, haemorrhage, infections), with a profound effect on the level of consciousness, severe mental retardation, major congenital anomalies of the central nervous system, severe myasthenia gravis, spinal muscular atrophy, or other severe neurologic disease, ongoing neuromuscular blockade which precludes COMFORT-B scoring.

10. Patients with intracranial pressure (ICP) monitoring or with suspected increase in ICP
11. Patients with treatment-induced whole-body hypothermia.
12. Patients with pheochromocytoma.
13. Patients with prolonged QT interval or with significant risk for prolonged QT interval.
14. Patient not expected to survive next 48 hours or not committed to full medical care.
15. Female patients who are pregnant or breast-feeding.
16. Previous participation in the study (a patient can only participate once).
17. Known participation in any other clinical study that included drug treatment within three months of the first administration of the IMP.
18. Any for the study relevant medical history, or ongoing clinically significant disease, disorder or laboratory result which, in the opinion of the Investigator, precludes participation in the study for medical or ethical reasons.

INVESTIGATIONAL MEDICINAL PRODUCT

- The test product is inhaled isoflurane (Piramal) administered through the AnaConDa-S. The start infusion dose-rate at 2.0 mL/hr and should be titrated stepwise of 0.5-1.0 mL/hr to achieve and maintain the target sedation depth. The maintenance dose of isoflurane should not exceed 1.0% of the end-tidal volume.
- The comparator is intravenous midazolam. The start infusion dose-rate is 50-100 µg/kg/hr and should be titrated stepwise to achieve the target sedation depth, by decreasing/increasing 20-50 µg/kg/hr. The maintenance dose of midazolam should not exceed 300 µg/kg/hr.

DURATION OF TREATMENT

Patients will be exposed to inhaled isoflurane or IV midazolam up to 48 hours (±6 hours) during the study treatment period. If need for sedation remains after this, the physician may continue sedative treatment according to standard practice**.**

STATISTICAL METHODS

The study is designed in accordance with the estimand approach. The main statistical analysis will be conducted on the full analysis set (FAS) population and will be a mixed effects analysis of variance model with treatment group as fixed effect and country as a categorical random effect. Patients evaluable for efficacy (i.e. included in FAS) are those who received IMP and have at least 6 hours sedation period and at least 3 blinded COMFORT-B-assessments performed.

The primary endpoint will be the percentage of time of adequately maintained sedation on study treatment and will be calculated as the sum of time when the COMFORT-B assessment is considered a success, i.e. when the result of the blinded COMFORT-B assessment is equal to target sedation depth or maximum 1 point outside, divided by the total amount of time the patient is sedated. Relevant intercurrent events to the primary endpoint calculation, mainly the

use of rescue sedation, and the approach on how these will be handled in the analysis, including assigning failure time and censoring have been defined.

The main statistical analysis will follow a stepwise testing scheme, initially performing the superiority test, followed by a potential non-inferiority test. This follows a closed testing procedure, which means that the significance level will be kept at 5% in the potential second step. The claims of the study regarding the primary efficacy endpoint can be derived from the 95% confidence interval of the (isoflurane- midazolam)-difference in percentage of time with adequate sedation depth in absence of rescue sedation as follows:

- Isoflurane is superior to midazolam if the entire 95% confidence interval lies above 0.
- If isoflurane is not shown to be superior to midazolam, then isoflurane is non-inferior to midazolam if the entire 95% confidence interval lies above the pre-defined non-inferiority margin of -15% (relative difference).
- If isoflurane is not shown to be non-inferior to midazolam, i.e. the entire 95% confidence interval does not fall above -15% (relative difference), then the alternative hypothesis of isoflurane being non-inferior to midazolam can thus not be accepted.
- If the entire 95% confidence interval falls below -15% (relative difference), then the null hypothesis of isoflurane being inferior to midazolam will be considered accepted.

Descriptive statistics will be presented for all efficacy and safety variables. Continuous variables will be summarised by descriptive statistics (sample size [n], mean, standard deviation, minimum, median, and maximum value) by treatment group. This will be done for both actual values and change from baseline values. Categorical variables will be summarised in frequency tables showing number of patients, frequency and percentage of occurrence by treatment group. Graphical presentations will be used as appropriate and individual patient data will be listed.

Sample size calculation

The study will be powered for superiority with a non-inferiority test as a second step in the stepwise testing procedure. With a one-sided test at the 2.5% significance level, a total of 90 evaluable patients (randomised 2:1 isoflurane:midazolam) will give 80% power to detect superiority if the true difference in the primary variable is approximately 22.2 percentage points greater for isoflurane (79.8%) than with midazolam sedation (57.6%), assuming a common standard deviation of 35% percentage points.

It is expected that no more than 100 patients will be randomised into the study to get 90 patients evaluable for efficacy of the primary endpoint (as it is expected that up to 10% of the randomised patients will be excluded from the FAS analysis population, i.e. not evaluable for efficacy).

Assuming a 57.6% effect size for midazolam, the non-inferiority margin would be 49% (57.6% - 0.15*57.6%). A total of 66 to 87 evaluable patients will then give 80 to 90% power to detect non-inferiority if the effect size for isoflurane is mid-range of estimated superiority (17.5%).

2. LIST OF ABBREVIATIONS

The following abbreviations and special terms are used in this clinical study protocol (CSP).

Abbreviation	Explanation
AE	Adverse event
ADE	Adverse device effect
ADR	Adverse drug reaction
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AnaConDa	Anaesthetic conserving device
B-	Blood
BMI	Body mass index
BP	Blood pressure
CDASH	Clinical Data Acquisition Standards Harmonization
COMFORT-B	Comfort Behavior Scale
CRO	Clinical research organisation
CSP	Clinical study protocol
CSR	Clinical study report
CTFG	Clinical Trials Facilitation Group
DSMB	Data Safety Monitoring Board
eCCl	Estimated creatinine clearance
ECG	Electrocardiography
ECMO	Extracorporeal membrane oxygenation
eCRF	Electronic case report form
EEG	Electroencephalography
ESPNIC	European Society of Paediatric and Neonatal Intensive Care
$EtCO_2$	End tidal carbon dioxide
FAS	Full analysis set
F_{et}	End-tidal concentration
FiO_2	Fraction of inspired oxygen
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
GMP	Good Manufacturing Practice
hCG	Human chorionic gonadotropin
HME	Heat- and moisture exchanger
HR	Heart rate
ICF	Informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceutical for Human Use
ICP	Intracranial pressure
ICU	Intensive care unit
IEC	Independent ethics committee
IMP	Investigational medicinal product
ITT	Intention-to-treat
IV	Intravenous

Abbreviation	Explanation
IUS	Intrauterine hormone-releasing system
MAC	Minimal alveolar concentration (measured in expired air)
MAP	Mean arterial pressure
MedDRA	Medical dictionary for regulatory activities
$PaCO_2$	Partial carbon dioxide pressure
PDCO	Paediatric committee of the European Medicines Agency
PEEP	Positive end-expiratory pressure
PICU	Paediatric intensive care unit
PIM	Paediatric index of mortality
Pinsp	Set inspiratory pressure
PIP	Paediatric investigational plan
PP	Per protocol
pRIFLE	Paediatric Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease
PS	Pressure support
PT	Preferred term
S-	Serum
SADE	Serious adverse device effect
SADR	Serious adverse drug reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SDTM	Study Data Tabulation Model
SmPC	Summary of product characteristics
SOP	Standard operating procedure
SOS-PD	SOPHIA Observation Withdrawal Symptoms-Paediatric Delirium scale
SpO_2	Peripheral capillary oxygen saturation
SUSAR	Suspected unexpected serious adverse reaction
UADR	Unexpected adverse drug reaction
USADE	Unanticipated serious adverse device effect
VIS	Vasoactive-Inotropic Score
v/v	Volume per volume
w/v	Weight per volume

3. DEFINITIONS OF TERMS

The following definitions are used in this clinical study protocol.

Terms	Definition
Bradycardia	Heart rate -20% of the baseline value.
Censored time	Time interval that is omitted in the calculation of the primary endpoint due to deviation from the treatment regimen defined in the CSP.
Coordinating Investigator	An investigator assigned the responsibility for the coordination of investigators at different centres participating in a multicentre trial (ICH E6 R2))
Estimand	A precise description of the treatment effect reflecting the clinical question posed by the trial objective. It summarizes at a population-level what the outcomes would be in the same patients under different treatment conditions being compared (ICH E9 (R1)).
Estimator	Method of analysis to compute an estimate of the estimand using study data.
Evaluable patient (included in FAS)	A patient that has received IMP, has had a study treatment period of at least 6 hours and at least 3 blinded COMFORT-B assessments performed after the initial IMP titration phase (up to +2 hours).
Failure time	Time interval in the calculation of the primary endpoint where assessed sedation depth is not equal to the target sedation depth (i.e. COMFORT-B scores are outside the ranges specified for "Success" time) or a specific type of intercurrent event has occurred.
Hypertension	Mean arterial blood pressure +20% of the baseline value.
Hypotension	Mean arterial blood pressure -20% of the baseline value.
Hypoxia	Oxygen saturation below 88% for more than 5 minutes. For patients with baseline oxygen saturation of 92% or lower due to e.g. underlying disease, a drop of 10% or more for more than 5 minutes.
ICU-free days	Days alive and not in ICU in a defined follow-up period after randomisation.
Intercurrent event	Event occurring after treatment initiation that affects either the interpretation or the existence of the measurements associated with the clinical question of interest.
Legal guardian	Parent(s) of participating patient, legally appointed guardian(s) or legally acceptable representative(s), as defined by national and local laws and regulations who consent(s) on behalf of the participating underage patient.
Mechanical ventilation	Invasive ventilator support to an intubated or tracheotomized patient.
Per protocol rescue sedation	Specified sedative agents which will be allowed for use during study treatment, defined in Section 10.3.1.
Pre-study sedation period	Sedation prior to randomisation. The time when the patient received sedatives and analgesics during the current ICU-admission, prior to starting the IMP.
Renal insufficiency	Class *Injury* or worse according to the pRIFLE classification, i.e. eCCl decrease of 50% compared to baseline and a urinary output of <0.5mL/kg/hr for 16 hours.
Study discontinuation	Patient discontinues their participation from the study prematurely (no intercurrent event to primary objective).
Study drug	Product under investigation, i.e. isoflurane and midazolam. Used in this study protocol synonymously with IMP.
Study treatment period	Time from start of IMP administration to end of IMP administration.
Success time	Time interval in the calculation of the primary endpoint where assessed sedation depth equals the target sedation depth, or is maximum 1 point outside target interval, in absence of intercurrent events.
Tachycardia	Heart rate +20% of the baseline value.
Treatment discontinuation	Patient discontinues study treatment prematurely (intercurrent event to the primary objective).
Ventilator-free days	Days alive and without invasive ventilation support during a defined follow-up period after randomisation.

4. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

4.1 Investigators

A list of investigators and site personnel will be available in the files at the Sponsor and at the site.

4.2 Sponsor

Sedana Medical AB
Vendevägen 89
SE-182 32 Danderyd, Sweden

4.3 Sponsor medical representative

Peter Sackey, MD, PhD, Chief Medical Officer
Sedana Medical AB
[redacted]

4.4 Contract research organisation

The project management, monitoring, data management, statistical analysis, safety reporting and study report is outsourced to the contract research organisation (CRO) [redacted]

[redacted]

4.5 Financing and insurance

Sedana Medical AB, Sweden, is the Sponsor of the study and is funding the study. All financial details are provided in the separate contracts between the Institution, and the Sponsor or designee. The Sponsor will compensate the Institution for their work in the study. However, the compensation will not be affected by the outcome of the study.

The Sponsor or designee maintains appropriate insurance coverage for clinical studies and will follow applicable local compensation regulations. All patients in the study can retrieve financial compensation in the case of harm as a consequence of study participation.

5. ETHICAL AND REGULATORY CONSIDERATIONS

5.1 Statement of compliance

This study will be conducted in compliance with this clinical study protocol (CSP), the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice (GCP) (ICH Harmonised Tripartite Guideline), applicable regulatory requirements, and in accordance with the latest revision of the Ethical Principles for Medical Research Involving Human Subjects (World Medical Association Declaration of Helsinki).

5.2 Ethics and regulatory conduct of the study

The Sponsor or designee will, prior to site initiation, obtain favourable opinion/approval to conduct the study from all appropriate regulatory agencies/competent authorities in accordance with any applicable country specific regulatory requirements. In addition, independent ethics committee(s) (IEC) will review the CSP, the written patient information and informed consent form (ICF), any other written materials given to the patients and their legal guardian(s) and other study-specific documentation (as required). All applicable ethical and regulatory approvals must be available before a patient is exposed to any trial-related procedure, including screening for eligibility.

Local regulations regarding obtaining informed consent and assent for inclusion of underage patients should be followed. It is the responsibility of the Investigator to obtain signed informed consent/assent according to applicable regulations from all patients and legal guardian(s) prior to any study related activities, refer to Section 9.2 for details on this process. The ICF will incorporate (or be accompanied by a separate document incorporating) information on the processing of the patient data within the scope of this study which complies with relevant data protection and privacy legislation.

The patient and their legal guardian(s) will be free to discontinue the study at any time, without any consequences for the patient's further care and without the need to justify their decision (See Section 9.6).

5.3 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the Investigators and the Sponsor. Changes to the CSP and/or the ICF and assent forms must be approved by the Sponsor, IEC and if applicable, the regulatory authority before implementation. In case of acute safety risks changes may be implemented without prior approval. In this case any implemented change must be properly documented by the Investigator and the Sponsor must be immediately notified.

If an amendment to the CSP substantially alters the study design, or increases the potential risk to the patients, written informed consent and assent must be obtained again for currently enrolled patients and legal guardian(s) (as applicable). Information must also be provided to new patients and legal guardian(s) prior to their entry into the study.

6. INTRODUCTION

The clinical study design, objectives, major endpoints, major inclusion/exclusion criteria, statistical hypotheses and power calculations has been approved by the Paediatric Committee (PDCO) of the European Medicines Agency as part of the Paediatric Investigational Plan (PIP), [redacted]

6.1 Background

Current methods of sedation in mechanically ventilated paediatric patients often involve co-administration of intravenous (IV) midazolam and opioids, and sometimes ketamine and α2-adrenergic agonists such as clonidine or dexmedetomidine (Kudchadkar et al. 2014). Propofol is occasionally used for short-term procedural sedation in the paediatric intensive care unit (ICU) (Laudenbach et al. 2008, Kruessel et al. 2012) but should not be used for prolonged sedation in paediatrics (UK Sedation Guidelines, Playfor et al. 2006). For continuous sedation in the intensive care unit (ICU) propofol is approved from the age of 16 years (Summary of product characteristics, SmPC, Diprivan™ 10 mg/mL).

One of the most commonly used, and the only sedative approved for continuous use in paediatric intensive care patients in Europe and the US, is midazolam. (Playfor et al 2006, Kudchadkar et al. 2014, Label Midazolam). This benzodiazepine, however, is associated with a number of issues, particularly problems with tolerance (Tobias 2000, Grant et al. 2013, Eifinger et al. 2013) and withdrawal syndrome, prolonged sedation on discontinuation, and hypotension with bolus dosing (Playfor et al 2006).

Isoflurane is a well-characterised drug used for maintenance of anaesthesia in adults and children. Isoflurane was approved in the 1980s and there is extensive clinical experience of its use for this indication. Dose recommendations for approved indications of isoflurane are similar for adult and paediatric patients: average end-tidal concentrations (Minimal Alveolar Concentration, MAC values) required for pain-free anaesthesia during surgical stimulation in adults (22-30 years) and children (1-5 years) are 1.28% and 1.60% (isoflurane administered in 100% oxygen), respectively (Isoflurane Piramal SmPC UK 2019). For sedation, the end-tidal anaesthetic gas concentration is typically slightly more than one-third of the MAC, i.e. slightly above MAC-awake, and 0.3 to 0.5 expired vol.% provides acceptable sedation in adults (Meiser et al. 2005, Sackey et al. 2004). In a paediatric pilot study (Sackey et al. 2005) using the AnaConDa, adequate sedation was achieved in two children with end-tidal isoflurane concentrations of 0.3% to 0.4%, while in a child with refractory status epilepticus, antiepileptic effect was achieved at a higher exposure, 0.9%. In a study with 15 patients, in which isoflurane was used as a rescue medication the end tidal concentration was 0.9 ± 0.12 % (Eifinger et al. 2013).

Published data suggest that patients with status epilepticus require higher exposures than other ICU patients for appropriate effect, and in these patients sedatives are given to suppress epileptic neural activity as monitored by electroencephalography (EEG) rather than to achieve a specific level of sedation, and therefore evaluation of the degree of sedation on the COMFORT scale, the primary efficacy endpoint in the current study, may be inappropriate. Patients with ongoing epileptic activity therefore are unsuitable for inclusion in this sedation efficacy and safety study.

The α2-adrenergic agonists (clonidine, dexmedetomidine) act centrally by stimulating α_2-adrenergic receptors and producing a reduction in sympathetic tone. This counteracts hypertension and tachycardia but also produces mild sedation without causing respiratory depression and exerts anxiolytic effects that are comparable with those of low to moderate doses of benzodiazepines (Wolf et al. 2014). The α2-adrenergic agonists reduce the requirement for other sedative agents and

improve haemodynamic and sympathoadrenal stability. Adverse effects associated with the use of clonidine include bradycardia and hypotension. Clonidine has also been associated with withdrawal syndrome; termination after prolonged administration has been associated with hypertension and seizures, and abrupt discontinuation after prolonged administration should be avoided (Playfor et al 2006). While used in clinical practice, the approved indications for clonidine do not include paediatric sedation (SmPC Catapres™) and for dexmedetomidine only sedation in adults is included in the label (SmPC Dexdor™).

6.1.1 The AnaConDa – A volatile sedative evaporation device

The AnaConDa is a small, disposable volatile anaesthetic agent delivery and reflection system, developed for the administration of isoflurane and sevoflurane to mechanically ventilated patients, without the need for an anaesthesia machine. The AnaConDa (100 mL) and AnaConDa-S (50 mL) are EC-certified according to European Medical Device Directive 93/42/EEC (as risk classification IIa products according to Annex IX) and has been on the market since 2005 and 2017, respectively. The inspiratory placement of AnaConDa has been CE-marked for patients with tidal volumes of 30-200 mL since 2019. The AnaConDa is approved for the delivery of isoflurane and sevoflurane to invasively ventilated patients. The 50 mL AnaConDa-S will be used for all paediatric patients in this study.

The AnaConDa is integrated into the ventilator circuit in place of the commonly used passive heat- and moisture exchanger (HME), between the patient and the Y-piece. It contains a miniature vaporiser and conserving medium consisting of an interwoven lipophilic active carbon filter that also exerts heat and moisture exchanging properties. The volatile anaesthetic is administered continuously via a syringe pump into the miniature vaporiser. The anaesthetic exhaled by the patient enters the reflection system, is predominantly stored in the active carbon filter, and redirected into the inspiratory air.

The unique evaporator is a porous plastic rod with a large surface area which will vaporise isoflurane or sevoflurane due to the volatility of both agents. The evaporator rod evaporates the anaesthetic at a rate proportional to the flow rate setting on the syringe pump. The patient end-tidal concentration (F_{et}) can be adjusted by increasing or decreasing the syringe pump rate while monitoring the F_{et} value on the Gas Monitor. The syringe is a standard 50/60 mL version but with a unique connector system to prevent unintentional IV administration of volatile sedatives. The unique syringe connector only allows for connection to the AnaConDa agent line.

Approximately 90% of the anaesthetic/sedative agent delivered will be reflected when the AnaConDa is placed at the Y-piece of the respiratory circuit. When the AnaConDa is placed on the inspiratory port of the ventilator there will be no reflection of the anaesthetic/sedative agent. A total volume of 1.2 mL of fluid anaesthetic agent is required for prefilling the system. A sampling port from the AnaConDa allows the expired gas concentration to be continuously displayed on the gas monitor. Once the monitor detects the anaesthetic agent in the expired gas, the infusion rate can be titrated to achieve the desired level of sedation.

In contrast to an anaesthesia machine there is no need for additional equipment to maintain operation of the AnaConDa besides the use of standard ICU equipment. There is also no CO_2 absorber to manage which is a critical element of an anaesthesia machine. The AnaConDa is intended for single use and needs to be replaced every 24 hours or when needed, e.g. in patients with mobilisation of significant airway secretions.

6.1.1.1 Implications of adding dead space to the breathing circuit

When a device such as an AnaConDa or a heat and moisture exchanger is placed nearest the Y-piece in the breathing circuit it adds volume to the circuit that the patient rebreathes, so-called dead space. The physician must determine if the patients breathing capacity (tidal volume) is sufficient for the patient to tolerate the additional dead space. If patient tidal volume is too low or insufficient for any reason, then the patient is at risk of rebreathing CO_2 and an alternative placement must be used (see Section 6.1.1.2).

6.1.1.2 Device for the clinical study

For this paediatric clinical study, the AnaConDa-S is to be used in all patients randomised to isoflurane.

The 50 mL AnaConDa-S was developed as a smaller dead space device to mitigate the risk of hypercarbia that the first AnaConDa implied. It is also the preferred device for young adults or adult patients with reduced lung volume. The equivalence evaluation shows that the 50 mL device is comparable to the original 100 mL device for all performance criteria. Preliminary clinical experience and bench testing suggests that this device, when attached in the standard mode at the Y-piece of the respiratory circuit, is unsuitable for patients with tidal volumes below 200 mL. This is due to the 50 mL dead space of the AnaConDa-S, which can cause rebreathing when used in patients with tidal volumes below 200 mL, previously also demonstrated for the original 100 mL AnaConDa (Sackey et al. 2005; Jung et al. 2007; Eifinger et al. 2013; Hoemberg et al. 2012; Palacios et al. 2016; Mencia et al. 2018; Nacoti et al. 2018). In the current study therefore, the alternative placement, on the inspiratory side of the breathing circuit will be used for treatment of patients with tidal volumes 30-200 mL. When placed on the inspiratory limb, the AnaConDa-S will not add any dead space to the breathing circuit. All sites will receive training in the use of AnaConDa-S for both placements prior to the study.

6.2 Research hypothesis

In a systematic review of published studies evaluating the degree of sedation in paediatric patients in the ICU (Vet et al. 2013), 15 studies reported the incidence of degree of sedation as a proportion of observations. Optimal sedation was ascertained in 15 to 93% of observations, undersedation in 0 to 22%, and oversedation in 0 to 82% of observations. In these 15 studies, patients were optimally sedated in 57.6% of the observations, undersedated in 10.6% of the observations, and oversedated in 31.8% of the observations.

Oversedation delays recovery, as greater sedative consumption is associated with longer duration of ventilation as well as extubation failure. Oversedation also induces tolerance and withdrawal syndrome. Undersedation, on the other hand, may lead to increased distress and adverse events (AE) such as unintentional extubation or displacement of catheters (Vet et al. 2013). A difference of 15% or more in the proportion of time critically ill children are adequately sedated is therefore a clinically relevant difference. Similarly, if a new sedative is to be considered non-inferior to the current standard therapy, the proportion of time critically ill children are adequately sedated should not be more than 15% lower than with current standard therapy.

Based on the published data of isoflurane in the adult population and the case series in paediatric intensive care, where isoflurane has consistently been efficacious in providing adequate sedation when intravenous sedation failed, isoflurane is expected to render a larger proportion of time in the sedation interval characterised as adequate sedation in the paediatric population.

The estimated superiority is in the range of 15-20% percentage units and the study is designed to show superiority. The study is also powered for non-inferiority with a pre-defined non-inferiority margin of 15% (relative difference).

6.2.1 Medical need

The standard sedative, midazolam, is associated with relatively long wake up times after discontinuation. An increasing number of publications demonstrate that midazolam or benzodiazepines in general cause severe withdrawal and delirium symptoms (Mody et al. 2016, Mody et al. 2018, Meyburg et al. 2018, Smith et al. 2017, Traube et al. 2017). When used for long-term administration, accumulation of midazolam and its active metabolite in plasma is also a concern for patients with renal or hepatic insufficiency (Playfor et al 2006). There is no other drug approved for continuous sedation in mechanically ventilated paediatric ICU patients. Therefore, there is a medical need for an alternative sedative in paediatric ICU patients.

6.2.2 Potential benefits of isoflurane for sedation in children

Isoflurane is a promising alternative providing adequate sedation in children when conventional sedation failed, as described in several published case series (Arnold et al. 1993, Kelsall et al. 1994, Kita et al. 1995, Sackey et al. 2005, Cooper et al. 2007, Ariyama et al. 2009, Hoemberg et al. 2012, Eifinger et al 2013, Mencia et al. 2018).

Published data indicates that with isoflurane sedation in adults, autonomic functions such as temperature and blood pressure control, respiration and intestinal motility are well preserved (Meiser et al. 2005). These features facilitate modern therapeutic concepts such as early enteral feeding and augmentation of spontaneous breathing. Awakening after inhalational sedation is quick and predictable, extubation can be planned and organised, and the time during which the patient needs very close observation is short (Kong et al 1989, Sackey et al 2004). Long-term follow-up from a randomised trial in adults comparing midazolam and isoflurane/AnaConDa sedation concluded that sedation of ICU patients with isoflurane may result in fewer delusional memories or hallucinations from the ICU compared with intravenous midazolam (Sackey et al. 2008).

Volatile anaesthetics are mainly eliminated by exhalation, and elimination is thus independent of renal and hepatic function, potentially making isoflurane particularly suitable for critically ill children with hepatic or renal insufficiency (Hoemberg et al. 2012). Potential therapeutic benefits in children are assumed to be similar to those demonstrated in adults, involving faster wake-up times, earlier mobilisation, and potentially less AEs than with the current common use of intravenous sedation, as single sedatives or a cocktail of multiple sedatives (Ávila-Alzate et al. 2020, Vet et al. 2016). In addition, published case reports indicate that isoflurane may be effective in sedating epileptic children and children with severe bronchoconstriction, i.e. asthma, and in children for whom spontaneous breathing is beneficial (Johnston et al. 1990, Maltais et al 1994, Restrepo et al. 2005, Turner et al. 2012, Zeiler et al. 2015).

6.3 Rationale for study design, doses and control groups

A randomised age-stratified active-controlled open label design with a blinded assessor is used in this study.

Intravenous midazolam is the most commonly used sedative in invasively ventilated paediatric patients in Europe (see Section 6.1). It is approved for use in paediatric intensive care sedation, and therefore the most appropriate active control for the present study.

European guidelines on sedation in critically ill children recommend that "Doses of sedative agents should be titrated to produce the desired level of sedation" (Consensus Guidelines on Sedation and Analgesia in Critically Ill Children, UK 2006). These guidelines also recommend that the level of sedation be regularly assessed and documented using a sedation assessment scale, wherever possible using a validated scoring system such as the COMFORT Behavior (COMFORT-B) scale (Ista et al 2005, Harris et al. 2016).

Sedated critically ill patients are unable to self-report, which requires observational parameters assessed by healthcare professionals. The desired level of sedation for an individual will vary according to the underlying pathophysiological process and the need for certain therapeutic, invasive or investigative procedures. The primary endpoint of this study involves evaluation of adequately maintained sedation in each patient by investigators using the COMFORT-B scale. The COMFORT-B scale is widely used in paediatric intensive care units to assess young children's pain and distress and is recommended by the European Society of Paediatric and Neonatal Intensive Care (ESPNIC) (Harris et al. 2016). The scale was developed for observations of distress in children receiving ventilation in an intensive care environment (Ista et al 2005). The COMFORT-B scale includes six behavioural items (Maaskant et al. 2016, Appendix A). For clinimetric properties, see Section 11.2.16.

The scheduled assessments of COMFORT-B scale will be performed by an assessor not aware of the treatment arm or the prescribed target sedation depth interval. A blinded assessor is used in combination with randomisation to limit the occurrence of conscious and unconscious bias in the conduct of the study and interpretation of outcomes.

From a safety perspective there are indications (Arnold et al. 1993, Ariyama et al. 2009, Kelsall et al. 1994) that younger patients exposed to isoflurane for longer periods (>24h) may develop transient neurological side effects, such as psychomotor events. These transient symptoms have consistently appeared during or in the first 24 hours after cessation of isoflurane sedation (Arnold et al. 1993, Ariyama et al. 2009, Kelsall et al. 1994, Sackey et al. 2005). The recruitment algorithm is designed to allocate more power to sensitive patient groups for safety evaluation. The study will randomise no more than 100 patients in order to achieve approximately 90 evaluable patients, based on randomisation 2:1 – twice as many patients will receive isoflurane - to inform the risk of isoflurane-specific AEs such as those reported by Ariyama et al. 2009. For purposes of safety evaluation, to ensure that a majority of patients are exposed in the younger ages, and to ensure sufficient numbers for safety evaluation across age groups, a structured recruitment is employed with a targeted number of patients for each age group (Table 1).

Table 1 Number of treated patients, by age group

Age group*	**Minimum number of treated patients**
3 to 7 (less than 8) years	At least half of all treated patients
8 to 11 (less than 12) years	At least 20 treated patients Maximum 25 treated patients
12 to 17 (less than 18) years	At least 20 treated patients Maximum 25 treated patients
Total	**No more than 100 treated patients**

*age at randomisation

The randomisation will be stratified by the three age groups and type of ICU admission (planned or unplanned mechanical ventilation). Type of ICU admission is used as proxy for study treatment duration. The actual study treatment duration will not be known with certainty at the time of randomisation. However, it is expected that most of the patients enrolled in the study due to planned surgery and need for postoperative mechanical ventilation will have a shorter sedation need compared to patients admitted in an unplanned situation. In most cases, it is expected that the need for sedation in these patients would be 12-24 hours, while patients with unplanned intubation and mechanical ventilation may require a longer sedation period. Therefore, the reason for ICU admission (planned mechanical ventilation or unplanned mechanical ventilation) for enrolled patients will be used as a stratification factor with the aim to balance the treatment allocation between patients with shorter duration of sedation versus longer duration of sedation.

Based on the above discussions, the following rules for when to stop enrolment per age group have been defined and are described in Table 2.

Table 2 Rules for when to stop enrolment per age group

Age group	Rule(s)
Age 3 to 7 (less than 8) years	• Enrolment should not be stopped before at least half of all treated patients have been randomised into this subgroup
8 to 11 (less than 12) years	• Enrolment should not be stopped before at least 20 treated patients have been randomised into this subgroup • Enrolment should be stopped if maximum 25 treated patients have been randomised into this subgroup.
12 to 17 (less than 18) years	• Enrolment should not be stopped before at least 20 treated patients have been randomised into this subgroup • Enrolment should be stopped if maximum 25 treated patients have been randomised into this subgroup.
Total	Enrolment should not be stopped before at least 90 patients in total have been confirmed as evaluable for the primary endpoint.

When the number of evaluable patients defined for a target group is reached, that group will be closed for enrolment and investigators will be instructed to limit enrolment to the other, still open target groups.

In addition to the above-described stratification factors, the country where the patient is enrolled will also be a stratification factor in order to balance the number of patients receiving isoflurane vs midazolam across the clinical sites and to adjust for any differences in local clinical practice.

The dosing in the isoflurane group is expected to result in significantly lower end-tidal concentrations than those recommended for maintenance of anaesthesia for the paediatric age group concerned (e.g. Isoflurane Piramal SmPC UK 2015). Mean or median exposures achieved at the desired degree of sedation may constitute recommended levels in a future approved product label for paediatric patients.

Patients will be closely followed using standard ICU monitoring of vital functions (intermittent or continuous assessment of heart rate (HR) and peripheral arterial oxygen saturation, intermittent or

continuous assessment of systolic and diastolic blood pressure (BP)) (Section 11.2.10), depth of sedation (Section 11.2.16), documentation of parameters of mechanical ventilation (Section 11.2.13) and intermittent blood gas analysis (Section 11.2.15). Organ safety will be evaluated by laboratory assessment of renal and liver function, from baseline up to the end of the 48-hour post-study treatment monitoring (Section 11.2.14). After the 48-hour post-study treatment monitoring patients will be followed on a weekly basis until 30±2 days after end of study treatment (Section 11.1.3.2).

6.4 Benefit/risk and ethical assessment

Midazolam is the only labelled continuous intravenous therapy in Europe and the US for paediatric sedation in mechanically ventilated ICU patients. However, due to tolerance development to midazolam in a significant proportion of critically ill paediatric patients, dosage tends to escalate or polypharmacy is employed to manage severe agitation, hypertension, tachycardia and later withdrawal in mechanically ventilated children (Tobias 2000, Grant et al. 2013, Wolfe et al. 2014, Lebet et al. 2018, Taffarel et al. 2018,) despite the off-label status of other sedative drugs. Delirium related to the use of benzodiazepines, such as midazolam, is associated with prolonged ICU stay in paediatric patients (Smith et al. 2017).

Isoflurane has been used for induction and maintenance of anaesthesia in millions of adult and paediatric patients since the 1980s, during which time the concentrations significantly exceed the concentrations needed for ICU sedation, therefore the safety profile of the immediate effects of isoflurane is considered well-categorized. Advantages observed when using isoflurane for ICU sedation in adults include a more rapid onset of action, bronchodilation, rapid pulmonary clearance and minimal metabolism and fast wake-up time (Sackey et al 2004). In paediatric intensive care, isoflurane has been used successfully as a rescue therapy when intravenous sedation strategies have been exhausted (Arnold et al. 1993, Kelsall et al. 1994, Kita et al. 1995, Sackey et al. 2005, Cooper et al. 2007, Ariyama et al. 2009, Hoemberg et al. 2012, Eifinger et al 2013). Thus, there is reason to believe that isoflurane sedation may be better than intravenous midazolam in terms of maintaining adequate sedation with minimal additional concomitant medications.

Reversible psychomotor events are known to occur in a proportion of the youngest children exposed for longer periods of time to isoflurane (Ariyama et al. 2009, Kelsall et al. 1994, Sackey et al. 2005). A comparison of the efficacy and safety of these sedative agents for sedation in paediatric intensive care is therefore warranted and will be performed through this study.

On an individual level, isoflurane sedation in the intended dose for the intended duration is likely to be more efficacious than intravenous sedation with midazolam. The risks for severe immediate or prolonged side effects appears to be very small.

On a population level, an approved alternative for paediatric sedation in the ICU may improve the possibilities for physicians to provide better sedation and improved outcomes in this vulnerable population. Commonly observed adverse events when using midazolam for sedation in ICU such as delirium and withdrawal symptoms may potentially be avoided or reduced in patients on isoflurane. This, in combination with the rapid clearance and shorter wake-up times observed when using isoflurane in adults may mean a shorter ICU stay for paediatric patients, allowing a quicker turnaround of patients in the ICU.

Considering the long-standing clinical paediatric experience of isoflurane for induction and maintenance of anaesthesia since the 1980s, at exposures (MAC values) well exceeding those required for sedation, the clinical need for an alternative sedative for paediatric ICU sedation, and the published clinical experience of isoflurane for sedation in adult and paediatric patients, the benefits

of being exposed to investigational isoflurane sedation are consider to outweigh the risks for patients entering the current study.

7. STUDY OBJECTIVES AND ENDPOINTS

7.1 Primary objective

To compare the percentage of time adequate sedation depth is maintained within the individually prescribed target range in absence of rescue sedation as assessed according to the COMFORT-B scale, in isoflurane vs midazolam treated paediatric patients for an expected minimum of 12 hours.

7.1.1 Primary endpoint

Percentage of time of adequately maintained sedation within the COMFORT-B interval (light, moderate or deep sedation) prescribed at randomisation, monitored every 2 hours for a minimum of 12 hours (up to 48 hours ± 6 hours).

The primary endpoint assessment will start 2 hours after initiating study sedative treatment (or in the case of ongoing sedation, 2 hours after terminating ongoing sedatives). The primary endpoint assessment will be collected either until the study treatment is replaced with the standard treatment (at 48± 6 hours from study treatment initiation) or when the wake-up for extubation is started, whichever comes first. The COMFORT-B intervals are defined in Table 8.

7.2 Secondary objectives

7.2.1 Secondary objectives, efficacy

- Compare the use of opiates, and the development of tolerance to the sedative regimen as measured by the change in dose of study drug, opiates and other analgesics, over time in isoflurane- vs midazolam-treated patients.
- Compare the need for rescue sedatives and other sedatives in isoflurane- vs midazolam-treated patients.

7.2.2 Secondary objectives, safety

- Compare time from sedation termination to extubation in isoflurane- vs midazolam-treated patients.
- Compare the proportion of time with spontaneous breathing in isoflurane- vs midazolam-treated patients.
- Evaluate haemodynamic effect as indicated by inotropic/vasopressor agent administration in patients sedated with isoflurane compared with midazolam.
- Evaluate the frequency of withdrawal symptoms in isoflurane- vs midazolam-treated patients.
- Evaluate the frequency of delirium in isoflurane- vs midazolam-treated patients.
- Evaluate the frequency of neurological symptoms or psychomotor dysfunction during and up to 48 hours after discontinuation of isoflurane and midazolam treatment, and the association with duration of treatment, and total exposure (MAC hours and midazolam doses) over time.
- Compare the 30 days/hospital mortality in isoflurane- vs midazolam-treated patients.
- Compare ventilator-free days up to 30 days in isoflurane- vs midazolam-treated patients.
- Compare the time in ICU/hospital up to 30 days in isoflurane- vs midazolam-treated patients.
- Compare ICU-free days up to 30 days in isoflurane- vs midazolam-treated patients.

- Compare the safety profile in terms of experienced adverse events, safety laboratory values, blood gases, vital signs, body temperature and urinary output in isoflurane- vs midazolam-treated patients.

7.3 Secondary endpoints

7.3.1 Secondary endpoints, efficacy

- Dose of opiates, study drugs and other analgesics required, from first blinded COMFORT-B assessment (at +2 hours) to end of study treatment period, given per 24 hours.
- Mean dose of study drugs, opiates and other analgesics required, during the last 4 hours of study treatment, as compared to the first 4 hours of study treatment after first blinded COMFORT-B assessment.
- Mean dose of rescue propofol (mg/kg/24 hours) and mean dose of rescue ketamine/es-ketamine (converted to ketamine-equivalents mg/kg/24 hours), and mean dose of α2-adrenergic agonists (mg/kg/24 hours) to maintain the COMFORT-B score in the individually prescribed range, in isoflurane- vs midazolam-treated children (time window: from 2 hours after initiating study sedative treatment to end of sedative treatment).
- Number of doses of rescue sedation (propofol, ketamine, es-ketamine) given per 24 hours from first blinded COMFORT-B assessment (at +2 hours) to end of study treatment period.

7.3.2 Secondary endpoints, safety

- Time from end of study drug administration to extubation if study drug is terminated for extubation.
- Proportion of observations with spontaneous breathing efforts during study treatment.
- Need for additional inotropic/vasopressor agent and change in VIS score during study treatment period compared to baseline.
- Presence of withdrawal symptoms as assessed using the SOS-PD scale in patients exposed to more than a total of 96 hours sedation (including pre-study sedation period) until the end of the 48-hour post study treatment monitoring or ICU discharge, whichever comes first.
- Presence of delirium as assessed using the SOS-PD scale in patients admitted to the ICU for at least 48 hours (including period prior to study enrolment) until the end of the 48-hour post study treatment monitoring or ICU discharge, whichever comes first.
- Proportion of patients experiencing psychomotor dysfunction or neurological symptoms during sedation and/or in the 48 hours after discontinuation of isoflurane or midazolam treatment, in relation to duration of exposure to isoflurane or midazolam, and to cumulative midazolam mg/kg or isoflurane exposure (MAC hours).
- 30 days/hospital mortality.
- Ventilator-free days at 30 days from start of study treatment period.
- Time in intensive care unit/hospital at day 30 from start of study treatment period.
- Days alive and not in the ICU at day 30 from start of study treatment period.

- Proportion of patients with common as well as sedation-related adverse events, and frequencies of these adverse events from start of study treatment to end of 48-hour post study treatment monitoring.
- Frequency and intensity of adverse events from start of study treatment to day 30.
- Changes in vital signs, blood gases, body temperature and urinary output from baseline to end of study treatment.
- Changes in clinical chemistry and haematology parameters from baseline up to the 48-hour post-study treatment monitoring.

7.4 Exploratory objectives

- Determine the isoflurane dosage, end-tidal concentrations and infusion rates, and the midazolam dosage, required for adequate sedation in mechanically ventilated paediatric patients.
- Evaluate frequency and type of AnaConDa-S device deficiencies when used for sedating patients with isoflurane.

7.4.1 Exploratory endpoints

- The mean and median dose (MAC value and end-tidal concentration) of isoflurane and mean and median dose of midazolam required for achieving the target level of sedation, over time, by age group.
- Number of study drug bolus doses given per 24 hours during midazolam and isoflurane sedation of mechanically ventilated patients.
- Ventilator parameters (ventilation mode, tidal volume, minute volume, fraction of inspired oxygen (FiO_2), end tidal carbon dioxide [$EtCO_2$], total breathing rate, positive end-expiratory pressure [PEEP], set inspiratory pressure [Pinsp], level of pressure support [PS] above PEEP).
- Frequency and type of AnaConDa-S device deficiencies during isoflurane sedation.

8. INVESTIGATIONAL PLAN

8.1 Overall study design and flow chart

This is a therapeutic confirmatory (phase III), multicentre, open label with a blinded assessor, randomised, age-stratified, active controlled study vs. standard treatment. Patients will be assigned to a sedative treatment through randomisation in a 2:1 ratio to either inhaled isoflurane via the AnaConDa-S, or standard treatment (IV midazolam). The study treatment duration is expected to be a minimum of 12 hours and up to 48±6 hours with a follow-up period of 30±2 days.

Patients eligible for the study will involve two categories, either planned need for mechanical ventilation and ICU admission (e.g. due to a planned surgery) or need for mechanical ventilation in an unplanned situation. The study will be stratified by this category of reason for ICU admission as well as age group and country. No more than 100 patients at approximately 20 sites in Europe will be randomised, to reach 90 patients evaluable for efficacy.

Patients will be switched from ongoing sedative treatment to the randomised study treatment. Ongoing sedative treatment must not have exceeded 72 hours at the time of randomisation. When possible, informed consent for postoperative patients (and assent when appropriate) should be obtained prior to surgery. In some cases, when such patients are not identified before ICU admission, informed consent and assent may be obtained in the ICU. If the patient is unconscious at the time of inclusion, information about the study will be given and assent obtained as soon as the patient's condition allows. Informed consent will be obtained from the legal guardian(s) whether the patient is conscious or unconscious at the time of inclusion in accordance with local regulation. The patients admitted to the participating ICUs with unplanned need for mechanical ventilation will be identified via a pre-screening process (log), based on available clinical data prior to obtaining informed consent. Refer to Sections 9.1 and 9.2 for a detailed description of recruitment, screening and informed consent processes.

Following the informed consent procedure, patients will be formally screened and if all criteria are met, randomised using digital media to inhaled isoflurane by use of the AnaConDa-S, or intravenous midazolam in a 2:1 ratio. Shortly prior to randomisation, the Investigator/study team determines the sedation depth and prescribes the target sedation depth as either "light", "moderate" or "deep", based on the COMFORT-B scores (see Section 11.2.16, Table 8). Study treatment will be titrated to achieve and maintain the target sedation depth. The sedation depth will be assessed through blinded COMFORT-B assessments every two hours throughout the study treatment period and will, in the statistical analysis, be adjusted for potential confounding factors such as the need of rescue sedation, α2-adrenergic agonists, extra sedation agents administered due to therapeutic or diagnostic procedures and use of analgesia (incl. opiates). Therefore, administration of rescue and procedural sedation agents as well as analgesia will be recorded throughout the study treatment period including doses administered.

One change of the target sedation depth (light, moderate or deep) will be allowed for the welfare of the patient during the study treatment period. This optional single change of prescribed target sedation depth is not intended for shorter medical procedures, when additional sedation/analgesia is permitted to the clinically indicated level. The time and medical rationale for changing the prescribed sedation target should be recorded in the electronic case report form (eCRF).

The SOPHIA Observation Withdrawal Symptoms Scale – Paediatric Delirium (SOS-PD) will be evaluated in patients which have been admitted to the ICU for 48 hours or more (SOS-PD delirium module) and in patients which have been exposed a total of 96 hours of sedation or more, including the pre-study sedation period (SOS-PD withdrawal module). From these time points, the SOS-PD

delirium and withdrawal modules will be evaluated every 8 hours respectively during the remaining study treatment period until the end of 48-hour post-study treatment period or until the patient is discharged from ICU, whichever comes first.

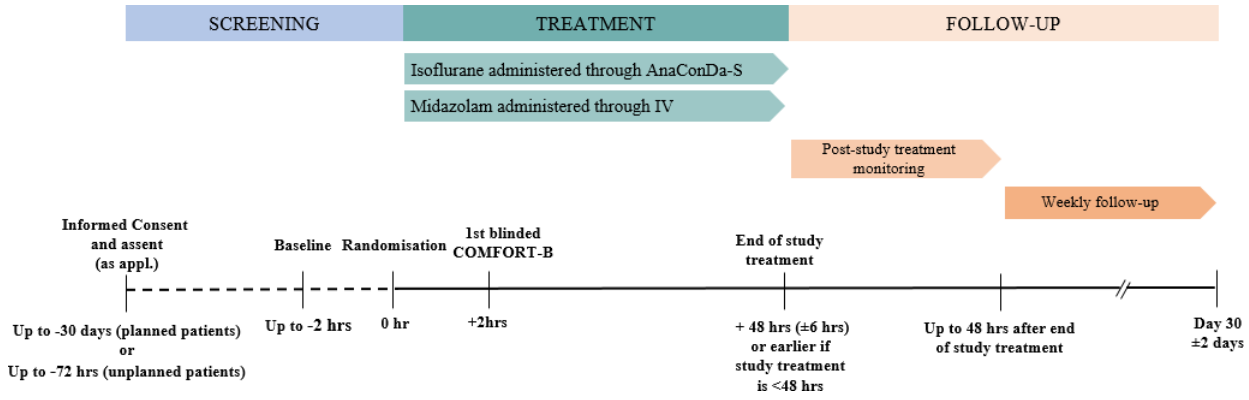
Vital signs and ventilator parameters will be collected every 2 hours. In addition, clinically significant abnormalities in any of these parameters occurring between these 2-hour-intervals will be recorded as an adverse event. Blood gases, body temperature and urinary output will be collected every 8 hours.

After the 48±6 hours of study treatment, the treating physician may continue sedation of the patient according to local practice. The patient will be followed closely for 48 hours after end of study treatment, including a follow-up safety laboratory assessment, or until the patient is discharged, if this is earlier than 48 hours after end of study treatment. After this initial 48-hour post-study treatment monitoring the patient will be followed up on a weekly basis up to 30±2 days after end of study treatment. Adverse events will be recorded weekly, either by examining the patient in the hospital or through contact with the patient/legal guardian(s) or caregiver if patient is discharged. On day 30, the Investigator will record, based on review of the patient's medical records, or by contacting the patient, legal guardian(s) or caregiver, the number of ventilator-free days, time in ICU/hospital and mortality.

In the case of identified neurological symptoms, such as psychomotor events (known event in young patients exposed for longer periods of time to isoflurane (Ariayma et al. 2009, Kelsall et al. 1994, Palacios et al. 2016, Sackey et al. 2005)) each event will be monitored at least daily in the first week after end of study treatment or until resolution. Persistent neurological or behavioural symptoms, as well as other ongoing AEs will be monitored until resolution or referral to a physician for follow-up.

A chart describing the flow of the study is provided in Figure 1. An overview of the assessments to be performed at each visit are given in Table 6. Details of the assessments are provided in Section 11.1 and 11.2. The rationale for the study design is discussed in Section 6.3.

Figure 1 Study flow chart



Describes the study flow, including the screening, treatment and follow-up periods.

9. SELECTION OF PATIENT POPULATION

9.1 Recruitment and screening

The participating patients will be recruited at the study sites and may be referred from other departments at the same hospital or from another hospital. Potential candidates will be patients of age 3-7, 8-11 or 12-17 years that require mechanical ventilation in either of the two categories:

a) Planned mechanical ventilation: Patients planned for ICU admission (postoperative patients), who will start study treatment in the ICU after leaving the operating room.
b) Unplanned mechanical ventilation: Patients admitted to ICU in an unplanned situation and requiring mechanical ventilation

The study sites are advised to designate a person responsible for regular pre-screening, based on available clinical or chart information, to identify potential study patients among patients admitted to the clinic/study site. Potential candidates and their legal guardian(s) will be contacted and invited to receive written and oral information about the study (see Section 9.2).

Investigators must keep a record (e.g. a patient pre-screening log) of all patients who entered the pre-study screening and were considered for enrolment even if they were not subsequently enrolled. This information is necessary to verify that the patient population was selected without bias (in accordance with ICH-GCP 8.3.20). The reasons for non-eligibility are to be defined in terms of one or more of the eligibility criteria.

A screening number will be allocated to each patient for whom informed consent (and assent as applicable) has been provided (refer to Section 9.2). The screening number will be generated automatically in the eCRF. The screening number will allow identification of patients irrespective of their possible eligibility for the study.

The recruitment will be monitored to ensure the inclusion of patients is balanced to the target patient groups defined in Section 6.3. When the number of patients defined for a target a group is reached, that group will be closed for enrolment and investigators will be instructed to limit enrolment to the other, still open target groups.

9.2 Informed consent and assent process

The informed consent and assent processes used in this study will follow applicable local regulations. Master patient information sheets, informed consent forms and assent forms will be created and adapted according to local regulations.

9.2.1 Informed consent from legal guardian(s)

The legal guardian(s) of patients considered to potentially be eligible to enter the study will be approached with information about the study. They will be provided with a written patient information sheet and informed consent form, the Investigator will discuss the study in detail with the legal guardian(s), describe its objectives and procedures as well as the possible benefits and risks involved. The Investigator will ensure they have ample time to ask questions about the study. They will be informed about the voluntary nature of the study and that they are free to decline participation or discontinue the study at any time without it affecting the care of their child. The legal guardian(s) will also be informed in writing of how the data from the study will be stored and analysed, maintaining confidentiality, in accordance with local data protection laws.

If the legal guardian(s) is willing to enter the patient in the study, they will be asked to sign and date the informed consent form in accordance with local regulations. A copy of the written information and the signed ICF will be provided to the legal guardian(s).

9.2.2 Assent and consent from patients

If appropriate and possible (if patient is not sedated at the time of recruitment) the patient will be approached to provide assent for participation in the study. For patients sedated at the time of recruitment the assent process will be completed as appropriate after the patient has awoken.

The Investigator will describe the study, its objectives, procedures and possible benefits and risks involved in a manner appropriate to the patient's age and level of understanding. If the patient is able to read, he/she will be provided with an age-appropriate assent form with information about the study and will be asked to sign and date this, if in accordance with local regulation. For children not yet able to read, the Investigator will together with legal guardian(s) read a verbal assent form to the patient.

If the child is able to understand the information given about the study and after consideration declines participation, this must be respected and the child will not enter the study, even if his/her legal guardian(s) have provided informed consent. If this decline of assent is communicated after the study treatment period due to the patient not being able to provide assent before enrolment, the patient's wishes will be respected and patient will be discontinued from the study, in accordance with Section 9.6.

All assent forms used will be witnessed by the legal guardian(s) and signed by the Investigator as applicable in accordance with local regulations. A copy of the signed assent forms will be provided to the patient or their legal guardian(s).

In some countries, children over a certain age and level of understanding are considered capable to provide informed consent. For these patients, the informed consent should be obtained according to the same process outlined for legal guardian(s) in Section 9.2.1 above. If such a patient is sedated at the time of recruitment the legal guardian(s) can provide informed consent as per local regulations.

9.3 Patient selection criteria

Each patient randomised should meet all of the inclusion criteria and none of the exclusion criteria for this study. Under no circumstances can there be exceptions to this rule.

9.3.1 Inclusion criteria

For inclusion in the study patients should fulfil the following criteria:

1. Paediatric patients at least 3 years to 17 (less than 18) years at the time of randomisation, admitted to an ICU/with planned ICU admission. Patients who will turn 18 years during the study (including follow-up) will not be included.

2. Expected mechanical (invasive) ventilation and sedation for at least 12 hours.

3. Informed consent obtained from the patient, patient's legal guardian(s) as required by local regulations. Where applicable, assent obtained from the patient to participate in the clinical study.

4. **Germany only:** Women of childbearing potential who are sexually active: The patient does already use a highly effective contraception method or agrees to use a highly

effective contraception method from signing the informed consent form (screening) until the operation/study treatment. According to Clinical Trials Facilitation Group (CTFG) recommendations, methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include:

- combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal)
- progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable)
- intrauterine device
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised partner
- sexual abstinence or absence of sexual relations with men

9.3.2 Exclusion criteria

Patients should not enter the study if any of the following exclusion criteria is fulfilled:

1. Ongoing seizures requiring acute treatment.

2. Continuous sedation for more than 72 hours at time of randomisation.

3. Less than 24 hours post cardiopulmonary resuscitation.

4. Uncompensated circulatory shock.

5. Known hypersensitivity to isoflurane or to other halogenated anaesthetics (such as halothane), benzodiazepines, non-investigational medicinal product(s) (analgesics, additional rescue sedatives that may be required during the study) or to any of their formulation ingredients.

6. Known or suspected genetic susceptibility to malignant hyperthermia.

7. Patients with acute asthma or obstructive lung disease symptoms requiring treatment at inclusion.

8. Patient with tidal volume below 30 mL or above 800 mL.

9. Inability to perform reliable COMFORT-B assessment in the opinion of the Investigator e.g. due to (not limited to):

   Severe traumatic brain injury, intracranial pathology (tumour, haemorrhage, infections), with a profound effect on the level of consciousness, severe mental retardation, major congenital anomalies of the central nervous system, severe myasthenia gravis, spinal muscular atrophy, or other severe neurologic disease, ongoing neuromuscular blockade which precludes COMFORT-B scoring.

10. Patients with intracranial pressure (ICP) monitoring or with suspected increase in ICP

11. Patients with treatment-induced whole-body hypothermia.

12. Patients with pheochromocytoma.

13. Patients with prolonged QT interval or with significant risk for prolonged QT interval.

14. Patient not expected to survive next 48 hours or not committed to full medical care.

15. Female patients who are pregnant or breast-feeding.

16. Previous participation in the study (a patient can only participate once).

17. Known participation in any other clinical study that included drug treatment within three months of the first administration of the IMP.

18. Any for the study relevant medical history, or ongoing clinically significant disease, disorder or laboratory result which, in the opinion of the Investigator, precludes participation in the study for medical or ethical reasons.

9.4 Method of assigning patients to treatment groups

9.4.1 Randomisation

An independent statistician at the CRO will create a computer-generated randomisation list and randomisation will be performed centrally through a digital media system prior to administration of the first dose of IMP. Each patient will receive a unique randomisation number generated in the digital media. The permuted block randomisation will be stratified by age group (3-7 years/8-11 years/12-17 years), ICU admission categories (Planned mechanical ventilation/Unplanned mechanical ventilation), and country.

9.5 Procedures for handling incorrectly included patients

Patients that do not meet the inclusion/exclusion criteria for the study should not, under any circumstances, be enrolled into the study.

Where patients that do not meet the eligibility criteria are enrolled in error, a discussion must occur between the Sponsor's Medical Representative/Medical Monitor and the Investigator regarding whether to continue or discontinue the patient from the study. The Medical Monitor is to ensure all such decisions are appropriately documented. In situations where an agreement cannot be reached, the patient should have their randomised therapy stopped and proceed to end of treatment assessments per definition of treatment discontinuation in below Section 9.6.4.

9.6 Premature discontinuation

9.6.1 Study discontinuation due to withdrawal of consent

Patients, or the patient's legal guardian(s) may decide to discontinue the patient's participation in the study at any time and for whatever reason without having to justify their decision or having it affect their right to clinical care according to local practice. If the reason for discontinuation is due to withdrawal of consent, the reason for withdrawal of consent should be documented, if possible.

9.6.2 Treatment discontinuation due to lack of efficacy

In case a patient cannot be adequately sedated with study treatment in accordance with Sections 10.2- 10.5, the patient should be discontinued from the study treatment and treated according to the attending physician's judgement.

However, the patient should continue in the study even if their study treatment is discontinued; all assessments required at the end of treatment visit and follow up visits will be conducted and recorded in the eCRF, if possible.

9.6.3 Other reasons for treatment discontinuation

Patients must be discontinued from the study treatment in case of:

- Emergence of an unacceptable adverse event, defined as malignant hyperthermia, severe refractory hemodynamic instability related to the IMP (severe hypotension) or severe psychomotor dysfunction due to the IMP.
- The participant experiences a medical emergency that necessitates permanent discontinuation of study treatment.
- Patient requiring continuous neuromuscular blockade after inclusion.

Patients may also be discontinued from the study treatment at any time at the discretion of the Investigator, due to any of following reasons:

- New risk to the patient as judged by the Investigator and/or the Sponsor.
- Severe non-compliance to the CSP as judged by the Investigator and/or the Sponsor.
- Patient was incorrectly enrolled (see Section 9.5).

In these cases, the patient should continue in the study even if their study treatment is discontinued; all assessments required at the end of treatment visit and follow up visits will be conducted and recorded in the eCRF, if possible.

9.6.4 Procedures for discontinuation of a patient from the study

If a patient discontinues study treatment prematurely, this will be considered to be a case of treatment discontinuation. This definition only includes cases as described in Sections 9.6.2- 9.6.3 above where the patient discontinues study treatment but still requires sedation. If the patient's need for sedation becomes shorter than the initially expected minimum of 12 hours, and the study treatment is stopped prematurely for this reason, the patient will be considered to have completed the study treatment period.

The date, time and reason(s) for treatment discontinuation and the presence of any adverse events will be documented in the eCRF. Unless the patient or their legal guardian(s) withdraws consent, the patient should continue in the study even if their study treatment is discontinued; all assessments required at the end of treatment visit and follow up visits will be conducted and recorded in the eCRF, if possible.

If a patient completes the study treatment period but discontinues from the study in the follow up period, or discontinues the study prior to initiating the study treatment, this is considered to be a case of study discontinuation. The patient is free to do so at any time and if so, no further follow-up or data collection will be performed for that patient.

If a patient discontinues the study treatment or the study, his/her enrolment/randomisation code cannot be reused. Patients who have discontinued their participation in the study cannot re-enter into the study.

9.6.5 Premature termination of study

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

Conditions that may warrant termination of the study include, but are not limited to the following:

- The discovery of an unexpected, serious, or unacceptable risk to the patients enrolled in the study.
- Recommendation from the Data Safety Monitoring Board (DSMB) to discontinue the study.
- A strategic decision on the part of the Sponsor to suspend or discontinue the study.

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

10. STUDY TREATMENTS

10.1 Study treatments

Patients in this study will be allocated to receive either isoflurane (Piramal) administered via the AnaConDa-S device or midazolam administered as an IV infusion. Both isoflurane and midazolam are referred to as Investigational Medicinal Product (IMP) in this CSP.

The isoflurane and midazolam provided for the purpose of this study may only be used during the study treatment period. If the need for sedation remains after the study treatment period, such medication will be provided by the clinic sites in accordance with their standard practice. The use of the IMP after the end of the study treatment period is prohibited.

Any other medications (except for the IMP) required for the care of the patients will be provided by the clinic sites.

10.1.1 Identity of investigational medicinal products

The identity of the IMPs is presented in Table 3.

Table 3 Identity of the investigational medicinal products

Investigational medicinal product	Dosage form and strength	Packaging	Manufacturer	Batch number
Isoflurane	Inhalation, 100% v/v	250 mL	Piramal	*
Midazolam	IV Solution for infusion, 1 mg/mL or 2 mg/mL	**	**	*

* The batch number will be recorded in the Study Master File and will be reported in the Clinical Study Report (CSR).

** The brand, company name and packaging will be recorded in the Study Master File and will be reported in the CSR.

10.1.2 Packaging and labelling

Commercially available isoflurane and midazolam will be provided by the Sponsor through an authorised Service Provider (PCI, Bridgend, United Kingdom).

Study-specific labelling of the IMP will be carried out at a central warehouse of the Service Provider. Labels will be prepared in accordance with Good Manufacturing Practice (GMP) Annex 13 and local regulatory guidelines.

10.1.3 Shipping, dispensing and accountability

The Service Provider will ship IMP kits to local warehouses, pharmacies or directly to the study sites, depending on local regulatory requirements.

All deliveries and dispensing activities should be documented by maintaining IMP accountability logs. The study site will maintain an IMP accountability log detailing the dates and quantities of IMP and other study products received, dispensed to, used by, each patient and products returned or destroyed at the end of the study. The study monitor will verify the IMP accountability throughout the study.

The IMPs provided for this study shall be used only as directed in the CSP and only during the study treatment period.

The Investigator is responsible for establishing routines for correct handling of the IMP at their site, to ensure that:

- IMP are correctly received, counted for and logged, by a designated person.
- Accurate records are maintained, accounting for the receipt and disposition of the IMP
- IMP are handled and stored safely, properly, and in agreement with the given handling and storage instructions.
- The IMPs are prescribed only by a person authorised to do so by the Investigator.
- The IMPs are only dispensed by designated site staff member(s) noted on the Site Delegation Log.
- Record dispensing of IMP to patients on the IMP Dispensing Log. At least the following will be documented; patient ID, the product and quantity dispensed, date of dispensing, and leftover IMP from the dispensed unit returned to storage after patient has completed the study. This record must be kept in addition to any IMP administration information recorded in the eCRF or the patients' medical chart/source documents.
- All unused IMP and empty containers are stored until they have been checked by the monitor.

After verifying drug accountability, study site personnel or the monitor will return all unused IMP and all empty bottles to the Sponsor or designee for destruction or destroy locally upon agreement with, and approval from, the Sponsor or designee.

10.1.4 Storage

All IMP should be kept in a secure place with limited access under appropriate storage conditions and in accordance with local regulations.

Isoflurane should be stored in room temperature (not above 30°C). The bottles should be kept tightly closed.

Midazolam should be stored in room temperature (under 25°C). The product is light sensitive and should be stored in its original package.

Temperature logs should be kept for the storage room where the IMPs are stored. The min/max temperature should be noted daily (working days sufficient unless automatic temperature readings are available).

10.1.5 AnaConDa-S Supply, Storage and Accountability

The AnaConDa-S devices for use in patients randomised to isoflurane treatment will be manufactured under the responsibility of Sedana Medical Ltd., Twomilehouse, Naas, Co. Kildare, Ireland. The AnaConDa-S devices will be provided in their commercial packaging and labelled with a Unique Device Identifier. The Instructions for Use will be provided within the packaging. The AnaConDa-S devices provided for this study will only be used during the study treatment period. The use of the AnaConDa-S devices will be accounted for during the trial using a device accountability log.

In addition to the AnaConDa-S, the study sites will be provided with auxiliary supplies required for the use of the AnaConDa-S, such as the unique AnaConDa syringes, gas sampling connector, Isoflurane filling adapter, FlurAbsorb and FlurAbsorb accessory kit. The AnaConDa-S and auxiliaries should be stored in room temperature.

10.2 Doses and treatment regimens

10.2.1 Target sedation depth based on COMFORT-B interval

Before randomisation, the Investigator or designee will first assess COMFORT-B and thereafter prescribe the desired degree of sedation for each patient as one of three COMFORT-B intervals – light, moderate or deep sedation (refer to Section 11.2.16). This will be considered the target sedation depth to reach and maintain and will guide the treating ICU staff in their titration of study treatment to the patient. See Section 10.2.6 for description on change of target sedation depth.

10.2.2 Initiation of study treatment

Dose-titration to reach the desired target sedation depth is performed during initiation of study treatment and is expected to be completed within 2 hours of initiating study treatment. During this initial titration of study treatment, the patient's sedation level is assessed continuously by the Investigator or designee who is un-blinded to the study treatment allocation. A minimum of one non-blinded COMFORT-B score should be assessed during the titration phase.

Each dose and time point for modifying the dose or the dose-infusion rate should be recorded. The occurrence of symptoms potentially related to the dose-titration, and the duration of these symptoms, must be recorded as adverse events in accordance with Section 11.2.20.

All other continuous or intermittent sedatives, including α2-adrenergic agonists or propofol, must be terminated at the time of initiating study treatment. Depending on how long the patient has been sedated prior to enrolment and their current clinical status, the investigator may terminate the ongoing sedatives up to 30 minutes prior to initiating study treatment (for example deep midazolam sedation for almost 3 days). For a full description of medications prohibited during the study treatment period, refer to Section 10.4.

The initiation and dosage of inotropic/vasopressor agents in patients is based on clinical signs such as heart rate, blood pressure, volume status, diuresis, together with other monitoring indices, clinical

chemistry and the patients ongoing and anticipated clinical course. The complex information in these parameters informs the clinical decision of the Investigator regarding the choice and dosage of these medications. Use of isoflurane in hypovolaemic, hypotensive and debilitated patients has not been extensively investigated. Caution should be exercised when administering isoflurane to such patients. A lower dose may be considered in these patients. The administration of inotropic/vasopressor agents should be recorded in eCRF as described in Section 11.2.19.1.

10.2.3 Isoflurane administered via AnaConDa-S

- Active compound: Isoflurane
- Dosage form: Inhalation
- Packaging: 250 mL bottles containing 100% v/v

Isoflurane is supplied in 250 mL bottles containing 100% v/v of isoflurane. Isoflurane should be brought to 18-25 °C before administration in order to avoid gas volume expansion upon warming (so called auto pumping).

The AnaConDa-S is set up according to the instruction for use together with a gas monitor and elimination system and should be used according to the instructions for use provided.

10.2.3.1 Dose-titration of isoflurane

The syringe is filled with 50 mL of isoflurane and put into the syringe pump. Sedation will start after priming of the AnaConDa-S, with 1.2 mL isoflurane. The syringe pump is started at an initial dose-rate of isoflurane (Table 4) and in the case of ongoing sedative treatment, all other sedatives are simultaneously turned off (may be turned off up to 30 minutes prior to initiating isoflurane).

During initiation and until the prescribed target sedation depth is achieved and considered stable, sedation depth will be assessed regularly, and isoflurane dosage will be titrated stepwise by increasing/decreasing the infusion rate (Table 4) to achieve the prescribed target sedation depth. The dose-titration is expected to be completed within 2 hours of initiating study treatment. During this period, a minimum of one COMFORT-B assessment should be performed.

Table 4 Isoflurane/AnaConDa-S dose-rate and dose-titration schedule

Age groups/tidal volume	Start infusion dose-rate [1]	Dose-titration schedule
Patients with small tidal volumes (30-200 mL) or deemed in need of active humidification, AnaConDa-S to be placed on the inspiratory limb of the ventilator	2.0 mL/hour	Titrated stepwise based on achievement of target sedation depth, by decreasing/increasing 0.5 - 1.0 mL/hr Bolus 0.2 - 0.3 mL
Patients with tidal volumes 200-800 mL, standard AnaConDa-S placement at the Y-piece of respiratory circuit [2]	2.0 mL/hour	Titrated stepwise based on achievement of target sedation depth, by decreasing/increasing 0.5 - 1.0 mL/hr Bolus of 0.2 - 0.3 mL

[1] Before initiating the starting dose infusion, the AnaConDa-S is to be primed with 1.2 mL isoflurane.

[2] For patients with tidal volumes of 200-800 mL, the placement at the Y-piece can be used, unless active humidification is considered necessary, or if the Investigator has concerns regarding added dead space.

After the initial dose titration is completed (within 2 hours after start of study treatment), further titration and bolus doses (refer to Table 4) are allowed, if necessary, to maintain the prescribed target sedation depth. The maintenance dose of isoflurane should not result in exceeding 1.0% end-tidal isoflurane.

The AnaConDa-S device will be exchanged after 24 hours of study treatment or earlier. The time point for change of device should be recorded in the eCRF.

10.2.4 Intravenous midazolam

- Active compound: Midazolam
- Dosage form: Intravenous solution for infusion
- Dosage strength: 1 mg/mL or 2 mg/mL

10.2.4.1 Dose-titration of midazolam

In patients already sedated with midazolam-infusion at the time of randomisation, the same midazolam dose-rate will be continued at the time of start of study treatment unless adjustment is required to reach the target sedation depth set at baseline. If the target sedation is lighter than the most recent result of COMFORT-B assessment, the midazolam infusion should be reduced. If the target sedation is deeper than the most recent result of COMFORT-B assessment, midazolam infusion should be increased.

In patients on ongoing sedative treatments other than midazolam at the time of randomisation, study treatment will start by initiating an IV infusion of midazolam as specified in Table 5, and other sedatives are simultaneously turned off (may be turned off up to 30 minutes prior to initiating midazolam). Patients not already sedated at time of randomisation will be given midazolam according to the dose-titration schedule below.

Table 5 Midazolam dose-rate and dose-titration schedule

	Start infusion dose-rate	Dose-titration schedule
All patients	50-100 µg/kg/hr	Titrated stepwise based on achievement of target sedation depth, by decreasing/increasing 20-50 µg/kg/hr IV bolus of 50-100 µg/kg

After the initial dose titration is completed (within 2 hours after start of study treatment), further titration and bolus doses (refer to Table 5) allowed, if necessary, to maintain the prescribed target sedation depth. The maintenance dose should not exceed 300 µg/kg/hr (0.3 mg/kg/hr) midazolam.

10.2.5 Study treatment maintenance

Throughout the study treatment period, the dose of study treatment (isoflurane or midazolam) should be titrated as needed, to maintain the prescribed target sedation depth interval, based on the bi-hourly blinded COMFORT-B assessments performed. This is primarily done by increasing or decreasing the infusion rate of the study treatment and/or administering bolus doses of study treatment. Four bolus doses of study treatment per hour are allowed to be given, if necessary, to maintain the prescribed target sedation depth.

If the maintenance dose of the study treatment is insufficient to reach or maintain prescribed target sedation depth a rescue sedative agent should be given in accordance with Section 10.3.1.

10.2.5.1 Analgesic treatment

All study patients should receive adequate analgesia throughout the study at the discretion of the Investigator. Continuous opiate infusions are allowed throughout the study treatment period and may be adjusted as clinically appropriate (if the patient appears to be in pain, or otherwise appears to benefit from opiate dose increase/reduction). Unless given and documented for medical procedures as described in 10.3.2 and 10.3.3, bolus doses of opiates will be considered and handled as rescue sedation in the analysis.

Ketamine or es-ketamine may not be used for sedation treatment maintenance as they are considered rescue or procedural sedation drugs (Section 10.3).

10.2.5.2 Postoperative patients

Postoperative patients are recommended to receive non-opioid analgesics (not including ketamine or es-ketamine) during the study period (unless contraindicated in an individual patient).

10.2.6 Change in target sedation depth

If medically indicated, the Investigator or designee may change the prescribed target sedation depth once during the study treatment period. The time and medical rationale for this change should be documented in the eCRF. This should be done a minimum of 1 hour before the next scheduled blinded COMFORT-B assessment.

10.2.7 Wake-up for extubation

The process of wake-up for extubation (i.e. removal of endo-tracheal tube) may be performed according to clinical practice. Other additional sedatives, analgesics or antipsychotics may be used at the discretion of the Investigator from this time point. Use of such medications should be recorded as described in Section 11.2.19.1. The Investigator should document in the eCRF when the wake-up period is considered initiated. Generally, this time point should coincide with the time when the Investigator stops IMP administration or reduces it significantly (>25%), with the aim to extubate the patient. During the wake-up period, no blinded COMFORT-B assessments will be performed. The time of initiating wake-up and the actual time of extubation should be recorded in the eCRF if wake-up for extubation is initiated during the study treatment period. If the wake-up for extubation is terminated and the patient remains intubated, the time for ending the attempt and the reason for non-extubation should be noted.

10.3 Rescue sedation, procedural medication and other medications

Whenever possible, the study patient's sedative requirements should be met using only the randomised study treatment, which may be titrated as necessary in accordance with Section 10.2. If the maintenance dose of study treatment is insufficient to reach or maintain prescribed target sedation, action may be taken as outlined in Section 10.3.1. If the patient requires additional analgesia or other sedative or anaesthetic agent due to a medical procedure, action may be taken as outlined in Sections 10.3.2-10.3.3.

The reason for administering additional sedative agents must be recorded in the eCRF as either to facilitate a medical procedure, or as rescue sedation.

10.3.1 Per protocol rescue sedation

Rescue sedation is defined as sedative agents other than the IMP that is allowed in case of inadequate sedation due to e.g. observed acute agitation or immediate risk of extubation which is not controlled

by administration of study treatment maintenance dose, bolus doses of study treatment and co-treatment with analgesic agent.

Rescue sedation alternatives allowed in this study are:

- bolus propofol 1-2 mg/kg, maximum 2 doses per hour (infusions of propofol are strictly prohibited)
- bolus ketamine 1-2 mg/kg
- bolus es-ketamine 0.5-1 mg/kg

Administration of sedative agents for the reason defined in this section should be recorded as "rescue sedation" in eCRF.

10.3.2 Medication for minor ICU procedures

The infusion rate of study treatment may be increased prophylactically, in anticipation of increased requirement due to, or during, a planned minor procedure in the ICU e.g. changing in dressings, napping, washing, re-positioning patient, bronchoscopy, intravenous line placement, tube or radiological examination.

Besides prophylactic increase of the IMP, the following other medications are permitted during such procedure:

- bolus propofol 1-2 mg/kg, maximum 2 doses per hour (infusions of propofol are strictly prohibited)
- bolus ketamine 1-2 mg/kg
- bolus es-ketamine 0.5-1 mg/kg
- short-acting opioids
- neuromuscular blocking agents

Administration of these medications may be repeated as needed during the procedure.

Medications administered for this purpose should be recorded as “procedural medication” in the eCRF.

10.3.3 Additional sedation or anaesthesia for surgical or diagnostic procedure outside the ICU

In the case of a need for deeper sedation or anaesthesia arises during the study treatment period, due to e.g. a surgical or diagnostic procedure such as radiological examination or surgery, study treatment may be temporarily increased or replaced/augmented by other medications as indicated and unrelated to the COMFORT-B assessments. The time for implementing the deeper sedation or anaesthesia for the procedure must be recorded in the eCRF.

Anaesthesia and analgesia for these purposes should be given according to local clinical practice. Medication administered for this purpose should be recorded as “procedural medication” in the eCRF.

After the procedure, study treatment should be re-initiated as soon as possible in the ICU and study treatment should be titrated to reach the target study sedation depth prescribed for the patient. Study treatment must be re-initiated within 2 hours of the end of the procedure/anaesthesia administration and return to the ICU.

10.4 Treatment restrictions during study treatment

For patients receiving sedative treatment at the start of the study these sedative agents must be discontinued during start of administration of the IMP. Patients included in the study should not have received more than 72 hours of sedation at the time of randomisation, in order to reduce confounding of study results by other treatments.

10.4.1 Prohibited medications

The following medications may influence the study outcomes and are prohibited throughout the study treatment period:

- Chloropromazine
- Chloral hydrate
- Barbiturates
- Other benzodiazepines than midazolam
- Gamma-hydroxybuturate
- Melatonin
- α2-adrenergic agonist boluses (for infusion of α2-adrenergic αs, refer to Section 10.4.2)
- Gabapentin, unless the patient had this before ICU admission
- Haloperidol or other neuroleptics, unless the patient had these before ICU admission

Continuous infusions of neuromuscular blocking agents are not allowed for treatment maintenance but may be used as indicated for medical procedures as described in 10.3.2.

10.4.2 α2-adrenergic agonist infusions

Treatment with α2-adrenergic agonists must be discontinued prior to initiating study treatment.

Although it is unlikely, it cannot be excluded that eligible study patients treated with α2-adrenergic agonists at the time of entry into the study (maximum 72 hours), may develop withdrawal symptoms such as hypertension or tachycardia. As a first step, this should be managed by the IMP which may be titrated as necessary in accordance with Sections 10.2. However, in the case of hypertension (defined as mean arterial pressure (MAP) increase from baseline >20%) or tachycardia, (defined as HR increase from baseline >20%) observed in the first 12 hours that cannot be resolved despite an increase of study treatment dose of at least 50% compared with the first study treatment steady-state dose reached after dose-titration (+2 hours after study treatment initiation), an infusion of the same α_2-adrenergic agonist can be restarted at a maximum of 50% of the pre-randomisation dose. Thereafter, the dose of the α2-adrenergic agonists should be reduced, or the infusion stopped as soon as considered clinically feasible.

Administration of α2-adrenergic agonists will be documented as concomitant medication in the eCRF and will be handled statistically in line with the description in Section 13.5.3.4.

For patients not receiving α2-adrenergic agonists before randomisation, their use is not permitted during study treatment.

10.5 Treatment discontinuation

In case a patient cannot be adequately sedated with study treatment and rescue sedation administration is required more than 4 times in 2 hours, the patient should be discontinued from the study treatment and treated according to the attending physician's judgement. For patients with treatment discontinuation, the time from discontinuation will be considered failure time, see Section 13.5.3.4.

10.6 Other concomitant medication

Other medications not described above that are considered necessary for the patient's safety and well-being, may be given at the discretion of the Investigator.

10.7 Post-study treatment

After the patient completes study treatment, at 48±6 hours after initiating study treatment at the latest, the sedation may continue according to local practice as deemed necessary by the treating physician. The isoflurane, midazolam and AnaConDa-S provided for the study may not be used after the completion of the study treatment period.

10.8 Treatment compliance

The administration of IMP will be done by personnel at the site. All doses given and changes in dose of IMP, rescue sedation, procedural medication, α2-adrenergic agonists and analgesics will be recorded in the eCRF in order to assess the compliance with the study treatment regimen (see Section 11.2.19.1).

10.9 Blinding of study treatment for blinded assessors

At site initiation, the Investigator will identify a group of individuals who will serve as blinded assessors throughout the study. These individuals will be delegated the task of the blinded COMFORT-B assessment to be performed every 2 hours during the study treatment period of each patient. Measures will be taken to ensure the treatment allocation is not available to blinded assessors during the study. A site-specific blinding plan will be created in order to document the site team management and relevant considerations for maintaining the blinding at each specific site, ensuring that the team of blinded assessors remain blinded to the patient's treatment allocation throughout the study treatment period. The blinded assessors will also be blinded to the prescribed target sedation depth which is based on the COMFORT-B intervals.

The blinded assessors will be familiar with the clinical environment of the ICU. Specific measures will be taken to ensure the treatment allocation is not known to the blinded assessor upon entry to the room where the patient is being treated, including visually obscuring the AnaConDa-S and the study treatment being administered. A detailed blinding instruction will be provided to each study site.

11. SCHEDULE AND DEFINITIONS OF ASSESSMENTS

The schedule of assessments is presented in Table 6. The visits are described in detail in Section 11.1 and the assessments are defined in Sections 11.2

Table 6 Schedule of assessments

	SCREENING		**TREATMENT**						**FOLLOW-UP**	
	Day -30 to Day -1		**0 – 48±6 hours**							
	≤-30 days (planned patients) Or ≤-72 hrs (unplanned patients)	≤-2 hrs	0 hr	0-2 hrs titration phase	*Every 2 hrs (±30 min)*	*Every 8 hrs (±1 hr)*	24±6 hrs	48±6 hrs Or earlier if study trt <48 hrs	Up to 48 after end of study treatment	Up to 30 days after end of study trt Weekly FU visit/contact: Day 9±2 days, 16±2 days, 23±2 days and 30±2 days
Procedure		**Baseline**	**Randomisation/ Start of IMP**					**End of study treatment**	**Post-study treatment monitoring**	**Follow up visits or phone contact**
Informed consent and assent (as appl.)	X									
Eligibility criteria	X	X [a]								
Demography	X									
Medical and surgical history		X								
PIM3 [b]		X								
Physical examination		X						X		
Body weight & height [c]		X								
ECG		X								
Pregnancy test (if appl.) [d]		X								
Vital signs		X			X [e]			X		
Urinary output [f]						X		X		
Body temperature		X				X		X		
Ventilator parameters [g]		X			X			X		
Safety lab samples (clin chemistry and haematology)		X					X	X	X [h]	
Blood gases		X				X [i]		X		
Prescription of target sedation depth [j]		X								
Randomisation			X							
IMP administration [k]			X----------					X		
COMFORT-B by blinded assessor					X [l]					
SOS-PD delirium module [m] (if appl.)		X	X			X		X	X	
SOS-PD withdrawal module [n] (if appl.)						X		X	X	
Concomitant medications, incl. rescue sedation [o]		X	X ----------					X		X [n]
Non-pharmacological interventions			X							X
Adverse events (AEs) [p]			X ----------							X
AnaConDa-S device deficiencies			X ----------					X		
Daily follow up of neurological and psychomotor AEs (if appl.)									X	X

	SCREENING		TREATMENT						FOLLOW-UP	
	Day -30 to Day -1		0 – 48±6 hours							
	≤-30 days (planned patients) Or ≤-72 hrs (unplanned patients)	≤-2 hrs	0 hr	0-2 hrs titration phase	*Every 2 hrs (±30 min)*	*Every 8 hrs (±1 hr)*	24±6 hrs	48±6 hrs Or earlier if study trt <48 hrs	Up to 48 after end of study treatment	Up to 30 days after end of study trt Weekly FU visit/contact: Day 9±2 days, 16±2 days, 23±2 days and 30±2 days
Procedure		**Baseline**	**Randomisation/ Start of IMP**					**End of study treatment**	**Post-study treatment monitoring**	**Follow up visits or phone contact**
Time in ICU, ventilator-free days, mortality [q]										X

[a] Re-check of eligibility criteria.
[b] The results of PIM3 will be collected, either as assessed at ICU admission per clinical practice or, at the latest, as assessed at baseline.
[c] Body weight (in kg) and height (cm) should be measured where possible or be estimated. Weight and height available in patient's records can be used if measured within the last 30 days.
[d] If possible, a urine dipstick should be used preferentially. The urine test should have a sensitivity of at least 20 mIU/mL for human chorionic gonadotropin (hCG). The investigator must assess the result of the pregnancy test before determining if the patient can enter the study. Assessment from the same calendar day may be used.
[e] Any clinically significant abnormality in vital signs that occurred since the previous assessment will be recorded as an AE (including time point, variable and value) (Section 11.2.10). The 2-hourly assessments should be performed after the patient has been undisturbed for the last 5 minutes.
[f] Volume of urinary output (in mL) will be assessed and recorded, every 8 hours during the study treatment period, starting from baseline and ending at the end of study treatment
[g] Ventilator parameters include end-tidal concentration of isoflurane (%vol) where appl., ventilator mode, minute volume, fraction of inspired oxygen (FiO_2), end tidal carbon dioxide ($EtCO_2$), total breathing rate, positive end-expiratory pressure (PEEP), inspiratory pressure (Pinsp) and level of pressure support (PS) above PEEP. The 2-hourly assessments should be performed after the patient has been undisturbed for the last 5 minutes.
[h] Safety laboratory parameters should be assessed once in the 48-hour post study treatment monitoring period, if patient is still in ICU at 18 hours after end of study treatment (see Section 11.2.14). Analyses performed within a 18-48-hour window after end of study treatment period per standard practice can be used.
[i] Any clinically significant abnormality in blood gases observed since the previous assessment will be recorded as an AE (including time point, variable and value) (Section 11.2.15).
[j] COMFORT-B target sedation depth interval re-evaluation and change is allowed once during the study.
[k] Initial titration of IMP administration will be performed using the COMFORT-B scale to reach the target sedation depth, this is expected to be completed within 2 hours of initiating IMP administration. In this initial titration step, the COMFORT-B scale assessed by a unblinded assessor will guide the continued titration or tapering of IMP administration. The maintenance dose and any dose changes of IMP, including boluses; isoflurane (mL/hr and end-tidal concentration Vol%), or midazolam (µg/kg/hr) will be recorded in the eCRF.
[l] The first blinded COMFORT-B assessment will be performed at +2 hours (±30 mins) after initiation of study treatment. The 2-hourly assessments should be performed after the patient has been undisturbed for the last 5 minutes.
[m] Assessment of delirium symptoms using the delirium module of the SOS-PD scale will start when a patient has been admitted to ICU for a total of 48 hours (including period admitted to ICU prior to enrolment), or earlier if clinically indicated. Thereafter the SOS-PD delirium module will be assessed at least every 8 hours until end of 48-hour post-study treatment monitoring period or ICU discharge whichever comes first.
[n] Assessment of withdrawal symptoms using the withdrawal module of the SOS-PD scale will be started in patients exposed to a total of 96 hours sedation (including pre-study sedation period), or earlier if clinically indicated. Thereafter the SOS-PD withdrawal module will be assessed at least every 8 hours until end of 48-hour post-study treatment monitoring period or ICU discharge whichever comes first.
[o] Concomitant medications incl. use of any rescue sedation and inotropic/vasopressor agents (to calculate the VIS score) are recorded from baseline until the end of the 48-hour post-study treatment monitoring. After this, only selected concomitant medications will be recorded if the patient is still in the ICU (refer to Section 11.2.19.1).
[p] Recording of AEs starts at initiation of IMP administration and will continue to the end of the 48-hour post-study treatment monitoring. After this, the patient's general condition will be assessed at the weekly follow up visits/contact. AEs identified during study treatment that are still ongoing will be followed up and any severe AEs, SAEs or AEs assessed to have possible or probable causality to the IMP with onset after the 48-hour post-study treatment monitoring will be recorded (see Section 12.3.1). Severe AEs and SAEs occurring in the follow-up period should be reported immediately.
[q] Data to determine total time in ICU, number of ventilator-free days and mortality will be recorded from the patient's medical records at day 30 after end of study treatment period.

11.1 Study schedule

11.1.1 Screening ≤-30 days (planned patients) or ≤-72 hours (unplanned patients) to 0 hour

Patients admitted to the ICU or planned to be admitted to the ICU (postoperative patients) will be pre-screened based on available clinical data prior to obtaining informed consent to identify patients suitable for participation.

Collection of informed consent as outlined in Section 9.1 will be performed before any study specific procedures are performed.

At screening the following data will be collected:

- Informed consent and assent as applicable according to local regulations
- Check of eligibility criteria (inclusion/exclusion criteria, as specified in Section 9.3)
- Demography

All baseline assessments should be completed before the patient proceeds to randomisation.

11.1.1.1 Baseline (up to 2 hours before randomisation)

Within 2 hours prior to randomisation the following assessments must be completed:

- Re-check of eligibility criteria
- Medical and surgical history
    - Reason for admission to ICU should be specified
- PIM3 (Paediatric Index of Mortality) (assessment performed at ICU admission may be used)
- Physical examination (assessments from the same calendar day can be used)
- Body weight and height
- ECG (assessments from the same calendar day can be used)
- Pregnancy test (if applicable) (The investigator must assess the result of the pregnancy test before determining if the patient can enter the study. Assessment taken within 24 hours of randomisation may be used)
- Vital signs
- Body temperature
- Ventilator parameters
- Safety laboratory samples (assessments from the same calendar day can be used)
- Blood gases
- Un-blinded COMFORT-B pre-randomisation (see Section 11.2.16)
- Prescribe target COMFORT-B (sedation depth; light, moderate or deep)
- SOS-PD scale, if applicable
    - Assessment of delirium module of the SOS-PD will start in patients at 48 hours after ICU admission, including any period prior to enrolment in the study, or earlier, if clinically indicated. Thereafter, it will be assessed at least every 8 hours until the end of the 48-hour post-study treatment monitoring period or ICU discharge, whichever comes first.
- Concomitant medication (all medications ongoing at baseline as outlined in Section 11.2.19)
    - Start date and time of the ongoing sedative treatment should be collected to confirm eligibility.
    - Use of inotropic/vasopressor agents will be recorded in order to calculate the VIS score at baseline.

11.1.2 Study treatment period (0 – 48±6 hours)

After initial dose titration of study treatment, the Investigator will regularly assess the adequacy of the prescribed sedation depth and may re-evaluate the prescribed target sedation depth. If medically indicated, the Investigator and the clinical team may make one change in target sedation depth once during the study treatment period. If changed, the time and medical rationale for changing the prescribed sedation target should be recorded in the eCRF.

In case of interruptions in the sedation, due to wake-up test/neurological assessments, medical or diagnostic procedures during study treatment period (see Sections 10.3.2 and 10.3.3) the time of the next COMFORT-B assessments should be adjusted in according to Section 11.2.16.1.

Throughout the study treatment period, data describing the IMP administration will be recorded in the eCRF:

- Initial IMP dose titration (in accordance with Section 10.2.2).
- The maintenance dose and any dose changes of IMP, including boluses; isoflurane (mL/hr and end-tidal concentration Vol%), or midazolam (µg/kg/hr)
- AnaConDa-S device deficiencies

During the study treatment period and up to the 48 hours follow-up after end of study treatment, the following should be continuously monitored and recorded in the eCRF:

- Concomitant medications, including any rescue sedation or procedural medication and use of inotropic/vasopressor agents for calculation of the VIS score throughout the study treatment period (in accordance with Section 11.2.19).
- Non-pharmacological interventions (see Section 11.2.19.1)
- Adverse events, including events defined as common adverse events, sedation-related adverse events, neurological and psychomotor adverse events and any other adverse events (see Sections 11.2.20 and 12.2.7)

For patients on isoflurane treatment, the AnaConDa-S device should be replaced no later than after 24 hours of use.

If the wake-up of the patient for extubation is initiated during the study treatment period, the date and time of initiating the wake-up period and actual time of extubation should be recorded (refer to Section 10.2.7 for study definition on initiation of wake-up).

11.1.2.1 Randomisation

- Randomisation using the digital media system and allocation to either isoflurane or midazolam treatment arms (see Section 9.4.1).
- Stop of ongoing sedative regimen and α2-adrenergic agonists. Depending on how long the patient has been sedated prior to enrolment and their current clinical status, the investigator may terminate the ongoing sedatives up to 30 minutes prior to initiating study treatment.

11.1.2.2 Initiation of IMP administration (0-2 hours)

- Initiation of IMP administration in accordance with Section 10.2 (start time of IMP means the start of the study treatment period i.e. **hour 0**). Titration of IMP dose until target sedation depth is reached, expected to be completed within maximum 2 hours.
- In this initial titration step the COMFORT-B scale is assessed by an unblinded Investigator or designee at a minimum of one documented COMFORT-B.

11.1.2.3 1st Blinded COMFORT-B assessment at +2 hours (±30 minutes)

The first blinded COMFORT-B assessment will be performed at 2 hours (±30 minutes) after initiation of study treatment (0 hour) at which point the target level of sedation is expected to have been reached.

- 1st COMFORT-B assessment by blinded assessor (see Section 11.2.16)

The following will also be assessed:

- Vital signs
- Ventilator parameters

11.1.2.4 Assessments every 2 hours (±30 minutes)

The following will be assessed:

- COMFORT-B by blinded assessor
- Vital signs
- Ventilator parameters

The assessments should be performed after the patient has been undisturbed for the last 5 minutes.

11.1.2.5 Assessments every 8 hours (±1 hour)

The following will be assessed:

- Urinary output
- Blood gases
- Body temperature
- SOS-PD scale, if applicable
    - Assessment of delirium with the SOS-PD delirium module will start in patients at 48 hours after ICU admission, including any period prior to enrolment in the study, or earlier, if clinically indicated. Thereafter, delirium will be assessed at least every 8 hours until the end of the 48-hour post-study treatment monitoring period or ICU discharge, whichever comes first.
    - Assessment of withdrawal with the SOS-PD withdrawal module will be started in patients exposed to a total of 96 hours of sedation (including pre-study sedation period), or earlier, if clinically indicated. Thereafter, withdrawal will be assessed at least every 8 hours until end of the 48-hour post-study treatment monitoring period or ICU discharge, whichever comes first.

11.1.2.6 Assessments 24 ±6 hours (for patients still on study treatment)

The following will be assessed:

- Safety laboratory assessments

11.1.2.7 End of study treatment 48±6 hours or earlier if study treatment period is <48 hours

At 48±6 hours of the study treatment period, or earlier if study treatment stops before 48 hours, the following assessments will be performed. The end of study treatment assessments should be performed for all patients where study treatment has been initiated.

- Physical examination
    - Assess patient's gastrointestinal motility and record in AE and concomitant medication forms in eCRF, as applicable;
        - Did the patient pass stool during the study period?

  - Did the patient receive medication or a procedure to enhance evacuation of stool during the study?
- Vital signs

- Urinary output
- Body temperature
- Ventilator parameters
- Safety laboratory assessments
- Blood gases
- SOS-PD scale, if applicable
  - Assessment of delirium module in patients which have been admitted to the ICU for 48 hours or more, including any period prior to enrolment in the study.
  - Assessment of withdrawal module in patients which have been exposed to a total of 96 hours of sedation or more (including pre-study sedation period).

11.1.3 Follow-up period

After end of study treatment, any treatments ensuring the patient's welfare in accordance to local clinical practice will be allowed. This includes medications during weaning and extubation, use of these medications should be recorded in the eCRF (see Section 10.2.7)

Patients with neurological or psychomotor symptoms identified during the study treatment period or in the 48-hour post-study treatment monitoring will be assessed daily for one week. If not resolved after this, the patient will be referred to a specialist physician (see Section 12.2.7.3).

11.1.3.1 Post-study treatment monitoring up to 48 hours after end of study treatment

The patient will be followed closely during a 48-hour post-study treatment period. During this time the following will be assessed:

- Safety laboratory parameters should be assessed once between 18 to 48 hours after end of study treatment if the patient is still in ICU at 18 hours after end of study treatment (see Section 11.2.14). Analyses performed within the 18-48-hour window per standard practice can be used, if multiple analyses are performed within the window, the last analyses performed will be reported.
- SOS-PD scale, if applicable (see Section 11.2.17).
  - Assessment of delirium module of the SOS-PD will start in patients at 48 hours after ICU admission, including any period prior to enrolment in the study, or earlier, if clinically indicated. Thereafter, it will be assessed at least every 8 hours until the end of the 48-hour post-study treatment monitoring period or ICU discharge, whichever comes first.
  - Assessment of withdrawal module will be started in patients exposed to a total of 96 hours of sedation (including pre-study sedation period), or earlier, if clinically indicated. Thereafter, the SOS-PD withdrawal module will be assessed at least every 8 hours until end of the 48-hour post-study treatment monitoring period or ICU discharge, whichever comes first.
- Adverse events
- Concomitant medications (see Section 11.2.19.1)

11.1.3.2 Weekly follow-up until 30 days after end of study treatment

After the 48-hour post-study treatment monitoring patients will be assessed for the below list of parameters on a weekly basis. If the patient is still in the hospital, the patient and their records will be examined by the Investigator. If the patient has been discharged, the Investigator or designee will

contact the patient, their legal guardian(s) and/or the caregiver to obtain data. The patient, their legal

guardian(s) and/or caregiver will be instructed to be observant of any signs and symptoms and document these as they occur in order to improve the recall of these events at the weekly follow-up. They will be offered an optional paper-based diary to use as an aid for documenting signs and symptoms during the follow up period. The Investigator will instruct the patients, their legal guardian and/or caregiver to report any severe or serious adverse events (SAE) immediately to the investigator in order to ensure these are reviewed in real-time and reported further as per Section 12.4.

The weekly follow-up will be done on day 9±2 days after end of study treatment, 16±2 days after end of study treatment 23±2 days after end of study treatment and 30±2 days after end of study treatment.

- Follow up of all ongoing adverse events as per Section 12.3.5. This includes the daily follow up on identified neurological and/or psychomotor symptoms, where applicable, per Section 12.2.7.3.
- Recording adverse events with onset after the end of the 48-hour post-study treatment period which are assessed as severe, serious or assessed to have possible or probable causality to the IMP. Examples of adverse events with possible or probable causality to the IMP include but are not limited to; muscle cramps or twitches, poor muscle control, abnormal movement, agitation, confusion, tremor, salivation, sleeping disturbances and phonation or speaking problems.
- Concomitant medications, if patient is still in the ICU, or discharged and re-admitted to another ICU (see Section 11.2.19.1).
- Non-pharmacological interventions (see Section 11.2.19.2).
- On **day 30**, the following will be recorded based on review of medical records or by contacting the patient, the legal guardian(s) or caregiver:
    - Mortality (is patient alive or deceased)
    - Number of days the patient has been mechanically ventilated (ventilator days), counted from day when study treatment was initiated. Time for extubation should be collected.
    - Time in ICU/hospital. Will be calculated based on information on whether the patient has been discharged from the ICU. If yes, the following data will be recorded:
        - Date and time for discharge
        - Where to the patient was discharged (intermediary care unit, general ward, another ward at the hospital, another hospital, rehabilitation unit, nursing home or home)
        - Whether patient has been re-admitted to an ICU or hospital
    - Completion of end of study form (define if patient completed study treatment period and if patient completed the study. Refer to definition on treatment and study discontinuation in Section 9.6.1)

11.2 Definitions of assessments

11.2.1 Screening and informed consent

The procedures used for screening and obtaining informed consent and assent are described in Sections 9.1, 9.2 and 11.1.1.

11.2.2 Eligibility criteria

Eligibility criteria should be checked during screening and verified at baseline before randomisation. The criteria are specified in Sections 9.3.1 and 9.3.2.

11.2.3 Demography

Demographic information will be collected at screening, including gender and age at randomisation. Race (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White) and ethnicity (Hispanic or Latino, Not Hispanic or Latino) will be collected where allowed per local regulation.

11.2.4 Medical and surgical history

Medical and surgical history should be obtained by interview at the baseline visit and include descriptions of all relevant diseases as judged by the Investigator.

Diseases that should be recorded include, but are not limited to, known allergy, current diseases, chronic diseases and neurologic diseases.

The reason for admission to the ICU should be recorded. E.g. if the patient is entering the study post-operatively, the type of surgery that has been performed should be recorded.

11.2.5 Paediatric index of mortality 3

The Paediatric Index of Mortality 3 (PIM3) (Straney et al. 2013) will be collected, either as assessed at ICU admission, per clinical practice or as assessed at the latest, at baseline.

11.2.6 Physical examination

A physical examination will be performed at baseline and at the end of the study treatment period and should include examination of the following:

- General condition
- Cardiovascular system
- Respiratory system
- Gastrointestinal system
- Neurological examination including general neurology (seizures)

In his/her assessment, the Investigator will consider results available from examinations such as ultrasound, radiology exam or laboratory tests and document his/her integrated assessment.

Each category will be evaluated and reported as “normal”, “abnormal not clinically significant” or “abnormal clinically significant”. Abnormal clinically significant findings at baseline must be reported in the medical history form of the eCRF and as adverse events if observed at end of treatment. For clinically significant abnormalities observed at baseline, the Investigator will document if, in his/her medical judgement, the finding is significant to a degree that precludes participation in the study or not.

11.2.7 Body measurements

Height (in cm) and body weight (in kg) should be recorded at baseline. Height and body weight will be measured if possible, or otherwise estimated. In case a height and body weight measured within the last 30 days prior to baseline is available in the patient's medical records, this can be recorded.

11.2.8 Electrocardiography

An ECG will be taken at baseline. If an ECG has been taken as part of standard practice the same calendar day, this ECG can be used. During the study the ECG will be performed as clinically indicated. The results will only be collected in case of observed worsening constituting an adverse event.

The results of the ECG examination will be assessed and reported as "normal", "abnormal not clinically significant" or "abnormal clinically significant". Abnormal clinically significant findings at baseline must be reported in the medical history form of the eCRF and as adverse events if observed after initiation of IMP. For clinically significant abnormalities observed at baseline, the Investigator will document if, in his/her medical judgement, the finding is significant to a degree that precludes participation in the study or not.

11.2.9 Pregnancy test

A pregnancy test will be performed at baseline in post-menarche female patients. If possible, a urine dipstick will be used preferentially. The urine test should have a sensitivity of at least 20 mIU/mL for human chorionic gonadotropin (hCG). If the urine test is positive, it must be followed by a quantitative analysis of hCG concentration in blood.

11.2.10 Vital signs

Vital signs will be assessed at baseline, followed continuously as per clinical routine and documented every 2 hours (to be performed after the patient has been undisturbed for the last 5 minutes) during study treatment period and at the end of study treatment. If an adverse event, defined as clinically significant out-of-range values in any of these parameters, occurs between these 2-hour-intervals it will be recorded. Vital signs assessments will include the following, all results will be recorded in the eCRF:

- Systolic and diastolic BP and MAP, measured by arterial transducer if available, or else by automated sphygmomanometer.
- HR measured by continuous ECG monitoring, if available. Otherwise pulse oximetry or palpation of the pulse over the radial artery may be used. HR should not be measured during coughing or nursing procedures.
- Oxygen saturation (SpO_2), measured by pulse oximetry.

At each assessment, the investigator will review and evaluate results and document whether results are considered "normal", "abnormal clinically significant" or "abnormal not clinically significant". Abnormal clinically significant findings at baseline must be reported in the medical history form of the eCRF and as adverse events if observed after initiation of IMP. Refer to Section 12.2.7.1 for definition of vital sign results considered clinically significant in the context of this study. However, the investigator may, according to their medical judgement, assess a result of a vital signs assessment as clinically significant even if it does not fulfil the study-specified criteria. For clinically significant

abnormalities observed at baseline, the Investigator will document if, in his/her medical judgement, the finding is significant to a degree that precludes participation in the study or not.

11.2.11 Urinary output

Volume of urinary output (in mL) will be assessed and recorded, every 8 hours during the study treatment period, starting from baseline and ending at the end of study treatment.

The result will be assessed and reported as "normal", "abnormal not clinically significant" or "abnormal clinically significant". Abnormal clinically significant findings must be reported as an adverse event if observed after initiation of IMP.

If the urinary output is below 0.5 mL/kg/hr over an 8-hour period, or the estimated creatinine clearance (eCCl) is decreased below 25% compared to baseline, this should be noted as a *risk* of acute kidney injury, in accordance with the pRIFLE classification (Akcan-Arikan et al. 2007, KDIGO Clinical Practice Guideline for Acute Kidney Injury 2012 and Kavaz et al. 2011). If patient's condition fulfils the pRIFLE criteria of the class of *Injury* or worse, this will be considered clinically significant and should be reported as an adverse event (refer to Section 12.2.7.1).

11.2.12 Body temperature

Body temperature will be documented at baseline, every 8 hours during study treatment period and at the end of study treatment and recorded in the eCRF.

The result will be assessed and reported as "normal", "abnormal not clinically significant" or "abnormal clinically significant". Abnormal clinically significant findings at baseline must be reported in the medical history form of the eCRF and as an adverse event if observed after initiation of IMP. If the body temperature is considered abnormal clinically significant at baseline, the Investigator will document if, in his/her medical judgement, the finding is significant to a degree that precludes participation in the study or not.

11.2.13 Ventilator parameters

Ventilator parameters will be assessed at baseline, every 2 hours during study treatment (to be performed after the patient has been undisturbed for the last 5 minutes), and at the end of study treatment. The following data will be recorded:

- End-tidal concentration of isoflurane for patients randomised to isoflurane (volume %)
- Ventilation mode, by selecting one of the below categories;
  a) Controlled: Fully controlled mechanical ventilation, no spontaneous breathing efforts
  b) Both controlled and spontaneous breaths
  c) Spontaneous with support: Solely supported ventilation (such as pressure support or NAVA) without any automatically given breaths (except back-up ventilation).
- Minute volume
- FiO_2
- $EtCO_2$
- Total breathing rate
- PEEP
- Pinsp
- PS above PEEP

Tidal volume will be calculated based on the recorded minute volume and the total breathing rate.

11.2.14 Safety laboratory assessments (clinical chemistry and haematology)

Blood sampling for assessment of clinical chemistry and haematology, will be performed at baseline, at 24 hours ±6 hours (if still on study treatment), at end of study treatment (48 hours ±6 hours) and once during the 48-hour post-study treatment period if the patient is still in the ICU at 18 hours after end of study treatment. For the last assessment in the post-study treatment period, analyses performed as part of standard practice within a time window of 18-48 hours after end of study treatment may be used, if multiple analyses are performed, the last analyses will be recorded. If the patient is discharged from the ICU earlier than 18 hours after end of study treatment, no further lab parameters will be mandated.

Blood samples may be collected from an arterial line, a peripheral vein or a central or peripheral venous cannula provided that mixing with any infusates is avoided by first discarding a small amount of blood prior to collection of the samples. Local hospital laboratories will be used to assess the safety laboratory parameters and samples will be analysed by routine analytical methods. The safety laboratory parameters assessed are presented in Table 7. All laboratory results will be recorded in the eCRF and samples will be destructed after analysis in accordance with the standard practice of the local hospital laboratories.

The investigator will review and evaluate the results and document whether they are considered "normal", "abnormal clinically significant" or "abnormal not clinically significant". Abnormal clinically significant findings at baseline must be reported in the medical history form of the eCRF and as an adverse event if observed after initiation of IMP. If an abnormal value is associated with corresponding clinical signs and symptoms, the sign/symptom should be reported as the adverse event and the associated laboratory result should be considered additional information and not reported as a separate adverse event. For clinically significant abnormalities at baseline, the Investigator will document if, in his/her medical judgement, the finding is significant to a degree that precludes participation in the study or not.

Any significant abnormalities observed will be followed according to clinical practice. Additional tests and other evaluations required to establish the significance or aetiology of an abnormal result or to monitor the course of an adverse event will be obtained when clinically indicated.

Table 7 Safety laboratory parameters

Clinical chemistry	Haematology
Serum (S)-alanine aminotransferase (ALT)	Blood (B)-haemoglobin
S-alkaline phosphatase (ALP)	B-total leucocyte count
S-aspartate aminotransferase (AST)	B-platelet count
S-bilirubin (total)	
S-creatinine	
S-creatine kinase	
S-potassium	
S-sodium	
S-urea	
S-glucose or B-glucose	

11.2.15 Blood gases

Blood gases will be assessed at baseline, every 8 hours during study treatment and at the end of study treatment. Samples for assessment of blood gases may be collected from an arterial line, capillary, a peripheral vein or a central or peripheral venous cannula, provided that mixing with any infusates is avoided by first discarding a small amount of blood prior to collection of the samples. The sampling

method and the results will be recorded in the eCRF. Local hospital laboratories will be used to assess the blood gas parameters and samples will be analysed by routine analytical methods. The samples will be destructed after analysis in accordance with the standard practice of the local hospital laboratories. The following parameters will be assessed:

- Partial carbon dioxide pressure (PaCO2)
- Lactate
- HCO3
- Base excess
- pH

At each assessment, the investigator will review and evaluate the results and document whether results are considered "normal", "abnormal clinically significant" or "abnormal not clinically significant". Abnormal clinically significant findings at baseline must be reported in the medical history form of the eCRF and as an adverse event if observed after initiation of IMP. If an abnormal value is associated with corresponding clinical signs and symptoms, the sign/symptom should be reported as the adverse event and the associated laboratory result should be considered additional information and not reported as a separate adverse event.

For clinically significant abnormalities at baseline, the Investigator will document if, in his/her medical judgement, the finding is significant to a degree that precludes participation in the study or not.

If abnormalities in blood gases are considered clinically significant and associated with or indicative of an adverse clinical event or condition in between the 8-hour assessments, this adverse event should be recorded (including time point, variable and value).

11.2.16 COMFORT Behavior scale

Depth of sedation will be assessed using the COMFORT-B scale, that is widely used in paediatric intensive care units to assess young patients' pain and distress. The scale was developed for continuous observation of distress in patients aged from birth to 18 years receiving ventilation in an intensive care environment (Ista et al 2005). The COMFORT-B scale has adequate reliability and construct validity with correlations between 0.68 and 0.84 for distress, between 0.42 and 0.94 for sedation and between 0.31 and 0.96 for pain, and adequately measure change (Maaskant et al. 2016).

The COMFORT-B scale used in this study includes six items to be assessed in mechanically ventilated children. The "crying" item will be excluded since mechanically ventilated patients cannot vocalise. The six items are Alertness, Calmness/agitation, Respiratory response, Physical movement, Muscle tone, and Facial tension. The evaluation form is displayed in Appendix A.

11.2.16.1 Procedure for COMFORT-B assessment

The assessors performing COMFORT-B evaluations must undergo training in the use of the instrument and its application in this study. After their training is completed, the Investigator will delegate the responsibility of blinded COMFORT-B assessment to qualified site staff.

At baseline, the investigator or designee will first perform an unblinded COMFORT-B assessment and thereafter prescribe the desired degree of sedation for each patient as one of three COMFORT-B intervals – light, moderate or deep sedation defined for this study (Table 8). This will be considered the target sedation depth for the patient and will be documented in the eCRF.

Table 8 Degree of sedation defined as intervals of the Comfort Behavior (COMFORT-B) scale

Degree of sedation (target sedation depth)	COMFORT-B score*
Light	17-22
Moderate	11-16
Deep	6-10

* Intervals based on the COMFORT-B scale agreed by the co-ordinating national investigators in Germany, France, Spain and Sweden, based on published data by Ista et al 2005, Ista et al 2009a, Amigoni et al 2012, Andersen et al 2015, Boerlage et al. 2015, and Dreyfus et al. 2017.

After initiation of IMP, sedation level assessments may be repeated frequently during the 2-hour dose-titration phase, in an unblinded manner, until the prescribed target sedation depth is reached. A minimum of one COMFORT-B assessment should be documented from this period. Thereafter, the evaluation will be performed by a blinded assessor every 2 hours (to be performed after the patient has been undisturbed for the last 5 minutes) until the end of study treatment (starting at +2 hours after IMP initiation and up to 48±6 hours). In accordance with the instructions defined for the instrument, the assessor should observe the patient for at least 2 minutes and score the behaviour using a worst-case scenario, i.e. the most distressed or painful behaviour shown during the observation period. The date, time and result (for every score item) of each COMFORT-B assessment performed shall be recorded in the eCRF.

If medically indicated, one change in the target sedation depth is allowed during the study treatment period. This target level does not apply when extubation is to be attempted, nor the short periods when diagnostic or therapeutic procedures or surgery are to be undertaken.

Interruptions in COMFORT-B schedule

In case of a temporary stop in sedation due to wake-up test/neurological assessment of patient, the COMFORT-B assessment should be postponed to at least 30 minutes after re-initiation of IMP administration. Similarly, in case boluses of additional sedative agents (other than IMP), opiates or neuromuscular blocking agents are needed during study treatment period due to a therapeutic or diagnostic procedures, the next COMFORT-B assessment should be postponed at least 30 minutes after last dose/end of infusion of opiates or sedative and no earlier than 60 minutes after the last dose of neuromuscular blocking agent. The subsequent COMFORT-B assessment will follow the original schedule of assessments every 2 hours, counted from the 1st blinded COMFORT-B assessment.

The COMFORT-B assessments will be stopped when the wake-up period for extubation is initiated (see Section 10.2.7)

11.2.16.2 Blinded assessor

The scheduled assessments of the COMFORT-B scale will be performed by an assessor not aware of the treatment arm or the prescribed target sedation depth interval (so called “blinded assessor”). This will be an individual who is not involved in the clinical care of the patient since the investigator, study coordinator and staff working bedside will not be blinded to the treatment allocation. The results of each blinded COMFORT-B assessment will be communicated to the investigator and study staff in order to evaluate if the patient is within the prescribed target sedation depth interval or otherwise titrate the study treatment as necessary. The blinded assessor delegated to perform the COMFORT-B will be

blinded to the treatment allocation for the extent of time he/she has the role of performing the scheduled COMFORT-B assessments.

11.2.17 SOPHIA Observation Withdrawal Symptoms-Paediatric Delirium Scale

The European Society of Paediatric and Neonatal Intensive Care (ESPNIC) recommends the use of the SOS-PD for evaluation of withdrawal and delirium symptoms (Harris et al. 2016).

Evaluation of withdrawal symptoms is relevant in patients who received sedatives and/or opioids for at least 4 days (or 96 hours) and start weaning. Patients could be at risk for delirium symptoms with or without signs of withdrawal and symptoms of delirium may be observed earlier, it is therefore recommended to start assessing the patient for symptoms of delirium using the SOS-PD scale already after 48 hours of ICU admission (Ista et al. 2018).

In this study, assessment of the delirium module of the SOS-PD will start in patients at 48 hours after ICU admission, including the period when they were admitted to ICU prior to enrolment. From this time point, the delirium module of the SOS-PD scale will be evaluated every 8 hours during the remaining study treatment period and until the end of the 48-hour post-study treatment period or until patient is discharged from ICU, whichever comes first. In accordance with the instructions of the SOS-PD instrument, the delirium module will not be assessed in patients who are very deeply sedated and not responding to stimuli. For patients where the sedation depth is precluding the SOS-PD delirium assessment, maintenance of the prescribed target sedation depth should be prioritized over assessing the SOS-PD delirium module.

Patients who have been exposed to a total of 96 hours of sedation (including pre-study sedation period) will be evaluated for withdrawal symptoms, using the withdrawal module of the SOS-PD scale. As for the delirium module, the withdrawal module of the SOS-PD scale will be evaluated every 8 hours during the remaining study treatment period and until the end of the 48-hour post-study treatment period or until the patient is discharged from ICU, whichever comes first.

If clinically indicated, due to e.g. suspicion of delirium, the assessment of the SOS-PD may be started earlier than the defined 48 hours after ICU admission and 96 hours of sedation. In case the patient has been given an intervention for delirium or withdrawal symptoms, the SOS-PD scale may be used more often than the defined every 8 hours, in accordance with the instructions defined for the instrument. The intervention administered will be collected in the concomitant medication form of the eCRF, in accordance with Section 11.2.18. In accordance with the instructions defined for the instrument, the assessor should score the signs and symptoms according to the worst moment during the observation period.

This tool includes observation of several clinical symptoms resulting in a score-range of 0 to 15 points for withdrawal symptoms and a score-range of 0 to 16/17 points for delirium symptoms. The cut off point for presence of withdrawal symptoms is ≥ 4. Presence of delirium is considered determined if part 1b is positive, and/or the score of step 2 is ≥ 4 or symptoms of hallucination are observed.

In the eCRF each score item is to be recorded, separately for delirium and withdrawal. The evaluation form is displayed in Appendix B.

11.2.18 IMP administration

Throughout the study treatment period data related to the administration of the IMP will be recorded, including doses, dose changes, start and stop times and occurrence of any interruptions in IMP administration (start and stop time of interruption) due to e.g. medical procedures outside the ICU.

The following will be documented regarding the placement of the AnaConDa-S:

- Placement in standard or inspiratory side
- Reason for choice:
  - Tidal volume (above or below 200 mL)
  - $PaCO_2$ values
  - Active humidification

11.2.19 Concomitant medications, rescue sedation and non-pharmacological interventions

For a full description on the allowed concomitant medications, incl. rescue sedation, refer to Sections 10.3 and 10.6. This section describes which concomitant medications should be recorded.

Based on recorded use inotropic/vasopressor agents, a VIS score (Gaies et al. 2010, McIntosh et al. 2017) will be calculated at baseline and throughout study treatment period, until the end of the 48-hour post-study treatment monitoring.

11.2.19.1 Recording of concomitant medications

The following medications will be recorded, including route of administration and indication:

At baseline:

- All ongoing medications
- The start date and time of the ongoing sedative treatment, incl. any analgesics and α2-adrenergic agonists should be recorded to confirm eligibility.

From the start of the study treatment to the end of the 48hour post-study treatment monitoring:

- Dose and all changes in dose of rescue sedation (ref. Section 10.3.1)
- Dose and all changes in dose of procedural medication (ref. Section 10.3.2 and 10.3.3)
- Dose and all changes in dose of any analgesia, including opioids
- Dose and all changes in dose of neuromuscular blocking agents
- Dose and all changes in dose of inotropic and vasopressor agents
- Dose and all changes in dose of continuous local anaesthetics
- Medication received to enhance evacuation of stool
- Interventions for treatment of identified delirium and withdrawal symptoms
- Dose and all changes in dose of any sedative agents (incl. α2- adrenergic agonists), opiates, antipsychotics, given during wakeup for extubation

All concomitant treatments which the patient was receiving at the time of any serious adverse event (SAE) should be recorded.

From the end of the 48-hour post-study treatment monitoring period until day 30:

While still in ICU, use of the following list of concomitant medications should be collected daily. The time of administration or dose given will not be recorded. After discharge from ICU, no concomitant medication will be collected except in case of an SAE.

- All sedative agents
- Opiates (other analgesics, such as NSAIDs and paracetamol should not be collected)

The following will not be recorded at any time during the study treatment or follow up period (except at the time of an SAE):

Standard ICU treatment including but not limited to:

- Antibiotics
- Nutritional solutions and supplements
- Electrolytes
- Hydrating infusions
- Antithrombotic medicine
- Antitussive medicine
- Stomach protection medicine
- Blood products

11.2.19.2 Recording of non-pharmacological interventions

From the start of study treatment until 30 days after the end of study treatment the following non-pharmacological interventions are to be recorded:

- All interventions used to treat an adverse event
- Renal replacement therapy (any mode)
- Extracorporeal Membrane Oxygenation (ECMO)
- Tracheostomy

11.2.20 Adverse events

Adverse events will be recorded from start of IMP until the end of the 48-hour post-study treatment monitoring. After this, the identified adverse events will be followed up weekly until 30 days after end of study treatment or until the until the medical condition of the patient is stable (see Section 12.3.5). In addition, any severe, serious or adverse events assessed to have a possible or probable causality to the IMP with onset after the 48-hour post-study treatment monitoring will be recorded at least weekly until 30±2 days after end of study treatment (see Section 12.3.1).

Events related to sedation depth, delirium and withdrawal will be reported separately and will not be reported as adverse events (see Section 12.2.1.1).

11.2.21 AnaConDa-S deficiencies

The AnaConDa-S will be used according to its instructions for use, and any deficiencies observed with the device should be reported. These will be reported by the Investigator immediately (within 24 hours) after becoming aware of it. For more details on reporting of deficiencies, refer to Section 12.2.8.

11.2.22 Handling of biological samples

All biological samples collected during this study will be analysed locally and destructed after analysis in accordance with the standard practice of the local hospital laboratories. No samples will be stored for the purpose of this study after the initial analysis.

12. ADVERSE EVENTS

12.1 Medical emergencies and contacts

The Investigator(s) are responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. A medical emergency usually constitutes an SAE and is to be reported as such, see Section 12.2.2.

12.2 Definitions of adverse events, adverse device effects and device deficiencies

The definitions and procedures for reporting AE, adverse device effects (ADE), SAE and serious adverse device effects (SADE) are presented in the sections below. It is of utmost importance that all staff involved in the clinical investigation is familiar with the definitions and procedures and it is the responsibility of the Investigator to ensure this.

12.2.1 Adverse events

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g. nausea, chest pain), signs (e.g. tachycardia, enlarged liver) or the abnormal results of an investigation (e.g. laboratory findings, ECG). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs.

12.2.1.1 Study specific definitions of events assessed and recorded separately

In this study, events relating to the desired degree of sedation are recorded using the COMFORT-B scale. Therefore, events such as “agitation”, “distress”, “undersedated”, “oversedated”, which are efficacy-related, will not be recorded as adverse events. However, cases of severe agitation (i.e. if patient is combative and/or requires immediate intervention) will be reported as an adverse event as this is considered an adverse event of special interest (see Section 12.2.7).

Similarly, signs and symptoms of delirium or withdrawal will be assessed using the SOS-PD scale and will not be recorded as adverse events.

12.2.2 Serious adverse events

A SAE is an AE occurring during any study phase that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event

An important medical event is considered an AE which, based upon appropriate medical judgement, may jeopardise the patient and may require significant medical or surgical intervention to prevent one of the outcomes listed above. Examples of medically important events are intensive treatment in an emergency room for allergic bronchospasm or blood dyscrasias, convulsions that do not result in hospitalisation, development of drug dependency or drug abuse.

Planned hospitalisations or surgical interventions for a condition that existed before the patient signed the ICF do not constitute an SAE if it did not change in intensity or worsened during course of study.

For the purpose of this study, all patients are hospitalised at the start of study and hospitalisation is in itself not an SAE. However, if a prolongation of hospitalisation is necessary, the reason for this prolongation may constitute an SAE.

12.2.3 Adverse drug reaction

An adverse drug reaction (ADR) is any untoward and unintended response to an IMP related to any dose administered.

All AEs judged by either the reporting Investigator or the Sponsor as having a reasonable causal relationship (judged as possibly or probably related to IMP as per Section 12.3.3) to an IMP qualify as ADR. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

The term serious adverse drug reaction (SADR) is to be used when an SAE is assessed as related to any dose administered.

12.2.4 Unexpected adverse drug reaction

An unexpected adverse drug reaction (UADR) is an adverse reaction, the nature, or severity of which is not consistent with the applicable product information e.g. SmPC or Investigator's Brochure (IB). When the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered unexpected.

12.2.5 Suspected unexpected serious adverse reaction

A SUSAR is a suspected unexpected serious adverse reaction, which is assessed as having a reasonable possibility of causal relationships with the study drug.

12.2.6 Adverse device effects

An ADE is an AE related to the use of an investigational medical device.

This definition includes any event resulting from insufficiencies or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device.

This definition includes any event that is a resulting from a user error or from intentional misuse of the investigational medical device.

12.2.6.1 Serious adverse device effects

A serious adverse device effect (SADE) is an ADE that results in any of the consequences characteristic of an SAE and includes device deficiencies that might have led to any of these consequences if suitable action was not taken, if intervention was not made or if circumstances were less opportune.

12.2.6.2 Unanticipated serious adverse device effect

An unanticipated serious adverse effect (USADE), is a SADE, which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report.

An anticipated serious adverse device effect (ASADE) is an effect which by nature, incidence, severity or outcome has been identified in the risk analysis report.

12.2.7 Definition of adverse events of special interest

Adverse events of special interest in this study are defined in Sections 12.2.7.1 to 12.2.7.5.

12.2.7.1 Common adverse events

AEs defined as common adverse events will be evaluated as part of a secondary safety endpoint in this study. These are events commonly seen in the study population and the clinical significance of these will be considered in the context of this study and the clinical status of the patients. Signs and symptoms of these events will be considered clinically significant if observed as described in this section and should thus be reported as adverse events. These are defined based on consensus among the national coordinating investigators for this study, as the following:

- Hypertension (increase 20% of baseline values)
- Hypotension (decrease 20% of baseline values)
- Tachycardia (increase 20% of baseline values)
- Bradycardia (decrease 20% of baseline values)
- Hypoxia (Oxygen saturation below 88% for more than 5 minutes. For patients with baseline oxygen saturation of 92% or lower due to e.g. underlying disease, a drop of 10% or more for more than 5 minutes.)
- Renal insufficiency (class *Injury* or worse as assessed according to the pRIFLE classification, i.e. eCCl decrease of 50% compared to baseline or a urinary output of <0.5mL/kg/hr for 16 hours (Akcan-Arikan et al. 2007, KDIGO Clinical Practice Guideline for Acute Kidney Injury 2012 and Kavaz et al. 2011))
- Nausea
- Vomiting
- Hyperglycaemia

12.2.7.2 Sedation-related adverse events

AEs defined as sedation-related adverse events are expected to occur in patients mechanically ventilated in the ICU. These are important to collect, all cases observed should be reported. These are defined, based on the systematic review performed by Grant et al. 2013 and a few additions judged as relevant in consensus among the national coordinating investigators, as the following:

- Obstipation
- Unplanned endotracheal tube extubation
- Unplanned removal of invasive tube or catheter
- Extubation failure (re-intubation needed within 24-48 hours of extubation)
- Ventilator associated pneumonia
- Catheter associated bloodstream infection
- Stage two pressure ulcers
- New tracheotomy
- Severe agitation (combative, requiring immediate intervention)
- Post-extubation stridor with chest-wall retractions at rest

The adverse events also listed by Grant et al. 2013 of "inadequate sedation management" and "clinically significant iatrogenic withdrawal" are not included within this definition as they are handled separately in this study, see Sections 12.2.1.1 and 12.2.7.4.

12.2.7.3 Neurological and psychomotor adverse events

All cases of neurological and psychomotor dysfunction should be collected as adverse events. These are defined as:

- Systemic or localized tremor
- Chorea
- Hallucinations
- Dystonia
- Seizures
- Other abnormal movements

Assessment of potential neurological and psychomotor symptoms will be done during the study treatment period and in the 48-hour post-study treatment period Any such AEs should be monitored daily for up to 1 week, or until resolved if earlier than end of the 1 week. If not resolved after 1 week, the patient will be referred to a paediatric neurologist for continued follow-up. These consultations will be monitored for up to 30 days after end of study treatment and time of resolution or current status, if unresolved be documented.

12.2.7.4 Delirium and withdrawal

Symptoms of delirium and withdrawal will be assessed using the SOS-PD scale (refer to Section 11.2.17) and will not be reported as separate adverse events.

12.2.7.5 Organ function parameters

Renal and hepatic function tests (S-creatinine, S-urea, S-Bilirubin, S-AST, S-ALT) as well as urine output will be assessed from baseline until 48 hours after end of treatment. Clinically significant worsening in any of these parameters as judged by the investigator will be reported as adverse events.

Patients with results indicating compromised organ function will be monitored until resolution or referral for clinical follow-up and management as clinically indicated.

12.2.8 Device deficiencies

A device deficiency is defined as inadequacy of an investigational medical device related to its identity, quality, durability, reliability, safety or performance. This may include malfunctions, use error, or inadequacy in the information supplied by the manufacturer.

Deficiencies observed with the AnaConDa-S will be recorded by the Investigator in the eCRF immediately (within 24 hours of becoming aware of it). The information recorded will include a description of the device deficiency as well as information about whether there was any injury to the patient or user as a result of the deficiency. If an injury occurred, an ADE or SADE should also be recorded in the eCRF as described in Section 12.3.

If no SADE occurred as a result of the device deficiency, the Investigator shall assess whether or not the device deficiency *could have led* to an SADE if:

- Suitable action had not been taken;
- Intervention had not been made; or

- Circumstances had been less fortunate.

All device deficiencies reported in the eCRF will lead to an email alert sent to predefined recipients, which will include the Sponsor. The Sponsor will also review all device deficiencies during the study and determine and document in writing whether they could have led to an SADE.

If the Investigator or Sponsor judges that the device deficiency could have led to an SADE, the device deficiency will be subject to similar safety reporting procedures as for SAEs, described in Section 12.4.

Reporting of deficiencies to competent authorities will follow the Sponsor's standard operating procedure (SOP) for incident reporting and the applicable regulation on device vigilance, i.e. MEDDEV 2.12-1 rev. 8 and Medical Device Regulation 2017/745 (MDR). In case requirements are discrepant, the requirements stipulated by the MDR will be followed.

12.3 Recording and reporting of adverse events

12.3.1 Collection of adverse events and adverse device effects

In this study the AE reporting period starts from the time of the first dose of IMP. This approach is selected since the enrolled patients are expected to display a large amount of adverse signs and symptoms in the period before the IMP is initiated which are unrelated to the treatment of IMP or device. By initiating AE reporting at start of IMP administration, the AE reporting is expected to capture events relevant for the evaluation of the study treatments. Events believed to be related to a protocol-mandated procedure, occurring prior to first dose of IMP, i.e. pre-treatment events should be reported in the Medical history log in the eCRF.

Any AEs occurring during the period from first dose of IMP until end of the 48-hour post-study treatment period should be recorded.

From the 48-hour post-study treatment period and until the end of the follow-up period at 30±2 days after the end of study treatment, the following will be recorded:

- All SAEs (regardless of causality to IMP)
- All severe AEs (regardless of causality to IMP)
- All other AEs that are assessed to have possible or probable causality to the IMP

ADEs should be reported during the time which the AnaConDa-S is used.

All patients will be carefully monitored for the occurrence of AEs during the study treatment and follow up period. The following should be recorded in the eCRF AE form:

- Brief description of the event (diagnosis).
- Start date (and time, if relevant).
- Stop date (and time, if relevant) (or resolution).
- Severity.
- Action taken regarding study drug/medical device.

- Assessment of causality.
- Seriousness.
- Outcome and date and time of outcome

Clearly related signs, symptoms and abnormal diagnostic procedure results should be grouped together and reported as a single diagnosis or syndrome whenever possible.

If a patient suffers from the same AE more than once and the patient recovers in between the events, the AEs should be recorded separately. If an AE changes in severity, a worst-case approach should be applied when recording the event, i.e. the highest severity and the longest duration of the event.

If the event is considered to be attributable to the device, this will be documented in the AE form in eCRF and will then be considered an adverse device effect.

12.3.2 Adverse event severity

Severity describes the intensity of an event. Regardless of the classification of an AE as serious or non-serious its intensity must be assessed by the Investigator. The grades of intensity are defined as follows:

- Mild: Clinical or diagnostic observations only; intervention not indicated.
- Moderate: Minimal, local or non-invasive intervention indicated.
- Severe: Intervention required, consider seriousness.

12.3.3 Adverse event causality assessment

Regardless of the classification of an AE as serious or non-serious (see above), its potential causal relationship with the IMP and/or with the medical device (device deficiency or user handling of the device) must be assessed by the Investigator and entered in the AE form. Causality will be assessed as:

- Probable: A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfil this definition.
- Possible: A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
- Unlikely: A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

12.3.4 Assessment of outcome

The Investigator must assess the outcome of an AE using the definitions below:

- Recovered: The patient has recovered completely, and no symptoms remain.
- Recovering: The patient’s condition is improving but symptoms still remain.

- Recovered with sequelae: The patient has recovered, but some symptoms remain (for example, the patient had a stroke and is functioning normally, but has some motor impairment).
- Not recovered: The patient's condition has not improved, and the symptoms are unchanged.
- Death: The patient's condition was fatal.

12.3.5 Follow-up of adverse events (serious and non-serious) with onset during the trial

During the study, the Investigator must follow-up on each AE/ADE until it is resolved or until the medical condition of the patient is stable.

After the patient's last visit (Day 30±2), the Investigator must follow-up on any AE/ADE collected during the study that is still ongoing until it is resolved, stabilized or referred to a clinical specialist, or until the Investigator judges that further follow-up is not necessary. For SAE/SADEs, the Investigator must report to the Sponsor all relevant new information, including, but not limited to information including discharge summaries, autopsy reports and medical consultation.

12.3.6 Collection of serious adverse events with onset after end of study

If an investigator becomes aware of an SAE after the end of the trial, and he/she assesses the SAE to have a possible or probable causality to the IMP, the investigator must immediately report the case to the Sponsor, regardless how long after the end of the study this takes place.

12.4 Reporting of serious adverse events and serious adverse device effects

Starting from the time of the first dose of IMP, all SAEs/SADEs must be reported to the Sponsor and or designee immediately, without undue delay but no later than within 24 hours after awareness of the event. All SAEs/SADEs have to be reported, whether or not they are considered causally related to the IMP and/or the investigational medical device.

The initial report should contain as much information as possible, but as a minimum the following information should be entered:

- Patient ID
- Description of event (diagnosis)
- Start date of the event (time if applicable)
- Study treatment
- Start date of the study treatment
- Start date of the use if medical device
- Causality assessment (study treatment and medical device)
- Seriousness criteria
- Severity

The full AE-form in the eCRF must be completed and any additional information requested by the Sponsor must be provided by the reporting Investigator as soon as possible, at the latest within 2

calendar days of the occurrence of the SAE. All AEs meeting the definition of serious will be immediately flagged to predefined recipients as soon as the event has been entered as "serious" and "saved" in the eCRF.

For reported deaths, the Investigator should supply the Sponsor and the EC (as applicable) with any additional requested information (e.g. autopsy reports and terminal medical reports).

12.4.1 Electronic serious adverse events reporting procedure

The SAEs/SADEs will be reported by the site electronically via the eCRF (Viedoc™). The site staff will enter all available information on the event (SAE or SADE) in the AE log for the specific patient. As soon as the event is saved as serious in the eCRF, an email alert will be sent to predefined recipients to highlight that an SAE has occurred.

A designated person at Sponsor or designee will review the SAE report to ensure that the report is complete and correct. If any important information is missing or is unclear, queries will be raised. Investigators or other site personnel will inform Sponsor or designee of any follow-up information on a previously reported SAE as soon as he or she becomes aware of it. Whenever an SAE is updated in the eCRF, a new email alert will be sent.

The designated person will contact the Medical Monitor to obtain his/her assessment of causality of the SAE.

If the SAE is judged as causally related to treatment by either the Investigator or Medical Monitor, expectedness will be assessed by the Medical Monitor. Expectedness will be assessed as "expected" or "unexpected". The reference documents for assessment of expectedness will be the isoflurane Investigator's Brochure and midazolam SmPC.

If any additional documentation is required, the Sponsor or designee will request this from the study site.

12.4.2 Paper reporting

In case the eCRF is out of order, or if no internet access is available at the study site, the SAE should be reported using a paper copy of the SAE Form, which will be available at the site in the Investigator Site File and should be completed manually. The completed, signed and dated report should, within 24 hours, be scanned and e-mailed to:

LINK Medical Safety Sweden
Attention: SAE SED002
email: safety@linkmedical.se

The study site should notify the site Monitor via phone or email about the submission of the SAE report. As soon as the site personnel have access to the eCRF, the SAE should be reported electronically as well.

12.5 Reporting of reportable events to CA and EC (SUSARs/SADEs)

12.5.1 Reporting of suspected unexpected serious adverse reactions

The term SADR is used whenever either the Investigator or Medical Monitor deems the SAE as possibly or probably related to the IMP. If a SADR is assessed as unexpected by the Medical Monitor, it is a SUSAR (see Sections 12.2.3 to 12.2.5).

Any reportable SUSAR, will be submitted to the competent authority, via the EudraVigilance database, and to the IEC(s) in accordance with local regulations and the Sponsor or designee's SOPs within the following timelines:

- 7 calendar days if fatal or life-threatening (follow-up information within an additional 8 days)
- 15 calendar days if non-fatal and non-life-threatening (follow-up information as soon as possible)

Timelines are counted from Day zero, i.e. the date of receipt by the Sponsor or designee.

The Sponsor or designee also has the obligation to, once a year throughout the clinical study (or on request), submit a safety report to the CA and the IEC taking into account all new available safety information received during the reporting period.

The Sponsor is responsible for informing the investigators concerned of relevant information about all potential SUSARs that could adversely affect the safety of patients.

12.5.2 Reporting of serious adverse device effect

Reporting of SAEs and SADEs associated with the use of the device to the EC will follow the Sponsor or designee's SOPs and the applicable regulation on device vigilance, i.e. MEDDEV 2.12-1 rev. 8 and MDR. In case requirements are discrepant, the requirements stipulated by the MDR will be followed.

12.6 Overdose

An overdose is a dose in excess of the dose specified in the IB or SmPC. An overdose should be monitored closely and treated as outlined in the SmPC. This should be recorded as follows:

- An overdose with associated AE is recorded as the AE diagnosis/symptoms on the relevant AE modules in the eCRF.
- An overdose without associated symptoms is only reported in the patient's medical records.

The IMP will be dispensed and administered in the ICU thus significant overdosing is not likely to happen.

12.7 Pregnancy

A pregnancy as such is not an AE, unless there is a possibility that the IMP has interfered with the efficiency of any contraceptive measures. However, if a patient becomes pregnant during the study treatment period, the investigator should report the pregnancy and pregnancy outcome to the Sponsor using a Pregnancy Report Form.

The pregnant patient will be followed until the end of the pregnancy. Any complication during the pregnancy should preferably be reported as an AE. The outcome of the pregnancy must be reported on the pregnancy report form.

In case of spontaneous abortion, stillbirth, congenital anomaly or birth defect, death or any other serious infant condition, must be reported and followed up as an SAE according to the procedures described in Section 12.4.

12.8 Data Safety Monitoring Board

An independent DSMB will be formed to monitor the benefit/risk ratio during the course of the study. The DSMB will evaluate all AESI, AEs, SAEs, ADEs, SADEs, and applicable patient characteristics. Additional data summaries may be requested as needed. They will not review efficacy endpoints or

dosing. The DMSB will comprise of at least 3 members with appropriate expertise who are all independent of the Sponsor. Study sites will be notified of any relevant safety findings that may jeopardise participants' safety. The DSMB will meet after the first 40 patients have completed the follow-up period. Subsequent meetings will occur at appropriate time when increments of 40 patients have completed the follow up period throughout the study duration. The independent DSMB will have the mandate to temporarily stop the enrolment on safety grounds. A charter will be established between the Sponsor or designee and the DSMB to outline the DSMB's responsibilities and procedures.

13. STATISTICAL METHODS

13.1 Statistical Analysis Plan

The principal features of the statistical analysis of the data are described in this section. Details on all statistical methods will be provided in the statistical analysis plan (SAP), which will be finalised before the first patient is enrolled into the study. In case of discrepancies in the description of the statistical analysis between this CSP and the SAP, the description in the SAP will prevail. Any deviations from the planned statistical analysis will be documented in the clinical study report (CSR).

13.2 General

The statistical analyses will be performed using SAS® Software version 9.4 or higher.

In general, descriptive statistics will be presented for all efficacy and safety variables. Continuous variables will be summarised by descriptive statistics (sample size [n], mean, standard deviation, minimum, median, and maximum value) by treatment group. This will be done for both actual values and change from baseline values. Categorical variables will be summarised in frequency tables showing number of patients, frequency and percentage of occurrence by treatment group. Graphical presentations will be used as appropriate and individual patient data will be listed.

All tests will be two-sided and performed at the 5% significance level unless stated otherwise.

13.2.1 Missing data

Methods for handling of missing data related to the primary endpoint is listed below in Section 13.5, whereas methods for handling of missing data related to the other endpoints will be specified in the SAP.

13.2.2 Multiplicity

As only one primary endpoint is selected, no adjustments for multiple comparisons will be made. The stepwise testing procedure of initially performing the superiority test, with a potential second step of the non-inferiority test, constitutes a closed testing procedure, which means that the significance level is preserved (i.e. at 5%) in the potential second step.

13.3 Description of analysis sets

13.3.1 Full analysis set

The full analysis set (FAS) will include all randomised patients who received IMP and have at least a 6-hour sedation period and at least 3 blinded COMFORT-B-assessments. The FAS will follow the intention-to-treat (ITT) principle, i.e. patients will be analysed according to the treatment group they were assigned to at randomisation. The main statistical analysis will be performed on this population.

13.3.2 Per protocol analysis set

The per protocol (PP) analysis set will include all patients in the FAS without any major protocol deviation affecting the primary analysis. In order to be included in the PP analysis set patients need to have been sedated for at least 12 hours, with at least 50% of the planned COMFORT B assessments performed. Furthermore, if two or more changes in prescribed target sedation depth should occur (one change is allowed), the patient will be excluded from the PP analysis set. Additional criteria for the determination of the PP population will be specified in the SAP. Supplementary analyses will be performed based on the PP analysis set, details will be described in the SAP.

13.3.3 Safety analysis set

The safety analysis set will include all patients who received IMP and will be analysed in accordance with actual treatment received.

13.4 Patient disposition, demographics and other baseline characteristics, medical and surgical history, prior and concomitant medication, and compliance

The disposition of patients, demographic data and relevant baseline characteristics (including medical history), prior and concomitant medication and compliance will be presented descriptively.

13.5 Analysis of the primary efficacy endpoint using an estimand approach

The primary objective for this study is to compare the percentage of time adequate sedation depth is maintained within the individually prescribed target range in absence of rescue sedation as assessed according to the COMFORT-B scale, in isoflurane vs midazolam treated paediatric patients for an expected minimum of 12 hours.

The intercurrent event “use of rescue sedation” is defined as any of the below:
i) use of per protocol rescue sedation, see Section 10.3.1;
ii) use of bolus doses of opiates, see Section 10.2.5.1;
iii) use of prohibited medications, including neuromuscular blocking agents that are not allowed for treatment maintenance, see Section 10.4.1;
iv) use of α2-adrenergic agonists infusions in a manner that leads to failure time in the calculation of the primary endpoint, see Section 10.4.2.

The administration of medications listed in ii)-iv) above will be handled similarly statistically as rescue sedation. The intercurrent event “use of rescue sedation” will refer to any of the defined 4 categories i)-iv) above when describing the statistical methods in this section of the study protocol.

The 5 attributes of the primary estimand are addressed in the below sub-sections.

13.5.1 Treatment conditions

Test product: isoflurane administered via the AnaConDa-S device, titrated to effect, inhalational vapour.

Comparator product: midazolam titrated to effect, continuous intravenous infusion.

For details, see Section 10.

13.5.2 Patient population

Paediatric patients at least 3 years to 17 (less than 18) years admitted to the ICU or with a planned ICU admission (e.g. postoperative patients) and expected to require mechanical ventilation and sedation for at least 12 hours.

For details, see inclusion/exclusion criteria in Section 9.3.

13.5.3 Endpoint

The definition of the primary endpoint is given in Section 7.1.1. A more detailed version, reflecting the text below describing the analysis strategy for the intercurrent events, is:

- Percentage of time of adequately maintained sedation, in absence of rescue sedation, within the COMFORT-B interval (light, moderate or deep sedation) prescribed at randomization (allowing for one change in prescribed target range) monitored every 2 hours for an expected minimum of 12 hours (up to 48 ± 6 hours).

The time-points of starting and stopping the study sedative treatment will be registered in the eCRF using a format similar to YYYY/MM/DD HH:MM.

The complete time between start and stop of the primary endpoint data collection period will be divided in several time intervals, each coded into one of the three possible categories: “Success”, “Failure”, or “Censored” as described in the below sub-sections. Using the composite endpoint approach, the percentage of time with adequate sedation depth without any rescue sedation is thus calculated for each patient as the total time coded as "Success" divided by the total time coded as "Success" or "Failure". Time coded as "Censored" will be omitted.

13.5.3.1 Definition of start time

For all patients, the start time of the data collection for the primary endpoint will be the first blinded COMFORT-B assessment (at +2 hours). Below, the three possible different scenarios at start of IMP are described:

1) For patients randomised to midazolam, and who are currently sedated with midazolam at the time of randomization, ongoing midazolam will be replaced with study specific midazolam (supplied by the Sponsor) at the same midazolam dose-rate. Thereafter dose can be titrated to reach the COMFORT-B target interval prescribed at baseline.

2) For patients randomised to midazolam, and who are receiving sedation other than midazolam at the time of randomization, study treatment will start by initiating an IV infusion of midazolam (as specified in Section 10.2.4.1), and all previous sedatives are simultaneously turned off (up to 30 minutes prior to initiating midazolam).

3) For patients randomised to isoflurane, study treatment will start by initiating isoflurane sedation (as specified in Section 10.2.3), and all other sedatives are simultaneously turned off (up to 30 minutes prior to initiating isoflurane).

13.5.3.2 Definition of stop time

The primary endpoint assessment will stop when the study treatment is stopped or reduced significantly (>25%) to prepare for extubation. This can be either due to wake-up for extubation or when study treatment is stopped and replaced with the standard treatment (at 48±6 hours from study start).

13.5.3.3 COMFORT-B assessments and intercurrent events (rescue sedation) related to primary endpoint

With the exception of COMFORT-B assessments performed before the start time of the data collection for the primary endpoint, a blinded assessor will make all COMFORT-B assessments at all subsequent time-fixed 2-hour assessments. All these assessments will be used to continually assess sedation depth as described Table 8 in Section 11.2.16.

After each COMFORT-B assessment, the results are shared with the study personnel and if the COMFORT-B assessment result means that the patient is outside their target sedation depth, the study treatment dose should be titrated according to Section 10.2.5.

Each of these COMFORT-B assessments will be coded for later analysis as “Success” or “Failure”.

Definition of “Success” in later analysis: Sedation depth at the scheduled measurement is equal to target sedation depth **or maximum 1 point outside**. This means that the ranges leading to “Success” code will be: Light 16-23, Moderate 10-17, Deep 6-11 (the lowest possible value would be 6). If a patient’s COMFORT-B score fits into two categories, and one these is the target sedation depth, the assessment will be coded as “Success”. Allowing the COMFORT-B evaluation to be 1 score outside the prescribed range is considered suitable given that the COMFORT-B assessment is a multidimensional scale and expected to exhibit smaller clinical variability between the bedside team rating and the blinded assessor. Regardless of this, staff informed about the current COMFORT-B score will titrate the study treatment with the aim to keep the patient within the prescribed interval.

Definition of “Failure”: Sedation depth at the scheduled measurement is not equal to target sedation depth (i.e. COMFORT-B scores are outside the ranges specified for the “Success” code above) or the patient has experienced a specific type of intercurrent event (rescue sedation). COMFORT-B scores outside the ranges specified for the “Success” code above will be connected with a 60-minute (±30 minutes of time of COMFORT-B assessment) long time interval coded as “Failure”. If the sedation depth is outside the range for success for two consecutive COMFORT-B assessments, performed at the scheduled time points, the full period between the two COMFORT-B assessments will be coded as “Failure”.

Use of rescue sedation will be connected with a 30-minute (±15 minutes of time of rescue sedation administration) long time interval coded as “Failure”. The coding of 30 minutes as failure time is considered suitable, based on an approximation of the duration of the clinical effect of the rescue sedation.

13.5.3.4 Further intercurrent events and other considerations related to primary endpoint

In addition to definition of success and failure times as described above, the term “Censored” is introduced.

Definition of a censored time-period: A time-period between the defined start and stop time, which will not be included in the calculation of the primary endpoint.

In addition to failure times, censoring is introduced in relation to the below listed situations, including intercurrent events other than the use of rescue sedation. Furthermore, the handling of missing data related to the primary endpoint is described below, as applicable.

1) Missing scheduled COMFORT-B assessments

A missing scheduled COMFORT-B measurement will be connected with a 120-minute (±60 minutes around the time-point when the COMFORT-B assessment was scheduled) long time interval coded as "Censored". The 120 minutes of time period censoring is considered suitable, based on the frequency of the scheduled COMFORT-B assessments (every 120 minutes).

If rescue sedation is administered during a time period for which the COMFORT-B assessment is missing, the assignment of failure time will prevail over the censoring due to missing data.

2) Temporary sedation stop due to wake-up test/neurological assessment

A temporary sedation stop due to wake-up test/neurological assessment will impact the calculation of the primary endpoint. No COMFORT-B assessments should be performed during such a stop, and the next COMFORT-B assessment after the wake-up must be made at earliest 30 minutes after re-initiation of study treatment to allow for titration of study treatment in order to reach the prescribed target sedation depth. The time from the start of the wake-up test/neurological assessment to the first COMFORT-B assessment will be coded as "Censored".

3) Change in prescribed target sedation depth

One change of the sedation target will be allowed for the welfare of the patient at any time during the study treatment period. The time and medical rationale for changing the prescribed sedation target should be recorded in the eCRF. One change of the sedation target will not impact the calculation of the primary endpoint. The treatment policy strategy is thus applied for this intercurrent event.

4) Medical diagnostic or therapeutic procedure in the ICU

Medical diagnostic or therapeutic procedures, such as changing in dressings, napping, washing or re-positioning patient, bronchoscopy, or intravascular access, may be needed during the study treatment period. For the conduct of such, the prophylactic administration of sedatives (the IMP sedative, propofol or ketamine), opioid and/or a neuromuscular blocking agents is allowed, see Section 10.3.2. These should be labelled "procedural medication" in the eCRF. Administration of such sedatives, opiate boluses and/or neuromuscular blocking agents will not impact the calculation of the primary endpoint. However, the next COMFORT-B assessment should not be performed during or earlier than 30 minutes after last dose/end of infusion of procedure-specific opiate/sedative and no earlier than 60 minutes after the last dose of neuromuscular blocking agent. These 30- and 60-minute time periods are considered clinically relevant to minimize residual effects of the procedural medication used.

5) Surgical procedure outside the ICU

Anaesthesia and analgesia for surgical procedures should be given according to standard of care. After the procedure, study treatment should be re-initiated as soon as possible in the ICU and study treatment should be titrated to the prescribed target sedation depth. After anaesthesia, the next COMFORT-B assessment should not be performed earlier than 60 minutes after re-initiation of study treatment. Administration of anaesthesia and analgesia for surgical procedures will impact the calculation of the primary endpoint. The time from the point of implementing the deeper sedation or anaesthesia to the first COMFORT-B assessment after re-initiation of study treatment will be coded as "Censored".

In case anaesthesia and analgesia is needed for surgical procedures, study sedation must be re-initiated within 2 hours of the stop of the procedure/anaesthesia. Later restart of study treatment will be considered a protocol deviation and time from 2 hours after the stop of the procedure until the next scheduled COMFORT-B assessment will be classified as failure time.

6) Treatment discontinuation

In case a patient cannot be adequately sedated with study treatment in accordance with Sections 10.2-10.5, this will be considered a failure of the study treatment. The Investigator should discontinue the study treatment and instead treat the patient according to current clinical practice and the attending physician's judgement, as described in Section 9.6.2. If a patient is discontinued from the study treatment because of study treatment failure, the time from study treatment stop up to 48 hours will be classified as "Failure".

In case anaesthesia and analgesia is needed for surgical procedures, study sedation must be re-initiated within 2 hours of the stop of the procedure/anaesthesia, otherwise the patient should be discontinued from the study treatment. Later restart of study treatment will be considered a protocol deviation and time from 2 hours after the stop of the procedure until the next scheduled COMFORT-B assessment will be classified as failure time. If a patient is discontinued from the study treatment, the time from stop of the procedure/anaesthesia up to 48 hours will be classified as "Failure".

All other reasons for treatment discontinuation that are not related to the efficacy of the study treatment, e.g. due to withdrawal of consent or any of the reasons exemplified in Section 9.6.3 will not be considered a failure. For these patients, the calculation of the time of adequate sedation depth will stop at the time of discontinuation. The results of the last COMFORT-B will carry forward up to 2 hours after the last COMFORT-B assessment until the time of discontinuation, any period existing after the +2 hours of the last COMFORT-B assessment until time of discontinuation will be considered missing, as per 1) above.

The composite endpoint strategy is thus applied for this intercurrent event.

7) α2-adrenergic agonists

A proportion of patients is expected to be receiving α2-adrenergic agonists at the time of study inclusion. In order to reduce confounding effects of previous sedation and potential withdrawal symptoms, sedation >72 hours is an exclusion criterion. However, it cannot be completely out ruled that a small proportion of patients may develop withdrawal symptoms. The following applies, in order to accommodate for potential α2-adrenergic agonist withdrawal symptoms: during the first 12 hours of study treatment, *re-starting* infusion of α2-adrenergic agonist up to a maximum of 50% dose prior to randomisation, if the patient develops signs of withdrawal (rebound tachycardia and hypertension, >20% worsening in any) that cannot be managed despite an increase of study treatment dose of at least 50% compared with the first study treatment maintenance dose reached after dose-titration (+2 hours after study treatment initiation), is allowed in the sense that it will not impact the calculation of the primary endpoint.

If the infusion dose of an α2-adrenergic agonist is increased above 50% of the dose prior to randomisation during the first 12 hours of the sedation period, the time of such use above 50% will be classified as "Failure".

Re-introduction of an α2-adrenergic agonist, more than 12 hours after study start, will lead to that the time from the start of this infusion period until it is terminated, is classified as "Failure". The composite endpoint strategy is thus applied for this intercurrent event.

Use of α2-adrenergic agonist bolus doses, is prohibited and will be connected with a 30 minute (±15 minutes of time of rescue sedation administration) long time interval coded as "Failure" in line with the principles for use of prohibited medications outlined above.

Finally, in the case where the patient has not received any α2-adrenergic agonist at the time of study inclusion, any use of α2-adrenergic agonists at any time during the study treatment period will be connected with "Failure" times according to the same principles as outlined above for α2-adrenergic agonist infusions or bolus doses. This means that for α2-adrenergic agonists infusions, the time from the start of this infusion period until it is terminated, will be classified as "Failure", whereas the use of α2-adrenergic agonists bolus doses will be connected with a 30 minutes (±15 minutes of time of rescue sedation administration) long time interval coded as "Failure".

8) Death

In case of patient death, the calculation of the time of adequate sedation depth will stop at the time of death. The results of the last COMFORT-B will carry forward up to 2 hours after the last COMFORT-B assessment until the time of death, any period existing after the +2 hours of the last COMFORT-B assessment until time of death will be considered missing, as per 1) above.

The composite endpoint strategy is thus applied for this intercurrent event.

13.5.4 Other intercurrent events

No other intercurrent events are identified for the primary estimand.

13.5.5 Population-level summary for the endpoint

The mean percentage of time with adequate sedation depth for isoflurane treated patients minus the mean percentage of time with adequate sedation depth for midazolam treated patients.

13.5.6 Main estimator

Aligned with the estimand, the main estimator, i.e. the main statistical analysis will be a mixed effects analysis of variance model with treatment group as fixed effect and country as a categorical random effect. The Age group (3-7 years; 8-11 years; and 12-17 years) and the reason for ICU admission (planned mechanical ventilation or unplanned mechanical ventilation) are not included as effects in the statistical model though covariates used for stratifying the randomisation typically are included in the model. In this case however, the randomisation is stratified because it is deemed important for the evaluation of safety, not for efficacy. As age and the duration of sedation are not known prognostic factors for the outcome in the primary endpoint, they are not included as effects in the statistical model. Least square means and the model-based estimate of the difference between the treatment groups, along with the corresponding symmetric 2-sided 95% confidence interval will be calculated.

The claims of the study regarding the primary efficacy endpoint can be derived from the 95% confidence interval of the (isoflurane- midazolam)-difference in percentage of time with adequate sedation depth in absence of rescue sedation as follows:

- Isoflurane is superior to midazolam if the entire 95% confidence interval lies above 0.
- If isoflurane is not shown to be superior to midazolam, then isoflurane is non-inferior to midazolam if the entire 95% confidence interval lies above the pre-defined non-inferiority margin of -15% (relative difference).
- If isoflurane is not shown to be non-inferior to midazolam, i.e. the entire 95% confidence interval does not fall above -15% (relative difference), then the alternative hypothesis of isoflurane being non-inferior to midazolam can thus not be accepted.
- If the entire 95% confidence interval falls below -15% (relative difference), then the null hypothesis of isoflurane being inferior to midazolam will be considered accepted.

This stepwise testing scheme follows a closed testing procedure, which means that the significance level will be kept at 5% in the potential second step involving the non-inferiority test.

The rationale for the clinical relevance of a relative difference in 15 percentage points in the proportion of percentage of time with adequate sedations depth is presented in Section 6.2.

The statistical analysis of the primary endpoint will be conducted on FAS.

13.5.7 Sensitivity analysis

To investigate the robustness of the main estimator results, the following sensitivity analyses of the same estimand will be performed. Details on these, and possibly other, sensitivity analyses will be given in the SAP.

1) Missing scheduled COMFORT-B assessments: Instead of censoring as described in Section 13.5.3.4, linear interpolation will be used.

2) Missing scheduled COMFORT-B assessments: Instead of only using censoring as described in Section 13.5.3.4, a combination of censoring and linear interpolation will be used in the sense that linear interpolation will be used if one value is missing between two observed values, whereas, if two or more sub-sequent values are missing, censoring will be applied.

13.5.8 Supplementary analysis

To investigate the robustness of the primary endpoint results, the following supplementary analyses of related estimands will be performed. Details on these, and possibly additional, supplementary analyses will be given in the SAP.

1) The statistical analysis of the primary endpoint, as defined in Section 13.5.3, will be conducted on a population of included patients that are 3-16 years old.

2) The statistical analysis of the primary endpoint, as defined in Section 13.5.3, will be conducted on the PP analysis set.

3) The statistical analysis of the primary endpoint, as defined in Section 7.1.1, will be evaluated using a treatment policy strategy, i.e. the intercurrent events as outlined in Sections 13.5.3.3 and 13.5.3.4 will, as a rule, be disregarded.

13.6 Analysis of the secondary efficacy endpoints

The statistical analysis of the secondary endpoints will be detailed in the SAP.

13.7 Safety analysis

Descriptive statistics will be used to present the safety outcomes in the two treatment groups as following the general principles given in Section 13.2, including incidences and severity of AEs and SAEs and incidences of AEs leading to treatment discontinuation.

All AEs will be coded according to MedDRA dictionary and grouped by preferred term (PT) and system organ class (SOC).

Information on AE intensity and relationship to IMP will be presented.

Laboratory and vital signs data will be presented descriptively.

Details of the safety analysis will be given in the SAP.

The safety analyses will use the safety analysis set.

13.8 Additional analyses

To support an eventual submission to the U.S. Food and Drug Administration (FDA), the complete set of statistical analyses described in sections 13.5 through 13.7 will also be conducted in a FAS population that excludes 17 year-old patients, consistent with the definition of paediatric patients in the U.S. as being less than 17 years of age.

13.9 Determination of sample size

The study will be powered for superiority with a non-inferiority test as a second step in the stepwise testing procedure. With a one-sided test at the 2.5% significance level, a total of 90 evaluable patients (randomised 2:1 isoflurane:midazolam) will give 80% power to detect superiority if the true difference in the primary variable is 22.2 percentage points greater for isoflurane (79.8%) than with midazolam sedation (57.6%), (Vet et al. 2013), Figure 2) assuming a common standard deviation of 35% percentage points.

It is expected that no more than 100 patients will be randomised into the study to get 90 patients evaluable for efficacy of the primary endpoint (as it is expected that up to 10% of the randomised patients will be excluded from the FAS analysis population, i.e. not evaluable for efficacy). Patients evaluable for efficacy (i.e. included in FAS) are those who received IMP and have at least 6 hours sedation period and at least 3 blinded COMFORT-B-assessments performed.

The rationale for the clinical relevance of a relative difference in 15 percentage points in the proportion of percentage of time with adequate sedations depth was presented in Section 6.2.

In previous observational studies of midazolam for sedation of critically ill patients where the COMFORT or COMFORT-B (Behaviour) scales were used, the incidence of optimal sedation ranged from 15% to 64%, most often assessed as the proportion of observations with optimal sedation score out of all observations (Table 9).

Table 9 Incidence of optimal sedation in critically ill patients in studies of midazolam using the COMFORT or COMFORT-B scales (observational studies or randomised controlled studies, RCT)

Study	Population Critically ill patients	n	Sedatives	Sedation scale	Definition optimal sedation	Incidence optimal sedation
Ista et al. 2009	0-3 yrs	131	Midazolam, morphine	COMFORT-B scale & NISS	COMFORT-B 11-22 with a NISS of 2	64% (2273/3573 obs)
Ista et al. 2005	0-18 yrs	78	Midazolam, morphine, ketamine, fentanyl	COMFORT-B scale & NISS	COMFORT-B 11-22 with a NISS of 2	48.8% (411/843 obs)
Froom et al. 2008	0-16 yrs	19	Midazolam, morphine, chloral hydrate	COMFORT	17–26	14.8% (4/27 obs)
De Wildt et al. 2005	2 days – 17 yrs	21	Midazolam	COMFORT	17–26	46.1% (244/497 obs)
Lamas et al. 2009	< 19 yrs	77	Midazolam, fentanyl (vecuronium)	COMFORT (& other)	18-40	18%
Wolfe et al. 2014	30 days to 15 years	120	Clonidine vs. midazolam (RCT)	COMFORT	COMFORT score 17–26 for ≥ 80% of the time	Midazolam: 30.5% (18/59) Clonidine: 34.4% (21/61)

COMFORT-B: behaviour, NISS: Nurse's Interpretation of Sedation, obs: observations, RCT: randomised controlled trial

Based on the above, indicating that isoflurane may be superior to IV sedation in the paediatric population, isoflurane is expected to render a larger proportion of time in the interval characterised as adequate sedation. The estimated superiority is in the range of 15-20% percentage units and the study will have a power of 80% to show superiority for a difference of 22.2% and 90 patients evaluable for efficacy. Assuming a 57.6% effect size for midazolam, the non-inferiority margin would be 49% (57.6% - 0.15*57.6%). A total of 66 to 87 evaluable patients will then give 80 to 90% power to detect non-inferiority if effect size for isoflurane is mid-range of estimated superiority (17.5%).

Sample size is also considered in relation to the safety analysis. As explained in Section 6.3, there are indications that the risk of neurological side effects is higher in young patients than in older patients and that a long sedation duration also increases the risk. Therefore, the randomisation is done in a stratum for age (3 age groups: 3-7 years; 8-11 years; and 12-17 years) and a stratum for reason for ICU admission (planned mechanical ventilation or unplanned mechanical ventilation) as detailed in Section 6.3.

A structured recruitment is employed to ensure that at least half of all patients are exposed in the youngest age group. It is expected that 1/3 of randomized patients will have a duration of treatment longer than 24 hours.

A hypothetical distribution for number of patients given a total sample size of 90 patients and the 2:1 randomization scheme is depicted below (Table 10). If dividing the data into two age groups, age less than 8 years and age 8 to 17 years, it is then expected that, within both age groups, approximately 10 and 5 patients in each treatment group respectively would have a duration of treatment longer than 24 hours. Having approximately 30 and 15 patients treated with isoflurane and midazolam respectively, in each of these two age groups, should allow for comparisons of safety by treatment group and age. Having approximately 10 and 5 patients treated with isoflurane and midazolam respectively, with longer duration of treatment within each of the two age groups, should allow for descriptive characterization of safety by treatment group, age and treatment duration.

Table 10 Hypothetical distribution for number of patients given a total sample size of 90 patients

Duration of study treatment	Age 3 to 7 years		Age 8 to 17 years		Total
	Isoflurane	Midazolam	Isoflurane	Midazolam	
≤24 hours	20	10	20	10	60
24 to 48 (±6) hours	10	5	10	5	30
Total	30	15	30	15	90
	45		45		

The probability is close to 80% to observe at least 5 events within 20 patients for an incidence of 30%. The distribution of events among four strata by age and treatment duration should then provide adequate information about the risk of for example psychomotor dysfunction events within patients treated with isoflurane (40 vs 20), by treatment group overall (60 vs 30), or by treatment duration overall (60 vs 30).

13.10 Interim Analysis

No interim analysis is planned to be performed within the study.

14. STUDY MANAGEMENT

14.1 Clinical monitoring

Before the initiation of the study at the study site, the study monitor will:

- Determine the adequacy of the facilities
- Discuss with the Investigator (and study personnel if applicable) their responsibilities with regards to GCP, protocol adherence and local regulations.

At an initiation meeting, the study monitor will review the study procedures with the Investigator (and the study personnel, as applicable) and document this in compliance with the ICH-GCP Guidelines.

During the study, the monitor will pay visits to the study site in order to:

- Provide information and support to the Investigator.
- Confirm that facilities remain acceptable.
- Confirm that the study team is adhering to the protocol.
- Confirm that data are being accurately recorded in the eCRF.
- Ensure that accountability checks for the IMP and device are being performed
- Conduct source data verification, which will require direct access to all original records for each patient (e.g. medical records). Definition of key variables to be source data verified will be described in the Monitoring Plan.

The frequency of the monitoring visits per site will be determined through a risk-based approach depending on recruitment rate, observed data quality and overall site performance. The monitor will be available (by phone and email) between visits if the investigator or other study personnel at the study site needs information, advice or help.

14.2 Audits and inspections

The purpose of an audit or inspection is to systematically and independently examine all study-related activities to document that they were conducted, recorded, analysed and accurately reported according to the study protocol, ICH-GCP including the Declaration of Helsinki and all other relevant regulations.

Audits or inspections may therefore be performed by authorised representatives of the Sponsor, the CRO or by a competent authority at the study site during or after the study. These visits may include source data verification and confidentiality documents are therefore created. This implies that auditors/inspectors will have the right to inspect the study sites at any time during and/or after completion of the study and will have direct access to source documents, including patients' medical records. By participating in this study, the Investigator agrees to this requirement.

The Investigator should contact the monitor and/or Sponsor immediately if they are contacted by a competent authority about an inspection at their study site.

14.3 Training of study personnel

The Investigator will maintain records of all individuals involved in the study (medical, nursing and other personnel). The Investigator will ensure that appropriate training relevant to the study is given to the personnel involved in the study, and that any new information of relevance to the conduct of the study is forwarded to the persons involved.

14.4 Protocol deviations

Deviations from the protocol should not occur. If a deviation occurs, the Investigator (or designee) will inform the monitor about the deviation and the implications of the deviation must be reviewed and discussed. The deviation will be logged by the monitor, including documentation of the reason and date for the deviation.

14.5 Study timetable

The recruitment period is planned to proceed until the required number of patients have been enrolled, where the intended first patient's first visit is in Q4 2020 and the intended last patient's last visit in Q1 2023.

14.6 Declaration of end of study

The end of the study is defined as the last patient's last visit.

The competent authority and the independent ethics committee will be informed that the clinical study has ended within 90 days of the end of the clinical study. If the study has to be terminated early, this period will be reduced to 15 days and the reasons will be clearly explained.

14.7 Study reporting

The data and information collected during this study will be reported in a CSR prepared by the Sponsor and submitted for comments and signature to the national coordinating investigators. The final study summary results shall be submitted to the regulatory authority as well as to the relevant IEC as required by local legislations, within 12 months after completion of the study. The trial and a summary of the trial results will 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.

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

14.8 Archiving

After the end of the study, the Investigator shall keep records of the study for the period mandated by local requirements or as specified in the clinical trial agreement. This includes any original source documents related to the study, including the Patient Identification List which provides the link between the named patient source records and pseudonymous eCRF data the original signed ICFs and detailed records of administration of IMP. The Sponsor should be contacted before any study related documentation is planned for destruction.

14.9 Patient confidentiality

The investigator will ensure that the confidentiality of the patients' data is preserved, any documents identifying the patient, such as the signed ICF will be maintained by the investigator in strict confidence. Patients will be assigned a unique identifier. Any records or datasets that are transferred to the Sponsor will contain the identifier only; patient names or any information which would make the patient identifiable will not be transferred.

The patient will be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the patient who will be required to give consent for their data to be used as described in the informed consent. The patient must be informed that his/her medical records may be examined by auditors or other authorized

personnel appointed by the Sponsor, by appropriate IEC members, and by inspectors from regulatory authorities.

14.10 Study agreements

The Investigator at the study site must comply with all the terms, conditions, and obligations of the clinical study agreement for this study. In the event of any inconsistency between this protocol and the clinical study agreement, the protocol shall prevail.

Agreements between the Sponsor and the Investigator must be in place before any study-related procedures can take place.

14.11 Publication

The Sponsor is the sole owner of the results from the study. No data can be shared or published before written approval has been received from the Sponsor.

By signing the protocol, the Investigator agrees that the results of the study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals by the Sponsor.

The information obtained during the conduct of this study is considered confidential and will be used by the Sponsor, for registration purposes and for the general development of the investigational product. Data will not be made available to any third party other than the authorised representatives of relevant authorities. Study progress and results of the project will be summarised in a CSR as described in Section 14.7. The report will also form the basis for scientific publications at the appropriate time.

All information supplied by the Sponsor in connection with this study shall remain the sole property of the Sponsor and is to be considered confidential information. No confidential information shall be disclosed to others without prior written consent from the Sponsor and shall not be used except in the performance of this study. The information obtained during this study may be made available to other physicians who are conducting other clinical studies with the investigational product, if deemed necessary by the Sponsor.

Results from the study may be published, and then authorship of a manuscript for publication in a peer-reviewed journal will be offered in compliance with the Vancouver guidelines.

The Sponsor determines when to publish. No other publications or presentations resulting from the study may be made until the study is completed and the primary study results have been published.

15. QUALITY CONTROL AND QUALITY ASSURANCE

This study will be conducted in compliance with this protocol, study specific procedures, SOPs at CRO, the Sponsor and the study site (as applicable), the ICH-GCP Guidelines, and the applicable regulatory requirements.

16. DATA HANDLING

16.1 Data management

The data management routines will be described in a data management plan and will include procedures for handling of eCRF, database set-up and management, data entry and verification, data validation, quality control of database, and documentation of the performed activities including information of discrepancies in the process. The database, data entry screens, and program will be designed in accordance with the protocol.

16.2 The web-based electronic case report form

Clinical data (including AEs and concomitant medications) will be entered into a 21 CFR Part 11-compliant eCRF (Viedoc™) provided by PCG Solutions AB (Uppsala, Sweden). The eCRF is a validated system, includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

Authorised study staff designated by the Investigator will complete data collection. Appropriate training will be completed with the Investigator and all authorised study site personnel prior to the study being initiated and any data being entered into the system for any study patient.

Entry errors occurring in the eCRF will be corrected electronically. Such corrections or modifications will be automatically tracked by an audit trail detailing the date and time of the correction and the name of the person making the correction.

16.2.1 The entering of data into the electronic case report form

All data must be entered in English. Study data should be entered into the eCRF within a reasonable time after the data become available. The investigator must submit a completed eCRF for each patient who signed the ICF.

The investigator must verify that all data entries in the eCRFs are accurate and correct. If some assessments are not done, or if certain information is not available, not applicable or unknown, this should be indicated in the eCRF and confirmed as missing and reason should be stated. The Investigator will electronically sign off the clinical data collected within the eCRF.

16.3 Source documents

The eCRF is essentially considered a data entry form and should not constitute the original (or source) records unless otherwise specified. Source documents are all documents used by the Investigator or hospital that relate to the patient's medical history, that verify the existence of the patient, the inclusion and exclusion criteria, and all records covering the patient's participation in the study. They include laboratory notes, memoranda, material dispensing records, patient files, etc.

The Investigator is responsible for maintaining source documents. Source documents should be preserved for the maximum amount of time permitted by local regulations. These will be made available for inspection by the monitor at each monitoring visit. Any supportive documentation submitted with the eCRF, such as laboratory or hospital records, should be clearly identified with the study ID and Patient Number. Any personal information, including the patient's name, should be removed or rendered illegible to preserve individual confidentiality.

16.4 User ID

The eCRF records will be automatically appended with the identification of the creator, by means of their unique UserID. Specified records will be electronically signed by the Investigator, to document his/her review of the data and acknowledgement that the data are accurate. This will be facilitated by means of the Investigator's unique UserID and password; date and time stamps will be added automatically at time of electronic signature.

16.5 Audit trail

All changes in the eCRF will be fully recorded in a protected audit trail, and a reason for the change will be required.

Once all data have been entered, verified, and validated, the database will be locked.

16.6 Data standards

Collection of data should be performed in the Clinical Data Acquisition Standards Harmonization (CDASH) format, according to the Clinical Data Interchange Standards Consortium (CDISC). The standards should be used to the extent possible and/or required for the specific study/project. The minimum requirement of the CDISC standard is to collect all core variables specified as 'Required' in the Study Data Tabulation Model (SDTM) format.

17. LIST OF REFERENCES

Ávila-Alzate et al. 2020
Ávila-Alzate Jennihe Alejandra, Gómez-Salgadob Juan, Romero-Martín Macarena, Martínez-Isasi Santiago, Navarro-Abal Yolanda, Fernández-Garcíag Daniel. Assessment and treatment of the withdrawal syndrome in paediatric intensive care units. Systematic review. Medicine (2020) 99:5; 1-13.

Amigoni et al. 2012
Amigoni A, Mozzo E, Brugnaro L, Gentilomo C, Stritoni V, Michelin E, Pettenazzo A. Assessing sedation in a pediatric intensive care unit using Comfort Behavioural Scale and Bispectral Index: these tools are different. Minerva Anestesiol 2012;78:322-9

Andersen et al. 2015
Andersen RD, Bernklev T, Langius-Eklöf A, Nakstad B, Jylli L. The COMFORT behavioural scale provides a useful assessment of sedation, pain and distress in toddlers undergoing minor elective surgery. Acta Paediatr 2015;104:904-9.

Akcan-Arikan et al. 2007
Akcan-Arikan A, Zappitelli M, Loftis LL, Washburn KK, Jeffersson LS and Goldstein SL. Modified RIFLE criteria in critically ill children with acute kidney injury. Kidney International 2007; 71:1028-1035.

Ariyama et al. 2009
Ariyama J, Hayashida M, Shibata K, Sugimoto Y, Imanishi H, O-oi Y, Kitamura A Risk factors for the development of reversible psychomotor dysfunction following prolonged isoflurane inhalation in the general intensive care unit. J Clin Anesth. 2009; 21:567-73.

Arnold et al. 1993
Arnold JH, Truog RD, Rice SA. Prolonged administration of isoflurane to pediatric patients during mechanical ventilation. Anesth Analg 1993;76:520-6.

Boerlage et al. 2015
Boerlage AA, Ista E, Duivenvoorden HJ, de Wildt SN, Tibboel D, van Dijk M. The COMFORT behaviour scale detects clinically meaningful effects of analgesic and sedative treatment. Eur J Pain. 2015;19:473-9.

Cooper et al. 2007
Cooper MK, Bateman ST. Cisatracurium in "weakening doses" assists in weaning from sedation and withdrawal following extended use of inhaled isoflurane. Pediatr Crit Care Med 2007;8:58-60.

de Wildt et al. 2005
de Wildt SN, de Hoog M, Vinks AA, Joosten KF, van Dijk M, van den Anker JN. Pharmacodynamics of midazolam in pediatric intensive care patients. Ther Drug Monit. 2005;27:98-102.

Dreyfus et al. 2017
Dreyfus L, Javouhey E, Denis A, Touzet S, Bordet F. Implementation and evaluation of a paediatric nurse-driven sedation protocol in a paediatric intensive care unit. Ann Intensive Care. 2017;36 doi: 10.1186/s13613-017-0256-7.

Eifinger et al. 2013
Eifinger F, Hünseler C, Roth B, Vierzig A, Oberthuer A, Mehler K, Kribs A, Menzel C, Trieschmann

U. Observations on the effects of inhaled isoflurane in long-term sedation of critically Ill children using a modified AnaConDa©-system. Padiatr 2013;225:206-11.

Froom et al. 2008
Froom SR, Malan CA, Mecklenburgh JS, Price M, Chawathe MS, Hall JE, et al. Bispectral Index asymmetry and COMFORT score in paediatric intensive care patients. Br J Anaesth. 2008;100:690-6.

Gaies et al. 2010
Gaies, MG, Gurney JG, Yen AH, Alberta H, Napoli, M L, Gajarski, R J, Ohye, R G, Charpie, J R, Hirsch, J C.Vasoactive-inotropic score as a predictor of morbidity and mortality in infants after cardiopulmonary bypass. Pediatr Crit Care Med 2010;11: 234-8.

Grant et al. 2013
Grant MJC, Balas MC, Curley MAQ. Defining Sedation-Related Adverse Events in the Pediatric Intensive Care Unit. Heart Lung. 2013; 42: 171–6.

Guidelines on a Medical Device Vigilance System (MEDDEV 2.12-1 rev.8)
Available from:
https://ec.europa.eu/docsroom/documents/32305/attachments/1/translations/en/renditions/native

Harris et al. 2016
Harris J, Ramelet AS, van Dijk M, Pokorna P, Wielenga J, Tume L, Tibboel D, Ista E. Clinical recommendations for pain, sedation, withdrawal and delirium assessment in critically ill infants and children: an ESPNIC position statement for healthcare professionals. Intensive Care Med 2016;42:972-86.

Hoemberg et al. 2012
Hoemberg M, Vierzig A, Roth B, Eifinger F. Plasma fluoride concentrations during prolonged administration of isoflurane to a pediatric patient requiring renal replacement therapy. Paediatr Anaesth 2012;22:412-3.

Ista et al. 2005
Ista E, van Dijk M, Tibboel D, de Hoog M. Assessment of sedation levels in pediatric intensive care patients can be improved by using the COMFORT “behavior” scale. Pediatr Crit Care Med 2005;6:58-63.

Ista et al. 2009
Ista E, de Hoog M, Tibboel D, van Dijk M. Implementation of standard sedation management in paediatric intensive care: effective and feasible? J Clin Nurs 2009;18:2511-20.

Ista et al. 2018
Ista E, van Beusekom B, van Rosmalen, J, Kneyber M C J, Lemson J, Brouwers A, Dieleman G C, Dierckx B, de Hoog M, Tibboel D, van Dijk M. Validation of the SOS-PD scale for assessment of pediatric delirium: a multicentre study. Critical Care 2018;22:309.

Jung et al. 2007
Jung C, Granados M, Marsol P, Murat I, Gall O. Use of sevoflurane sedation by the AnaConDa device as an adjunct to extubation in a paediatric burn patient. Burns. 2008 Feb;34(1):136-8. Epub 2007 Feb 28.

Johnston et al. 1990
Johnston RG1, Noseworthy TW, Friesen EG, Yule HA, Shustack A. Isoflurane therapy for status asthmaticus in children and adults. Chest. 1990 Mar;97(3):698-701.

Kavaz et al. 2011
Kavaz A, Özçakar ZB, Kendirli T, Öztürk BB, Ekim M, Yalçınkaya F. Acute kidney injury in a paediatric intensive care unit: comparison of the pRIFLE and AKIN criteria. Acta Pædiatrica. 2012; 101:126-129.

KDIGO Clinical Practice Guideline for Acute Kidney Injury, 2012
Work group Kidney Disease: Improving Global Outcomes (KDIGO). KDIGO Clinical Practice Guideline for Acute Kidney Injury. Kidney international supplements 2012:2(1)

Kelsall et al. 1994
Kelsall AW, Ross-Russell R, Herrick MJ. Reversible neurologic dysfunction following isoflurane sedation in pediatric intensive care. Crit Care Med 1994;22:1032-4.

Kita et al. 1995,
Kita, Sahara H, Yoshita Y, Shibata K, Ishise J, Kobayashi T. Status asthmaticus complicated by atelectasis in a child. Am J Emerg Med 1995 Mar;13(2):164-7**.**

Kong et al. 1989
Kong KL, Willats SM, Prys-Roberts C. Isoflurane compared with midazolam for sedation in the intensive care unit. BMJ 1989; 298:1277-80.

Kruessel et al. 2012
Kruessell M A, Udink ten Cate F E., Kraus A-J; Roth B, Trieschmann U. Use of propofol in pediatric intensive care units: A national survey in Germany. Pediatric Critical Care Med 2012;13(3):1-5.

Kudchadkar et al. 2014
Kudchadka Sapna R, Yaster Myron, Punjabi Naresh M. Sedation, Sleep Promotion, and Delirium Screening Practices in the Care of Mechanically Ventilated Children: A Wake-Up Call for the Pediatric Critical Care Community. Critical Care Med July 2014;Volume 42, Number 7, 1592-1600.

Label - Midazolam
Available from: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=1cc9ba6e-fa1b-405e-9de8-720c567b2a73

Lamas et al. 2009
Lamas A, Lopez-Herce J, Sancho L, Mencia S, Carrillo A, Santiago MJ, et al. Assessment of the level of sedation in children after cardiac surgery. Ann Thorac Surg. 2009;88:144-50.

Laudenbach et al. 2008
Laudenbach V and Nolent P. Sedation and analgesia in the paediatric intensive care unit. Annales Francaises d'anesthésie et de Réanimation 2008;27:623-632.

Lebet et al. 2018
Lebet RM, Asaro LA, Zuppa AF, Curley MAQ. Face and content validity of variables associated with the difficult-to-sedate child in the paediatric intensive care unit: A survey of paediatric critical care clinicians. Aust Crit Care 2018;31:167-173.

Maaskant et al. 2016
Maaskant J, Raymakers-Janssen P, Veldhoen E, Ista E, Lucas C, Vermeulen H. The clinimetric properties of the COMFORT scale: A systematic review. Eur J Pain 2016;20:1587-1611.

Maltais et al. 1994
Maltais F, Sovilj M, Goldberg P, Gottfried S B. Respiratory Mechanics in Status Asthmaticus: Effects of Inhalational Anesthesia. Chest Volume 106, Issue 5, November 1994: 1401-1406.

McIntosh et al. 2017
McIntosh A M, Tong S, Deakyne S J, Davidsson J A, Scott H F. Validation of the Vasoactive-Inotropic Score in Pediatric Sepsis. Pediatr Crit Care Med. 2017 Aug;18(8):750-75.

Medical Device Regulation (MDR)
Regulation 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices. Available from: http://data.europa.eu/eli/reg/2017/745/oj.

Meiser et al. 2005
Meiser A, Laubenthal H. Inhalational anaesthetics in the ICU: theory and practice of inhalational sedation in the ICU, economics, risk-benefit. Best Pract Res Clin Anaesthesiol. 2005 Sep;19(3):523-38.

Mencia et al. 2018
Mencía S, Palacios A1, García M, Llorente AM1, Ordóñez O1, Toledo B, López-Herce J. "An exploratory study of sevoflurane as an alternative for difficult sedation in critically ill patients" Pediatr Crit Care Med. 2018 Jul;19(7):e335-e341.

Meyburg et al. 2018
Meyburg J, Dill ML, Haken von R, Picardi S, Westhoff JH, Silver G, Traube C. Risk Factors for the Development of Postoperative Delirium in Pediatric Intensive Care Patients. Pediatr Crit Care Med 2018; 19:e514–e521.

Mody et al. 2016
Mody K. Benzodiazepines and transition to delirium in critically ill children. Crit. Care Med. 2016, 44:12 (Suppl).

Mody et al. 2018
Mody K, Kaur S, Mauer EA, Gerber LM, Greenwald BM, Silver G, Traube C. Benzodiazepines and development of delirium in critically ill children: estimating the causal effect. Crit Care Med. 2018 Sep; 46(9): 1486–1491.

Nacoti et al. 2018
Nacoti M, Colombo J, Fochi O, Bonacina D, Fazzi F, Bellani G, Bonanomi E. Sevoflurane improves respiratory mechanics and gas exchange in a case series of infants with severe bronchiolitis-induced acute respiratory distress syndrome. Clin Case Rep. 2018 Mar 25;6(5):920-925.

Paediatric Sedation Guidelines UK 2006
Stephen Playfor. Ian Jenkins. Carolyne Boyles. Imti Choonara. Gerald Davies. Tim Haywood , Gillian Hinson et al. For the United Kingdom Paediatric Intensive Care Society, Sedation Analgesia and Neuromuscular Blockade Working Group. Consensus guidelines on sedation and analgesia in critically ill children. Intensive Care Med 2006;32:1125–1136.

Palacios et al. 2016
Palacios A, Mencía S, Llorente AM, Cruz J, Toledo B, Ordóñez O, Olmedilla M, Lopez-Herce J.

Sevoflurane therapy for refractory bronchospasm in patients. Pediatr Crit Careed. 2016 Aug;17(8):e380-4.

Restrepo et al. 2005
Restrepo RD, Pettignano R, DeMeuse P**.** Halothane, an effective infrequently used drug, in the treatment of pediatric status asthmaticus: a case report. J Asthma. 2005 Oct;42(8):649-51.

Sackey et al. 2004
Sackey PV, Martling CR, Granath F, Radell PJ. Prolonged isoflurane sedation of intensive care unit patients with the Anesthetic Conserving Device. Crit Care Med 2004;32(11):2241-2246.

Sackey et al. 2005
Sackey PV, Martling CR, Radell PJ. Case reports. Three cases of PICU sedation with isoflurane delivered by the 'AnaConDa. Pediatric Anesthesia 2005;15: 879–885.

Sackey et al. 2008
Sackey PV, Martling CR, Carlswärd C, Sundin O, Radell PJ. Short- and long-term follow-up of intensive care unit patients after sedation with isoflurane and midazolam - a pilot study. Crit Care Med 2008;36:801-6.

Smith et al. 2017
Smith HAB, Gangopadhyay M, Goben CM, Jacobowski NL, Hamilton Chestnut M, Thompson JL, Chandrasekhar R, Williams SR, Griffith K, Ely WE, Fuchs DC, Pandharipande PP. Delirium and Benzodiazepines Associated With Prolonged ICU Stay in Critically Ill Infants and Young Children. Crit Care Med 2017; 45:1427–1435.

Straney et al. 2013
Straney L, Clements A, Parslow RC, Pearson G, Shann F, Alexander J, Slater A for the ANZICS Paediatric Study Group and the Paediatric Intensive Care Audit Network. Paediatric index of mortality 3: an updated model for predicting mortality in pediatric intensive care. Pediatr Crit Care Med 2013;14(7);673–681.

Taffarel et al. 2018
Taffarel P, Bonetto G, Barón FJ, Meregalli C. Sedation and analgesia in patients on mechanical ventilation in pediatric intensive care units in Argentina. Arch Argent Pediatr 2018;116:e196-e203.

Tobias 2000
Tobias JD. Tolerance, withdrawal, and physical dependency after long-term sedation and analgesia of children in the pediatric intensive care unit. Critical Care Medicine 2000;28:2122-32.

Traube C et al. 2017
Delirium and Mortality in Critically Ill Children: Epidemiology and Outcomes of Pediatric Delirium. Traube C et al.; Crit Care Med. 2017 May ; 45(5): 891–898.

Turner et al. 2012
Turner DA, Heitz D, Cooper MK, Smith PB, Arnold JH, Bateman ST. Isoflurane for life-threatening bronchospasm: a 15-year single-center experience. Respir Care 2012;57:1857-64.

Vet et al. 2013
Vet NJ, Ista E, de Wildt SN, van Dijk M, Tibboel D, de Hoog M. Optimal sedation in pediatric intensive care patients: a systematic review. Intensive Care Med.2013;39:1524.

Vet et al. 2016
Vet NJ, Brussee JM, de Hoog M, Mooij MG, Verlaat CW, Jerchel IS, van Schaik RH, Koch BC, Tibboel D, Knibbe CA, de Wildt SN; SKIC (Dutch collaborative PICU research network). Inflammation and Organ Failure Severely Affect Midazolam Clearance in Critically Ill Children. Am J Respir Crit Care Med. 2016 Jul 1;194(1):58-66. doi: 10.1164/rccm.201510-2114OC.

Wolf et al. 2014
Wolf A, McKay A, Spowart C, Granville H, Boland A, Petrou S, Sutherland A & Gamble C. Prospective multi-centre randomised, double-blind, equivalence study comparing clonidine and midazolam as intravenous sedative agents in critically ill children. The SLEEPS Study (Safety ProfiLe, Efficacy and Equivalence in Paediatric intensive care Sedation). Health Technol Assess 2014;18(71).

World Medical Association Declaration of Helsinki
World Medical Association Declaration of Helsinki; Ethical Principles for Medical Research Involving Human Subjects. 64th WMA General Assembly, Fortaleza, Brazil, October 2013.
Available from: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/

Zeiler et al. 2015
Zeiler FA, Zeiler KJ, Teitelbaum J, Gillman LM, West M. Modern inhalational anesthetics for refractory status epilepticus. Can J Neurol Sci 2015 Mar;42(2):106-15. doi: 10.1017/cjn.2014.121. Epub 2015 Jan 9.

Appendix A – COMFORT Behavior Scale

Comfort assessment

Behavior Scale

Date/time 1 | Date/time 2 | Date/time 3 | Date/time 4

Sticker with patient's name

		Place a mark 1	2	3	4	
Alertness	1.	☐	☐	☐	☐	deeply asleep (eyes closed, no response to changes in the environment)
	2.	☐	☐	☐	☐	lightly asleep (eyes mostly closed, occasional responses)
	3.	☐	☐	☐	☐	drowsy (child closes his/her eyes frequently, less responsive to the environment)
	4.	☐	☐	☐	☐	awake and alert (child responsive to the environment)
	5.	☐	☐	☐	☐	awake and hyper-alert (exaggerated responses to environmental stimuli)
Calmness/ Agitation	1.	☐	☐	☐	☐	calm (child appears serene and tranquil)
	2.	☐	☐	☐	☐	slightly anxious (child shows slight anxiety)
	3.	☐	☐	☐	☐	anxious (child appears agitated but remains in control)
	4.	☐	☐	☐	☐	very anxious (child appears very agitated, just able to control)
	5.	☐	☐	☐	☐	panicky (severe distress with loss of control)
Respiratory response (only in mechanically ventilated children)	1.	☐	☐	☐	☐	no spontaneous respiration
	2.	☐	☐	☐	☐	spontaneous and ventilator respiration
	3.	☐	☐	☐	☐	restlessness or resistance to ventilator
	4.	☐	☐	☐	☐	actively breathes against ventilator or coughs regularly
	5.	☐	☐	☐	☐	fights ventilator
Crying (only in spontaneously breathing children)	1.	☐	☐	☐	☐	quiet breathing, no crying sounds
	2.	☐	☐	☐	☐	occasional sobbing or moaning
	3.	☐	☐	☐	☐	whining (monotonous sound)
	4.	☐	☐	☐	☐	crying
	5.	☐	☐	☐	☐	screaming or shrieking
Physical movement	1.	☐	☐	☐	☐	no movement
	2.	☐	☐	☐	☐	occasional, (three or fewer) slight movements
	3.	☐	☐	☐	☐	frequent, (more than three) slight movements
	4.	☐	☐	☐	☐	vigorous movements limited to extremities
	5.	☐	☐	☐	☐	vigorous movements including torso and head
Muscle tone	1.	☐	☐	☐	☐	muscles totally relaxed; no muscle tone
	2.	☐	☐	☐	☐	reduced muscle tone; less resistance than normal
	3.	☐	☐	☐	☐	normal muscle tone
	4.	☐	☐	☐	☐	increased muscle tone and flexion of fingers and toes
	5.	☐	☐	☐	☐	extreme muscle rigidity and flexion of fingers and toes
Facial tension	1.	☐	☐	☐	☐	facial muscles totally relaxed
	2.	☐	☐	☐	☐	normal facial tone
	3.	☐	☐	☐	☐	tension evident in some facial muscles (not sustained)
	4.	☐	☐	☐	☐	tension evident throughout facial muscles (sustained)
	5.	☐	☐	☐	☐	facial muscles contorted and grimacing
Total score		☐☐		☐☐		

Appendix B - Sophia Observation Withdrawal Symptoms Scale and Delirium

Comfort assessment

SOS-PD scale

Sophia Observation withdrawal Symptoms-scale and Delirium

Date/time 1 | Date/time 2

Observer | Observer

Sticker with patient's name

Step 1a Withdrawal	1	2	Explanation
Heart rate	..... /min	..... /min	Enter highest rate in past 4 hours if available (electronic patient data management system), otherwise read the monitor or feel pulse.
Breathing rate	..... /min	..... /min	Enter highest rate in past 4 hours if available (electronic patient data management system), otherwise read the monitor or count breathing.
Baseline heart rate	..... /min	..... /min	Baseline is the mean value over the past 24 hours.
Baseline breathing rate	..... /min	..... /min	Baseline value is the mean value over the past 24 hours.

Step 1b Delirium*	1	2	Tick if yes
Parents do not recognize their child's behavior	☐ *	☐ *	Parents perceive their child's behavior as very different or unrecognizable in comparison with what they are accustomed to when the child is ill or in hospital; "this is not my child".

Step 2	Withdrawal 1	Withdrawal 2	Delirium 1	Delirium 2	
Tachycardia	☐	☐			Heart rate exceeds baseline by ≥ 15%.
Tachypnea	☐	☐			Breathing rate exceeds baseline by ≥ 15%.
Fever	☐	☐			Body temperature exceeded 38.4° C now or in past 4 hours.
Sweating	☐	☐	☐	☐	Without apparent reason.
Agitation	☐	☐	☐	☐	E.g.: irritable, restless, agitated, fumbling (trying to pull out catheters, venous lines, gastric tubes etc.).
Anxiety	☐	☐	☐	☐	Child shows anxious facial expression (eyes wide open, raised and tensed eyebrows). Behavior varies from panicky to introvert.
Tremors	☐	☐	☐	☐	Trembling, involuntary sustained rhythmic movements of hands and/or feet.
Motor disturbance	☐	☐	☐	☐	Involuntary movements of arm and/or legs; little muscle twitches.
Muscle tension	☐	☐	☐	☐	Clenching wrists and toes and/or hunched shoulders. Or: abnormal tensed position of head, arm and/or legs caused by muscle tension.
Attentiveness			☐	☐	If you (nurses) or parents fail to attract or hold the child's attention. Child is not aware of surroundings; living in "his own world"; Apathy.
Purposeful acting			☐	☐	If child has difficulty in doing things that normally are no problem; e.g. cannot grab pacifier or cuddly toy
Lack of eye contact			☐	☐	No or little eye contact with caregiver or parents.
Inconsolable crying	☐	☐	☐	☐	Inconsolable (shown by refusing food, pacifier or not wanting to play). Score silent crying in ventilated children as inconsolable crying.
Grimacing	☐	☐	☐	☐	Eyebrows contracted and lowered, nasolabial fold visible.
Sleeplessness	☐	☐	☐	☐	Child doesn't sleep more than one hour at a stretch; catnaps.
Hallucinations	☐	☐	☐	☐ *	Child seems to see, hear or feel things that were not there.
Disorientation			☐	☐	Only for children >5 years. Child doesn't know whether it is morning, afternoon or evening, is not aware where it is, does not recognize family or friends.
Speech			☐	☐	If speech is incomprehensible, unclear or child cannot tell a coherent story (not age appropriate).
Acute onset of symptoms			☐	☐	Acute change of symptoms compared to before hospital admission. The occurrence of symptoms strongly varies over the past 24 hours.
Fluctuations			☐	☐	
Vomiting	☐	☐			At least once in past 4 hours.
Diarrhea	☐	☐			At least once in past 4 hours.

Total score

SOS score	☐☐		Withdrawal score (max. is 15) Count ticked boxes
PD score*		☐☐	Delirium score (max. is 16/17) Count ticked boxes

*** Consult child-psychiatrist if: Step 1b is positive AND/OR Step 2 score is ≥ 4 or symptom with * is positive.**