Statistical Analysis Plan

Study Information:

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Author:	Malin Schollin

LINK Medical AB
Slottsgränd 2A
SE-753 09 Uppsala, Sweden

e-mail: malin.schollin@linkmedical.eu
mobile: +46 761636014

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1.1	Malin Schollin	20Feb2023	Updated based on amended protocol and comments from sponsor
2.0	Malin Schollin	02MAR2023	Final version 2.0

Table of Content

1 Acronyms and Abbreviations used in the Document

Acronyms & Abbreviations	
ADaM	Analysis Data Model
ADE	Adverse device effect
ADR	Adverse drug reaction
AE	Adverse event
ATC	Anatomical therapeutic chemical [classification system]
COMFORT-B	Comfort Behaviour Scale
CPMP	Committee for proprietary medicinal products
CRF	Case report form
CSP	Clinical study protocol
CSR	Clinical study report
DBL	Database lock
DDP	Data display plan
DSMB	Data Safety Monitoring Board
eCRF	Electronic case report form
EU	European Union
FAS	Full analysis set
GCP	Good Clinical Practice
hCG	Human chorionic gonadotropin
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICU	Intensive care unit
IMP	Investigational medicinal product
ITT	Intention-to-treat
IV	Intravenous
MAC	Minimal alveolar concentration
MAP	Mean arterial pressure
MedDRA	Medical dictionary for regulatory activities
N/A	Not applicable
PDCO	Paediatric committee of the European Medicines Agency
Per hour	Per clock hour
PICU	Paediatric intensive care unit
PIM3	Paediatric index of mortality 3
PIP	Paediatric investigational plan
PPAS	Per-protocol analysis set

Acronyms & Abbreviations	
SADE	Serious adverse device effect
SADR	Serious adverse drug reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SDTM	Study Data Tabulation Model
SOP	Standard Operating Procedure
SOS-PD	SOPHIA Observation Withdrawal Symptoms-Paediatric Delirium scale
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
WHO	World Health Organisation

2 Introduction

The Statistical Analysis Plan (SAP) is a complementary document to the Clinical Study Protocol and includes a more technical and detailed elaboration of the principal features of the proposed statistical analysis and presentations, and the way in which anticipated analysis problems will be handled.

If the SAP suggests changes to the principal features stated in the protocol, these should also be documented in a protocol amendment. Otherwise, it will suffice to record the changes in the SAP.

3 Study Objectives

3.1 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.

3.1.1 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 7.

3.2 Secondary Objectives, efficacy

1. 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.

2. Compare the need for rescue sedatives and other sedatives in isoflurane- vs midazolam-treated patients.

3.2.1 Secondary endpoints, efficacy

1. 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.

2. 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.

3. 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).

4. 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.

3.3 Secondary Objectives, safety

1. Compare time from sedation termination to extubation in isoflurane- vs midazolam-treated patients.

2. Compare the proportion of time with spontaneous breathing in isoflurane- vs midazolam treated patients.

3. Evaluate haemodynamic effect as indicated by inotropic/vasopressor agent administration in patients sedated with isoflurane compared with midazolam.

4. Evaluate the frequency of withdrawal symptoms in isoflurane- vs midazolam-treated patients.

5. Evaluate the frequency of delirium in isoflurane- vs midazolam-treated patients.

6. Evaluate the frequency of psychomotor dysfunction during and up to 48 hours after discontinuation of isoflurane or midazolam treatment, and the association with duration of treatment, and total exposure (isoflurane MAC hours and midazolam doses) over time.

7. Compare the 30 days/hospital mortality in isoflurane- vs midazolam-treated patients.

8. Compare ventilator-free days up to 30 days in isoflurane- vs midazolam-treated patients.

9. Compare the time in ICU/hospital up to 30 days in isoflurane- vs midazolam-treated patients.

10. Compare ICU-free days up to 30 days in isoflurane- vs midazolam-treated patients.

11. 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.

3.3.1 Secondary Endpoints, safety

1. Time from end of study drug administration to extubation if study drug is terminated for extubation.

2. Proportion of observations with spontaneous breathing efforts during study treatment.

3. Need for additional inotropic/vasopressor agent as defined by change in VIS score during study treatment period compared to baseline.

4. 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.

5. 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.

6. Proportion of patients experiencing psychomotor dysfunction 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).

7. 30 days/hospital mortality.

8. Ventilator-free days at 30 days from start of study treatment period.

9. Time in intensive care unit/hospital at day 30 from start of study treatment period.

10. Days alive and not in the ICU at day 30 from start of study treatment period.

11. 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.

12. Frequency and intensity of adverse events from start of study treatment to day 30.

13. Changes in vital signs, blood gases, body temperature and urinary output from baseline to end of study treatment.

14. Changes in clinical chemistry and haematology parameters from baseline up to the 48-hour post-study treatment monitoring.

3.4 Exploratory Objectives

1. Determine the isoflurane dosage, end-tidal concentrations and infusion rates, and the midazolam dosage, required for adequate sedation in mechanically ventilated paediatric patients.

2. Evaluate frequency and type of AnaConDa-S device deficiencies when used for sedating patients with isoflurane.

3.4.1 Exploratory Endpoints

1. 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.

2. Number of study drug bolus doses given per 24 hours during midazolam and isoflurane sedation of mechanically ventilated patients.

3. Ventilator parameters (ventilation mode, tidal volume, minute volume, fraction of inspired oxygen (FiO2), end tidal carbon dioxide [EtCO2], total breathing rate, positive end-expiratory pressure [PEEP], set inspiratory pressure [Pinsp], level of pressure support [PS] above PEEP).

4. Frequency and type of AnaConDa-S device deficiencies during isoflurane sedation.

4 Study Design

4.1 Overall study design

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 approximately 90 evaluable patients.

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.

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 6.15.1, Table 7). 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 that have been admitted to the ICU for 48 hours or more (SOS-PD delirium module) and in patients that 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.

4.2 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. 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 Behaviour (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 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 6.15.

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 evaluable 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 6.9), depth of sedation (Section 6.15), documentation of parameters of mechanical ventilation (Section 6.12) and intermittent blood gas analysis (Section 6.14). 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 6.13). 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 in the CSP).

5 Study 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.

5.1 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 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. The same number of subjects is needed using a two-sided test at the 5% significance level.

It is expected that no more than 100 patients will be randomised into the study to get approximately 90 patients evaluable for efficacy of the primary endpoint (as it is expected that, in average, 90% of the randomised patients will be included in the FAS analysis population, i.e. evaluable for efficacy).
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 3).

Table 3 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, and on studies of isoflurane as a successful rescue sedative, there are reasons to believe that isoflurane may be superior to IV sedation in the paediatric population and 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 percentage points and 90 patients evaluable for efficacy. Assuming a 57.6% proportion of adequate sedation for midazolam, the non-inferiority margin for the difference would be -9% units (0.15*57.6%) and non-inferiority would be proven if the lower end of the two-sided 95% CI for the difference between isoflurane and midazolam would be above -9% units. 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%).

The non-inferiority test will not be needed if superiority can be proven in the first testing step. As there will only be one confidence interval calculated, no multiplicity issues with switching from superiority to non-inferiority, if superiority cannot be proven, is foreseen. Also, the non-inferiority limit has been pre-defined and motivated in the CSP. See EMA

Committee for proprietary medicinal products (CPMP) Points to consider on switching between superiority and non-inferiority **(1)**.

Sample size is also considered in relation to the safety analysis. As explained in the CSP, 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 the CSP.
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 4). 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 4 Hypothetical distribution for number of patients given a total sample size of 90 patients

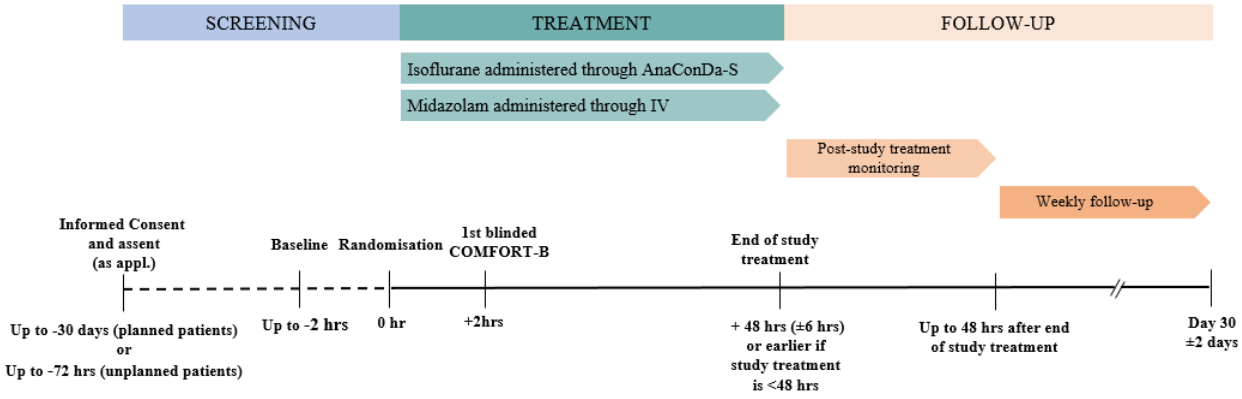
Duration of study treatment	Age 3 to 7 years		Age 8 to 17 years		Total
	Isoflurane	Midazolam	Isoflurane	Midazolam	
≤24 hours	20	10	20	10	60
24 to 48 (±6) hours	10	5	10	5	30
Total	30	15	30	15	90
	45		45		

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).

6 Assessments

A chart describing the flow of the study is provided in Figure 1.

Figure 1 Study flow chart



An overview of the assessments to be performed at each visit are given below in Table 4.

The assessments are defined in detail in the subsections below.

IMPORTANT NOTE: The Section numbers referred to in the footnotes in Table 4 constitute CSP section numbers.

SCHEDULE AND DEFINITIONS OF ASSESSMENTS

Table 5 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 hours 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]					

	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 hours 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**
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
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 in CSP). 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 in CSP). 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 in CSP).
[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 in CSP).
[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 in CSP). 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

6.1 Eligibility criteria

Eligibility criteria should be checked during screening and verified at baseline before randomisation.

6.2 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.

6.3 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.

6.4 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.

6.5 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.

6.6 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.

6.7 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.

6.8 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 29 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.

6.9 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. Documented HR should not be based on measurements 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. 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.

6.10 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.

6.11 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.

6.12 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 15 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.

6.13 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 5. 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 6 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	

6.14 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, a 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).

6.15 COMFORT-Behavioural (COMFORT-B) sedation scale

Depth of sedation will be assessed using the COMFORT-B scale, which 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.

6.15.1 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 7). This will be considered the target sedation depth for the patient and will be documented in the eCRF.

Table 7 Degree of sedation defined as intervals of the Comfort Behaviour (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.

6.15.2 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.

6.16 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 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 item “Disorientation” is only to be answered for children >5 years). 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 in the CSP.

6.17 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
  - Other

6.18 Concomitant medications, rescue sedation and non-pharmacological interventions

Also refer to Section 7.7.1. 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.

6.18.1 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 7.7.1.1)
- Dose and all changes in dose of procedural medication (ref. Section 7.7.1.2 and 7.7.1.3)
- 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 (any dose)
- 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

6.18.2 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

6.19 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 medical condition of the patient is stable. 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.

Events related to sedation depth, delirium and withdrawal will be reported separately and will not be reported as adverse events.

For details on assessments of adverse events, see CSP.

6.20 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.

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.

6.21 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 initial analysis.

6.22 Data Safety Monitoring Board

An independent Data Safety Monitoring Board (DSMB) will be formed to monitor the benefit/risk ratio during the course of the study. The DSMB will review all adverse events, vital signs, ventilator parameters and laboratory tests for all participants. 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. The members will be blinded to participant treatment assignments. 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 have completed the follow up period throughout the study duration. The independent DSMB will have the mandate to stop the study on safety grounds. A charter will be established between the Sponsor or designee and the DSMB to outline the DSMB's responsibilities and procedures.

7 Method of Analysis

7.1 General

All statistical analyses will be performed in accordance with the ICH E9 guideline for Statistical Principles for Clinical Trials **(2)**, using SAS® (Version 9.4 or higher, SAS Institute Inc., Cary, NC, USA). In addition, the primary endpoint analysis will be performed in accordance with ICH Harmonised Guideline for Addendum on estimands and sensitivity analysis in clinical trials to the Guideline on statistical principles for clinical trials E9(R1) **(3)**.

7.1.1 Presentation of Results

All results will be presented by treatment group and in total, unless stated otherwise. It should be clearly stated which unit applies to each presented variable.
Continuous data will be summarised using descriptive statistics, and the following parameters will be reported:

- number of patients with evaluable observations and missing observations
- arithmetic mean and standard deviation
- median
- first and third quartiles
- minimum and maximum.

Categorical data will be presented using absolute frequency and percentage. When the absolute frequency is zero, the percentage will not be presented. Unless stated otherwise, the denominator for percentage calculations will be the total number of patients in the applicable analysis set, including patients with missing data. For variables with missing values, the number and percentage of patients with missing values will be presented.

Significance tests will be two-sided and performed at the 5% significance level, unless stated otherwise. When reporting the results of significance tests, p-values will be presented.

All confidence intervals presented will be two-sided with a nominal confidence level of 95%, unless stated otherwise.

If assumptions connected to a specified statistical model can be questioned, a non-parametric method may be used as an alternative method, if considered needed.

Data will be presented using an appropriate number of decimal places, to ensure that undue precision is not implied (*e.g.* the number of decimals should not exceed the accuracy of the measuring instrument). Raw data will be presented with the same number of decimals as collected, and derived data with an appropriate number of decimals based on general practice, mathematical rationale or scientific rationale.

Minimum and maximum values will be presented with the same number of decimals as the analysed variable and the other descriptive statistics will be presented with one decimal more. Percentages and proportions will be presented with one decimal. Confidence interval bounds will be presented with the same number of decimals as the corresponding point estimate, and p-values will be presented with 4 decimals or as '<.0001'.

Mock tables and graphs are presented in the Data Display Plan (DDP), which is a supplementary document to this analysis plan. Individual patient data listings will be presented according to the ICH E3 guideline for Structure and Content of Clinical Study Reports **(4)**, unless stated otherwise.

In order to be prepared for the SAS programming after DBL, draft output as outlined in the DDP, is planned to be developed based on example data in the ongoing study.

7.1.2 Baseline

Unless stated otherwise, the baseline value for a parameter is defined as the last non-missing value before the first dose of the investigational medicinal product (IMP).

7.1.3 Analysis Relative Day

The analysis relative day for an assessment/value is defined as the time in days from the date of randomisation to the date of the assessment. The date of randomisation is considered as day 1, and earlier dates will correspond to a negative day.

7.1.4 Analysis Visit

An analysis visit is defined as a categorical variable used to classify values within an analysis variable into temporal or conceptual groups used for analyses.

The visits as defined in the case report form, CRF, will be used as analysis visits.

In general, data from unscheduled visits will be presented in data listings and used in analysis or summary tables as appropriate. An exception to this is data used to confirm eligibility in association with screening or randomisation where the last assessment will be considered in summaries of screening data.

7.1.5 Handling of Missing Data

For some endpoints, statistical analysis is based the observed values, whereas for other endpoints, missing data are handled using imputations. For details regarding handling of missing data for each endpoint, see description of study endpoints and the corresponding statistical analysis in sections below.

Missing items in the COMFORT-B and SOS-PD questionnaires will not be imputed. Thus, sum COMFORT-B and SOS-PD (withdrawal and delirium modules), cannot be computed if one or more items are missing.

For VIS scoring, no use of a listed drug should be entered as 0 in the formula for calculating VIS score, refer to Section 8.3.3. Missing values are not anticipated, since all vasopressors are noted in the medical chart.

Some of the items in the PIM3 questionnaire can be imputed as described in Straney et. al **(5)**. That is, in case of items in the PIM3 questionnaire that have an imputation strategy connected to it, as described in **(5)**, such items can be imputed accordingly. However, if items in the PIM3 questionnaire that do not have an imputation strategy connected to it, as described in **(5)**, such items will remain missing, and consequently no PIM3 score can be calculated in these situations.

Patients should be encouraged to stay in study after any possible study treatment discontinuation. In the case of study discontinuation after the study treatment period, data from such patients will in general be included in statistical analysis up to the time-point of study discontinuation. In survival analyses, such patients will be censored at the time-point of study discontinuation. In different analyses of count variables, e.g. ICU-free days, such patients will be excluded from the analysis.

Data listings will include the observed values. For derived variables, values based on imputed data can be presented in listings.

7.1.6 Interim Analyses

Not applicable (N/A).

7.1.7 Multiplicity

For the primary endpoint, a testing scheme following a closed testing procedure will be applied in the potential second step involving the non-inferiority test, see further details in Section 7.9.1.1.6.

For the secondary endpoints, no adjustments for multiple comparisons will be made. However, in Section 7.1.7.1 below, 2 Key Secondary efficacy endpoints are listed which are believed to be potentially supportive to a claim that opiates can be reduced over time with isoflurane vs midazolam, and would therefore constitute important additional and relevant clinical information related to the primary endpoint.

Furthermore, in Section 7.1.7.2 below, 3 Key Safety Endpoints are listed. The results from these will be considered to be potentially supportive to safety claims related to benefits reflected in these endpoints of isoflurane vs midazolam.

Regarding the remaining endpoints analyses, it should be noted that the probability of making a type I error increases with the number of statistical tests performed.

7.1.7.1 KEY SECONDARY EFFICACY ENDPOINTS

The first secondary efficacy endpoint listed in Section 7.9.2.1 below related to dose of opiates from first blinded COMFORT-B assessment to end of study treatment period, and the secondary efficacy endpoint listed in Section 7.9.2.2 below related to dose of opiates during the last 4 hours of study treatment, as compared to the first 4 hours of study treatment after first blinded COMFORT-B assessment, are considered Key Secondary Efficacy Endpoints.

7.1.7.2 KEY SAFETY ENDPOINTS

The first secondary safety endpoint, listed in Section 7.10.1.1 (time from end of study drug administration to extubation if study drug is terminated for extubation), and the second secondary safety endpoint, listed in Section 7.10.1.2 (proportion of observations with spontaneous breathing efforts during study treatment) and the third secondary safety endpoint, contained in Section 7.10.1.3 (change in VIS score during study treatment period compared to baseline) are considered Key Safety Endpoints.

7.1.8 Subgroups

Descriptive statistics of the primary endpoint will be presented for the following subgroups:
-Treatment duration (≤ 24h , > 24h).
-Age (youngest age group separately, the two other age-groups together).
-Sex.

Descriptive statistics of opiate requirements, i.e. see Sections 7.9.2.1 and 7.9.2.2, will be presented for the following subgroups:
-Medical/Surgical/Other. Here, “Surgical” equals Surgical/Trauma/Neuro-surgical in the eCRF variable ICU admission treatment category which has 5 response choices (Medical/Surgical/Trauma/Neuro-surgical/Other).

Descriptive statistics of adverse events will be presented for the following subgroups:
-Age (youngest age group separately, the two other age-groups together).
-Study treatment duration (≤24h/>24h).

7.2 Analysis Sets

The decision on the classification of patients to each analysis set will be taken at the clean file meeting, prior to DBL, and documented in the clean file report together with the reasons for excluding patients from analysis sets.

The analyses will be based on different analysis sets depending on the purpose of the analysis, i.e. for efficacy and safety respectively.

7.2.1 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.

For patients excluded from FAS, individual patient data will be listed.

Closely related to FAS, is the population of included patients that are 3-16 years old, i.e. the US Food and Drug Administration definition of a paediatric population to support a future US registration, see also Section 7.9.1.1.8 below. In addition to the presentation of the primary endpoint results for this US-defined paediatric age group, further efficacy endpoints may be presented.

7.2.2 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 (which should be interpreted as 12 hours of study sedative treatment from start of IMP), 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.

In summary, the criteria below constitute reason for exclusion from the PP analysis set:
* Two or more changes in prescribed target sedation depth.
* Less than 12 hours of study sedative treatment from start of IMP.
* Less than 50% of the planned COMFORT-B assessments performed.

The final decision as to which other protocol deviations should be considered as reason for exclusion from the PP analysis set should be made at the clean file meeting and documented in the clean file report.

The statistical analysis of the primary endpoint will be conducted on both the FAS and the PP analysis set. Depending on the number of patients excluded from the PP analysis set, the PP analysis set may play an important role in the evaluation of non-inferiority (2), see also Section 7.9.1.1.8.

7.2.3 Safety Analysis Set

The safety analysis set is defined as all patients who received at least one dose of the IMP.

The safety analysis set will include all patients who received IMP and will be analysed in accordance with actual treatment received, *i.e.* patients will be analysed 'as treated'.

Closely related to FAS, is the population of included patients that are 3-16 years old, i.e. the US Food and Drug Administration definition of a paediatric population to support a future US registration, see also Section 7.9.1.1.8 below. In addition to the presentation of the primary endpoint results for this US-defined paediatric age group, further safety endpoints may be presented.

7.3 Disposition of Patients

The following will be presented:
- Number of screened patients, in total.
- Number of screening failures, in total.
- Number of randomised patients, by treatment group and in total.

Based on the number of randomised patients, the following will also be presented, by treatment group and in total:

- Number and percentage of patients who did not receive any dose of IMP.
- Number and percentage of patients who received at least one dose of IMP.
- Number and percentage of patients who completed the study treatment.
- Number and percentage of patients who completed the study.
- Number and percentage of patients who discontinued the study treatment.
- Number and percentage of patients who discontinued from the study.
- Number and percentage of patients in each of the analysis sets.

The number of patients attending each study visit/time-points (Screening, Baseline, IMP administration, Study treatment period 0-24 h, Study treatment period 25-48 h, Study treatment period 49-54, End of study treatment, Post-study treatment monitoring, Follow up visits or phone contact Day 9, 16, 23, 30, Day 30/End of study) will also be

summarised. For the study treatment period the number of patients with an unblinded COMFORT-B assessment will be presented for each time point.

7.4 Protocol Deviations

Protocol deviations will be presented in data listings.

The number and percentage of randomised patients with at least one major protocol deviation leading to exclusion from the PP analysis set will be presented. For further details on how protocol deviation information will be used in the definition of the PP Analysis set, see Section 7.2.2 above.

7.5 Demographics and Baseline Characteristics

PIM3, see Section 6.4, and demographic data (age at randomisation, age category, sex, race, ethnicity, weight and height) will be presented by treatment and in total for all analysis sets using descriptive statistics.
Number and percentages of subjects for each reason for ICU admission, ICU admission treatment category and whether or not subjects were intubated via endotracheal tube or tracheostomy will be presented by treatment and in total for all analysis sets and the subgroups study treatment duration and age group. Also, prescribed target sedation depth and shift in sedation depth will be summarised the same way.

A frequency table on the overall evaluation (normal, abnormal not clinically significant, abnormal clinically significant) of electrocardiogram (ECG) will be presented for the baseline visit data by treatment and total for the safety analysis set.

7.6 Medical History and Concurrent Diseases

Medical history and concurrent diseases will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

For each system organ class and preferred term, the number and percentage of patients with at least one condition in that system organ class or preferred term will be presented. Medical history and concurrent diseases will be presented in separate tables, based on the safety analysis set.

Medical history is defined as events stopped prior to baseline. Concurrent diseases are defined as ongoing events and events stopped on or after baseline. If the start and/or stop date is partially unknown, the following imputation rules will be used for the purpose of classifying the events:

	Imputed start date	Imputed end date
Unknown year	Missing	Missing
Unknown month	1 January	31 December

Unknown day	First of month	Last of month

If it is not possible to classify the condition based on the reported and/or imputed start and end dates, it will be considered as concurrent. In data listings, the dates will be presented as reported.

7.7 Prior and Concomitant Medication

Medications will be coded according to the World Health Organization (WHO) Drug Dictionary and summarized by therapeutic subgroup (ATC level 2) and preferred name.

For each therapeutic subgroup and preferred name, the number and percentage of patients who used at least one medication of that therapeutic subgroup or preferred name will be presented. Prior and concomitant medications will be summarized in separate tables, based on the safety analysis set.

Concomitant medications will also be presented per WHO standardized drug grouping for the four standardized drug groupings ‘Analgesia producing opioids’, ‘Benzodiazepines’, ‘Antipsychotics’ and ‘Hypnotics and sedatives’.

If a reported medication cannot be coded with a preferred name, the lowest available higher-level dictionary term will be used instead in the summary tables. If a medication cannot be coded on a lower level than the therapeutic subgroup or the anatomical main group (ATC level 1), that medication will be presented as ‘Not codable’ under that therapeutic subgroup/anatomical main group.

Prior medication is defined as medication stopped prior to baseline. Concomitant medication is defined as ongoing medication or medication stopped on or after baseline. If the start and/or stop date is partially unknown, the following imputation rules will be used for the purpose of classifying the medication:

	Imputed start date	**Imputed end date**
Unknown year	Missing	Missing
Unknown month	1 January	31 December
Unknown day	First of month	Last of month

If it is not possible to classify a medication based on the reported and/or imputed start and end dates, it will be considered as concomitant. In data listings, the dates will be presented as reported.

For information related to the use of concomitant medications during the study treatment period and its’ consequences on the statistical analysis, see details below in sections 7.7.1 to 7.7.6.

7.7.1 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 in the CSP. 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 in the CSP. 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 in the CSP.

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

7.7.1.1 Per protocol rescue sedation

Rescue sedation is defined as sedative agents other than the IMP that are 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 is recorded in eCRF as "Type of concomitant medication"="Per-protocol rescue sedation".

A review of all reported concomitant bolus administrations with Preferred names Propofol, Ketamine, and Esketamine will be performed prior to data base lock. Based on this review additional recordings of the above three sedation alternatives, even if not recorded as "Type of concomitant medication"="Per protocol rescue sedation", may be considered as rescue sedation and defined as the intercurrent event "use of rescue sedation" (see Section 7.9.1). This will be documented in the clean file report prior to locking the database.

In addition, any recordings of bolus propofol, bolus ketamine and bolus es-ketamine with reason for administration = Procedural medication (minor procedure inside ICU) will not be considered as rescue sedation and therefore not defined as the intercurrent event "use of rescue medication".

7.7.1.2 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 is recorded as reason for IMP dose change/ reason for administration =”Procedural medication (minor procedure inside ICU)”.

7.7.1.3 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 is recorded as reason for IMP dose change=”Procedural medication (procedure outside ICU)”.

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.

7.7.2 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.

7.7.2.1 Prohibited medications

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

- Chlorpromazine
- Chloral hydrate
- Barbiturates
- Other benzodiazepines than midazolam
- Gamma-hydroxybutyrate
- Melatonin
- α2-adrenergic agonist boluses (for infusion of α2-adrenergic agonists, refer to Section 7.7.2.2)
- 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 7.7.1.2.

A review of all concomitant medications belonging to the following WHO standardized drug grouping/ATC/preferred names for classification of prohibited medication will be performed prior to database lock:

- SDG Analgesia producing opioids
- SDG Benzodiazepines
- SDG Antipsychotics
- SDG Hypnotics and sedatives
- NMBA (=ATC 'M03')
- Alpha2-adrenergic agonists = Preferred names {Clonidine, Dexmedetomidine}

The classification will be documented in the clean file report.

7.7.2.2 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 in the CSP. 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 α2-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 7.9.1.1.3.

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

A review of α2-adrenergic agonists = Preferred names {Clonidine, Dexmedetomidine} will be performed prior to database lock and the classification related to the intercurrent event "7) α2-adrenergic agonists" described in Section 7.9.1.1.3 will be documented in the clean file report.

7.7.3 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 7.9.1.1.3.

7.7.4 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.

7.7.5 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.

7.8 Compliance

Treatment in this study is continuous.

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 also Section 6.18.1).

7.9 Efficacy Evaluation

All analyses of efficacy endpoints will be performed on the full analysis set, and these analyses will be considered as the main analyses.

The per-protocol analysis set will be used for supplemental analyses of the primary endpoint.

7.9.1 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 with 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 7.7.1.1;
ii) Use of bolus doses of opiates, see Section 10.2.5.1 in the CSP;
iii) Use of prohibited medications, including neuromuscular blocking agents that are not allowed for treatment maintenance, see Section 7.7.2.1;
iv) Use of α2-adrenergic agonists infusions in a manner that leads to failure time in the calculation of the primary endpoint, see Section 7.7.2.2.

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 SAP.

The objective needs to be further specified so that it will be clear in terms of the 5 attributes related to the estimand (1, 2):

1) Patient population
2) Treatment administration (e.g. dose and frequency of dosing) for both the active and the comparator treatment
3) Primary Endpoint
4) Analysis strategy for other intercurrent events
5) Population-level summary for the primary endpoint

7.9.1.1 Estimand

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

7.9.1.1.1 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 in the CSP.

7.9.1.1.2 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 7.1 in the CSP.

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.

7.9.1.1.3 Endpoint

The detailed definition of the primary endpoint: Percentage of time of adequately maintained sedation, in absence of rescue sedation, within the COMFORT-B interval (light, moderate or deep sedation) prescribed at randomisation (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. A time interval not defined as “Failure” or “Censored” will be defined as “Success”. Using the composite endpoint strategy, 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.

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 randomisation, 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 randomisation, study treatment will start by initiating an IV infusion of midazolam (as specified in Section 10.2.4.1 in the CSP), 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 in the CSP), and all other sedatives are simultaneously turned off (up to 30 minutes prior to initiating isoflurane).

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).

COMFORT-B assessments and intercurrent events 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 7 in Section 6.15.1.

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 10.3.5 in the CSP.

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 of 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 120-minute (±60 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 bolus 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. For the use of infusion rescue sedation, the time from the start of this infusion period until it is terminated will be classified as “Failure”.

All mentioned intercurrent events above are to be handled with the Composite strategy which implies the occurrence of the intercurrent event is taken to be a component of the variable.

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 a number of 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 the first scheduled COMFORT-B measurement at +2 hours is missing it will be connected with a +60 minutes long time interval coded as “Censored” from the time-point when the COMFORT-B assessment as scheduled.

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 during such a stop will be used in the study analyses, and the next assessor-blinded 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

Short 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 7.7.1.2. 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 Section 10.2 in the CSP and Sections 7.7.1 to 7.7.3, 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 in the CSP. If a patient is discontinued from the study treatment because of study treatment failure (i.e reason for treatment discontinuation=Lack of efficacy), the time from study treatment stop up to 48 hours will be classified as "Failure". In addition, reasons given as other will be reviewed and assessed if to be considered as treatment failure. The classification of reason given as 'Other' will be documented in the clean file report.

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 in the CSP 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.

7.9.1.1.4 Other Intercurrent events

No other intercurrent events are identified for the estimand.

7.9.1.1.5 Population-level summary for the primary 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.

7.9.1.1.6 Main estimator analysis

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. An unstructured covariance structure will be used. 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 normality assumption will be investigated by studying the residual diagnostics plots. If the assumption of normality is seriously violated a transformation of data will be performed and tested for normality (e.g. log transform, rank transform). If the transformation(s) does

not yield normally distributed residuals, then a non-parametric test will be performed (e.g. Wilcoxon rank-sum test).

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 in the CSP.

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

7.9.1.1.7 Sensitivity analysis

To investigate the robustness of the main estimator results, the following sensitivity analyses of the same estimand will be performed.

1) Missing scheduled COMFORT-B assessments: Instead of censoring as described in Section 7.9.1.1.3, linear interpolation of the COMFORT-B scale will be used. If the last COMFORT-B assessment is missing, then linear extrapolation from last two COMFORT-B will be performed.

2) Missing scheduled COMFORT-B assessments: Instead of only using censoring as described in Section 7.9.1.1.3, 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.

3) A simpler analysis of variance model will be run with only treatment group used as a fixed explanatory factor.

7.9.1.1.8 Supplementary analysis

To investigate the robustness of the primary endpoint results, the following supplementary analyses of related estimands will be performed.

1) The statistical analysis of the primary endpoint, as defined in Section 7.9.1.1.3, will be conducted on the PP analysis set.

The statistical analysis of the primary endpoint will be conducted on both FAS and PP analysis set. Depending on the number of patients excluded from the PP analysis set, this analysis set may play an important role in the evaluation of non-inferiority.

2) The statistical analysis of the primary endpoint, as defined in Section 3.1.1, will be evaluated using a treatment policy strategy, i.e. the intercurrent events as outlined in section 7.9.1.1.3 except for missing COMFORT-B will, as a rule, be disregarded. Missing COMFORT-B will be censored as described in section 7.9.1.1.3.

A comparison between groups based only on the blinded two-hourly COMFORT-B assessments, i.e. application of the treatment policy strategy, will be performed.

3) A non-parametric method will be used as an alternative method.

Wilcoxon rank-sum test will be used as an alternative method, if considered more appropriate based on the distribution of the primary efficacy endpoint.

4) The statistical analysis of the primary endpoint, as defined in Section 7.9.1.1.3, will be conducted on all randomised patients.

5) The statistical analysis of the primary endpoint, as defined in Section 7.9.1.1.3, with the exception that it will be based on initial target sedation depth only, will be conducted.

6) The statistical analysis of the primary endpoint, as defined in Section 7.9.1.1.3, with the exception that use of α2-adrenergic agonists will disregarded in the analysis, i.e. connected to a treatment policy, will be conducted.

7.9.2 Analysis of the secondary efficacy endpoints

Throughout all endpoints described in this section, if the first blinded COMFORT-B assessment at +2 hours is missing for any reason then the planned timepoint (+2 hours after initiating study sedative treatment) will be used as the starting point.

7.9.2.1 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

Three endpoints are defined:

i) Dose of opiates (µg/kg/h, i.e. total dose/sedation time) from first blinded COMFORT-B assessment (at +2 hours) to end of study treatment period. Opiates are defined as concomitant medications belonging to WHO Standardized Drug Grouping = Analgesia producing opioids. The doses will be converted to Fentanyl IV equivalents as described in Section 8.2.1.

For each 24 hours period from first blinded COMFORT-B assessment (at +2 hours) (≤24 h, >24 h), treatment groups will be compared, based on observed data, i.e. less data is expected in the post 24 hours period. For each of these two periods, the dose of opiates will be compared between treatment groups using an analysis of variance model with treatment group as fixed effect and baseline opiate dose as covariate.

Opiate dose (total dose/sedation time) and change from baseline will be presented for the period prior and post 24 hours from first blinded COMFORT-B assessment, for the total study treatment period after first blinded COMFORT-B assessment, and for each 4-hour interval, by treatment, using descriptive statistics. In addition, presentations for the subgroup ICU admission will be provided.

ii) Dose of study drug from first blinded COMFORT-B assessment (at +2 hours) to end of study treatment period

Isoflurane and midazolam dosing over time from first blinded COMFORT-B assessment to end of study treatment period will be presented by target sedation depth (light, moderate, deep), by treatment, using descriptive statistics. Dose of study drug will be presented by 4 hours, by 12 hours, by 24 hours, and for the total study treatment period after first blinded COMFORT-B assessment.

The dose of study drug over time divided by minute volume and mean end tidal concentration (only for Isoflurane), for the same time intervals as for dose of study drug will be presented by treatment group using descriptive statistics.

iii) Doses of other analgesics from first blinded COMFORT-B assessment (at +2 hours) to end of study treatment period

The doses of analgesics taken from first blinded COMFORT-B assessment to end of study treatment period will be presented in data listings including treatment group information.

7.9.2.2 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

Patients with less than 8 hours on study treatment from first blinded COMFORT-B will be excluded from analysis of these endpoints.

Three endpoints are defined:

i) Dose of opiates (µg/kg/h, i.e. total dose/sedation time) during the last 4 hours of study treatment, as compared to the first 4 hours of study treatment after first blinded COMFORT-B assessment.

In addition, the change between period 2 (last 4 hours) and period 1 (first 4 hours) will be compared between treatment groups using an ANCOVA (analysis of covariance) model with treatment group as fixed effect and baseline opiate dose as covariate. Furthermore, an ANCOVA (analysis of covariance) model with treatment group as fixed effect and baseline opiate dose and "time between period 1 and period 2" as covariates, where the change in opiate dose between period 2 and period 1 is adjusted for the time difference between these two periods.

The opiate dose (total dose/sedation time) during the first 4 hours of study treatment, and the last 4 hours of study treatment, respectively, will be presented by treatment group using descriptive statistics. In addition, presentations for the subgroup ICU admission will be provided.

ii) Dose of study drug during the last 4 hours of study treatment, as compared to the first 4 hours of study treatment after first blinded COMFORT-B assessment

Isoflurane and midazolam dosing during the first 4 hours of study treatment, and the last 4 hours of study treatment, respectively, will be presented by target sedation depth (light, moderate, deep), by treatment, using descriptive statistics.

Isoflurane dose of study drug divided by minute volume and mean end tidal concentration will be presented by target sedation depth (light, moderate, deep), by treatment, using descriptive statistics.

iii) Doses of other analgesics during the last 4 hours of study treatment, as compared to the first 4 hours of study treatment after first blinded COMFORT-B assessment

The doses of analgesics will be presented in data listings including treatment group information.

7.9.2.3 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-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)

Three endpoints are defined:

i) Rescue propofol dose (mg/kg/h, i.e. total dose/sedation time) from first blinded COMFORT-B assessment (at +2 hours) to end of study treatment period

The dose of rescue propofol for the total study treatment period after first blinded COMFORT-B assessment will be compared between treatment groups using an analysis of variance model with treatment group as fixed effect.

Rescue propofol dose will also be presented by 12 hour periods, and by 24 hour periods, and for the total study treatment period after first blinded COMFORT-B assessment, by treatment, using descriptive statistics.

ii) Rescue ketamine/es-ketamine (mg/kg/h, i.e. total dose/sedation time) (es-ketamine converted to ketamine-equivalents) from first blinded COMFORT-B assessment (at +2 hours) to end of study treatment period

The dose of es-ketamine (mg/kg) will be converted to ketamine-equivalent dose by multiplying the es-ketamine dose by 2.

The ketamine-equivalent dose for the total study treatment period after first blinded COMFORT-B assessment will be compared between treatment groups using an analysis of variance model with treatment group as fixed effect.

Ketamine-equivalent dose will also be presented by 12 hour periods, and by 24 hour periods, and for the total study treatment period after first blinded COMFORT-B assessment, by treatment, using descriptive statistics.

iii) Dose of α2-adrenergic agonists (µg/kg/h, i.e. total dose/sedation time) from first blinded COMFORT-B assessment (at +2 hours) to end of study treatment period

α2-adrenergic agonists infusions dose will be presented by 12 hour periods, and by 24 hour periods, and for the total study treatment period after first blinded COMFORT-B assessment, by treatment, using descriptive statistics separately for Clonidine and Dexmedetomidine.

7.9.2.4 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

Number of per protocol rescue sedation (propofol, ketamine, and es-ketamine) doses will be presented by 12 hour periods, and by 24 hour periods, and for the total study treatment period after first blinded COMFORT-B assessment, by treatment, using descriptive statistics.

7.10 Safety Evaluation

All evaluations of safety data will be performed on the safety analysis set.

Descriptive statistics will be used to present the safety outcomes in the two treatment groups as following the general principles given in Section 7.1.1, 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.

7.10.1 Analysis of the secondary safety endpoints

7.10.1.1 Time from end of study drug administration to extubation if study drug is terminated for extubation

In the analysis, extubation will be regarded as an event. A Cox proportional hazards regression model will be used to compare time from end of study drug administration to extubation between treatment groups for the subgroup of patients with endo-tracheal tube where the study drug is terminated for extubation. The model will include treatment, sedation depth at end of study drug administration, and full stop of IMP (Y/N) as factors. If the wake-up for extubation is terminated and the patient remains intubated, the time to extubation will be censored at the time-point when the wake-up is terminated.

In addition, the log-rank test and Kaplan-Meier survival curves and estimates including quartiles, will be reported. The presentation will also include proportion of patients for each censoring event.

Time from end of study treatment period to extubation for the subgroup of patients with endo-tracheal tube where the study drug is terminated for extubation will be presented by treatment using descriptive statistics. The presentation will include separate presentation for patients extubated and patients censored showing descriptive statistics for the factors (sedation depth at end of study drug administration, and full stop of IMP (Y/N)).

7.10.1.2 Proportion of observations with spontaneous breathing efforts during study treatment

The individual proportions during the study treatment period will be compared between treatment groups using analysis of variance with treatment group as fixed effect.

The individual proportions of observations with spontaneous breathing efforts during study treatment will be presented by treatment, using descriptive statistics.

7.10.1.3 Need for additional inotropic/vasopressor agent defined as change in VIS score during study treatment period compared to baseline

The following endpoint is defined:

i) Change in VIS score during study treatment period compared to baseline

For each 24 hours period (prior and post 24 hours), treatment groups will be compared with respect to individual median VIS score, based on observed data, using the Wilcoxon rank-sum test.

The VIS score, and the change from baseline in the VIS score during study treatment period, will be presented for <24h and ≥24h, 2 to 4h and by 4h time intervals by using descriptive statistics.

7.10.1.4 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

For the subgroup of patients fulfilling the criteria for evaluation of withdrawal symptoms, i.e. patients exposed to more than a total of 96 hours sedation (including pre-study sedation period), the proportion of patients with presence of withdrawal symptoms will be presented for each follow-up time-point, by treatment, using descriptive statistics.

7.10.1.5 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

For the subgroup of patients fulfilling the criteria for evaluation of delirium, i.e. patients admitted to the ICU for at least 48 hours (including period prior to study enrolment), the proportion of patients with presence of delirium will be presented for each follow-up time-point, by treatment, using descriptive statistics.

7.10.1.6 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)

The following endpoints are defined:

i) Presence of psychomotor dysfunction during study sedation period and/or in the 48 hours after discontinuation of study treatment

The proportion of patients with presence of psychomotor dysfunction during study sedation period and/or in the 48 hours after discontinuation of study treatment will be presented, by treatment, using descriptive statistics. In addition, presentations by age group will be provided.

ii) Time from IMP start to first psychomotor dysfunction event (h).

The time from IMP start to first psychomotor dysfunction event for patients with psychomotor event will be presented using descriptive statistics.

iii) Cumulative study treatment dose up to first psychomotor dysfunction event (in mg/kg for midazolam and MAC hours for isoflurane) during the study treatment period

Cumulative study treatment dose up to first psychomotor dysfunction event will be presented for patients with psychomotor event will by treatment (in mg/kg for midazolam and in MAC hours for isoflurane) using descriptive statistics.

In addition, the same endpoints and analyses as described above but with cardiovascular events (as defined in Section 7.10.3.2) and hypotensive events (defined as adverse event with HLGT Decreased and nonspecific blood pressure disorders and shock) will be provided.

7.10.1.7 30 days/hospital mortality

The following endpoints are defined:

i) Mortality up to the day 30 visit

A Cox proportional hazards regression model will be used to compare time from randomisation to death between treatment groups. The model will include treatment as factor and baseline PIM3 as covariate. If the patient is alive at day 30 the survival time will be censored at day 30. Deaths registered after day 30 will be disregarded and survival time will be censored at day 30. Patients that are withdrawn prior to Day 30 will be censored at the time of study discontinuation.

In addition, the log-rank test and Kaplan-Meier survival curves and estimates will be reported.

ii) Hospital mortality up to the day 30 visit

A Cox proportional hazards regression model will be used to compare time from randomisation to death at hospital between treatment groups. The model will include treatment as factor and baseline PIM3 as covariate. If a patient dies outside the hospital, the survival time will be censored at the day the patient left the hospital the last time. If the patient is alive at day 30 the survival time will be censored at day 30. Deaths at hospital registered after day 30 will be disregarded and survival time will be censored at day 30. Patients that are withdrawn prior to Day 30 will be censored at the time of study discontinuation.

In addition, the log-rank test and Kaplan-Meier survival curves and estimates will be reported.

7.10.1.8 Ventilator-free days at 30 days from start of study treatment period

The number of ventilator-free days from day of start of study treatment up to day 30 will be presented by treatment using descriptive statistics. Patients that are withdrawn prior to Day 30 will be excluded from analysis.

7.10.1.9 Time in intensive care unit/hospital at day 30 from start of study treatment period

The following endpoints are defined:

i) The number of ICU-days

The number of ICU-days from day of start of study treatment up to day 30 will be presented by treatment using descriptive statistics. Patients that are withdrawn prior to Day 30 will be excluded from analysis.

ii) The number of hospital days

The number of hospital days from day of start of study treatment up to day 30 will be presented by treatment using descriptive statistics. Patients that are withdrawn prior to Day 30 will be excluded from analysis.

7.10.1.10 Days alive and not in the ICU at day 30 from start of study treatment period

The following endpoint is defined:

i) The number ICU-free days and alive

The number of ICU-free days and alive from day of start of study treatment up to day 30 will be presented by treatment using descriptive statistics. Patients that are withdrawn prior to Day 30 will be excluded from analysis.

7.10.1.11 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

Results will be presented by treatment using descriptive statistics. See Section 7.10.3 for details on which data that will be presented. In Sections 7.10.3.1.1 and 7.10.3.1.2 further details are given on which adverse events are considered as common adverse events and which are considered as sedation-related adverse events.

7.10.1.12 Frequency and intensity of adverse events from start of study treatment to day 30

Results will be presented by treatment using descriptive statistics. See Section 7.10.3 for details on which data that will be presented.

7.10.1.13 Changes in vital signs, blood gases, body temperature and urinary output from baseline to end of study treatment

The following endpoints are defined:

i) Changes in vital signs from baseline

For vital signs parameters, summary statistics will be presented for the baseline visit and for post-baseline time-points.

For post-baseline time-points, summary statistics on the change from baseline will also be presented.

Further, frequency tables on the clinical interpretation of the vital signs results (normal, abnormal not clinically significant, abnormal clinically significant) will be presented by time-point. For post-baseline time-points, shift-tables to illustrate the change from baseline in the clinical interpretation will be presented.

ii) Changes in blood gases from baseline

For blood gases parameters, see Section 6.14 above, summary statistics will be presented for the baseline visit and for post-baseline time-points. For post-baseline time-points, summary statistics on the change from baseline will also be presented.

Further, frequency tables on the clinical interpretation of the blood gases results (normal, abnormal not clinically significant, abnormal clinically significant) will be presented by time-point. For post-baseline time-points, shift-tables to illustrate the change from baseline in the clinical interpretation will be presented.

Each parameter will be summarised using standardised units and presented in a separate table. Test results reported in other units will be converted from their original units to the standardised units, using appropriate conversion factors. Data listings will include test results in both original and standardised units.

iii) Changes in body temperature from baseline

For body temperature, see Section 6.11 above, summary statistics will be presented for the baseline visit and for post-baseline time-points. For post-baseline time-points, summary statistics on the change from baseline will also be presented.

Further, frequency tables on the clinical interpretation of the body temperature results (normal, abnormal not clinically significant, abnormal clinically significant) will be presented

by time-point. For post-baseline time-points, shift-tables to illustrate the change from baseline in the clinical interpretation will be presented.

iv) Changes in urinary output from baseline

For urinary output (in mL), see Section 6.10 above, summary statistics will be presented for the baseline visit and for post-baseline time-points. For post-baseline time-points, summary statistics on the change from baseline will also be presented.

Further, frequency tables on the clinical interpretation of the urinary output results (normal, abnormal not clinically significant, abnormal clinically significant) will be presented by time-point. For post-baseline time-points, shift-tables to illustrate the change from baseline in the clinical interpretation will be presented.

Test results reported in other units will be converted from their original units to the standardised unit (mL) before analysis, using appropriate conversion factors. Data listings will include test results in both original and standardised units.

7.10.1.14 Changes in clinical chemistry and haematology parameters from baseline up to the 48-hour post-study treatment monitoring

The following endpoints are defined:

i) Changes in clinical chemistry and haematology parameters from baseline

Results will be presented by treatment using descriptive statistics. See Sections 6.13 and 7.10.4 for details on which data that will be presented.

7.10.2 Extent of Exposure

Efficacy and exploratory endpoints, see Sections 7.9.2.1, 7.9.2.2, and 7.11.1, will reflect exposure to study drugs. The duration of the study treatment period will also be presented.

7.10.3 Adverse Events

Adverse events will be coded according to MedDRA.

An overview of all adverse events will be presented, including the number and percentage of patients with at least one, and the total number, of the following:

- Adverse events.
- Serious adverse events.
- Adverse events leading to withdrawal of the IMP.
- Fatal adverse events.

- Adverse events of special interest, overall and broken down by type of adverse event of special interest (common adverse event, sedation-related adverse event, neurological and psychomotor adverse evet)
- Adverse events, broken down by severity.
- Adverse events, broken down by causality with IMP assessment.

The incidence of adverse events will be presented by system organ class and preferred term. For each system organ class and preferred term, the total number of adverse events as well as the number and percentage of patients with at least one adverse event in that system organ class or preferred term will be presented. The incidence of serious adverse events will be presented in the same way.

In addition, the incidence of adverse events of special interest, see Section 7.10.3.1 below, will be presented in the same way. Specifically, three categories of these are Common AEs, Sedation-related AEs, and Neurological and psychomotor adverse events. For each of these categories, the possible AEs to report in each of these categories are given as drop-down lists in the eCRF. In addition, cardiovascular adverse events, see section 7.10.3.2 below, will be presented separately.

Separate tables for the incidence of adverse events broken down by severity and the incidence of adverse events broken down by causality with IMP assessment will also be presented by system organ class and preferred term.

There will also be tables on the most frequently reported adverse events, on system organ class level and on preferred term level. The decision on the frequency cut-off for these tables will be taken during the analysis of the adverse events data in consultation with the author of the clinical study report and could be influenced by factors such as the overall number of adverse events, study design, and the nature of the indication. The frequency cut-off should be mentioned in a table note.

7.10.3.1 Definition of adverse events of special interest

Adverse events of special interest in this study are defined in Sections 7.10.3.1.1 to 7.10.3.1.5 in the CSP.

7.10.3.1.1 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

7.10.3.1.2 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 in the CSP.

7.10.3.1.3 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.

7.10.3.1.4 Delirium and withdrawal

Symptoms of delirium and withdrawal will be assessed using the SOS-PD scale (refer to Section 6.16) and will not be reported as separate adverse events.

7.10.3.1.5 Organ function parameters

Renal and hepatic function tests (S-creatinine, S-urea, S-Bilirubin, S-AST, and 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.

7.10.3.2 Cardiovascular events

Cardiovascular events are defined as SOS-PD symptom Tachycardia or the below listed MedDra terms:
HLGT Cardiac arrhythmias
HLGT Decreased and nonspecific blood pressure disorders and shock
HLGT Arteriosclerosis, stenosis, vascular insufficiency and necrosis
HLGT Vascular hypertensive disorders
HLT Cardiac and vascular procedural complications
PT Tachycardia

7.10.4 Laboratory

For the purpose of summary tables on laboratory test results, any value reported as below the lower limit of quantification or as undetectable will be considered as missing, and any

value reported as above the upper limit of quantification will be considered as being equal to the upper limit. In data listings, the reported value will be presented.

For continuous laboratory parameters, summary statistics on the laboratory test results will be presented by time-point. For post-baseline time-points, summary statistics on the change from baseline will also be presented.

For categorical laboratory parameters, frequency tables on the laboratory test results will be presented by time-point. For post-baseline time-points, the shift from baseline will also be presented.

For all laboratory parameters, frequency tables on the clinical interpretation of the laboratory test results (normal, abnormal not clinically significant, abnormal clinically significant) will be presented by time-point. For post-baseline time-points, the shift from baseline will also be presented.

Each parameter, see Section 6.13, will be summarised using standardised units and presented in a separate table. Test results reported in other units will be converted from their original units to the standardised units before analysis, using appropriate conversion factors. Data listings will include test results in both original and standardised units.

7.10.5 Physical Examination

For all body systems examined, frequency tables on the investigator's assessment (normal, abnormal not clinically significant, abnormal clinically significant) will be presented by time-point.

For post baseline time-points, the shift from baseline will also be presented.

7.10.6 Vital Signs

For vital signs parameters, summary statistics will be presented for the baseline visit and for post-baseline time-points.

For post-baseline time-points, summary statistics on the change from baseline will also be presented.

Further, frequency tables on the clinical interpretation of the vital signs results (normal, abnormal not clinically significant, abnormal clinically significant) will be presented by time-point. For post-baseline time-points, shift-tables to illustrate the change from baseline in the clinical interpretation will be presented.

7.11 Exploratory Evaluation

The analyses of the efficacy exploratory endpoints will be performed on the full analysis set, whereas the safety exploratory endpoints will be performed on the safety set. The first two exploratory endpoints below are considered efficacy endpoints, whereas the last two are considered safety endpoints.

7.11.1 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

The MAC hours of isoflurane will be presented by age-groups (3-7 years, 8-11 years, and 12-17 years), using descriptive statistics for the same time periods as described for the mean dose of study drug (Section 7.9.2.1). See also information on derivation of endpoints in Section 8.2.1.

7.11.2 Number of study drug bolus doses given per 24 hours during midazolam and isoflurane sedation of mechanically ventilated patients

Number of study drug bolus doses will be presented for the period prior and post 24 hours, and for the total study treatment period, by treatment, using descriptive statistics.

7.11.3 Ventilator parameters (ventilation mode, tidal volume, minute volume, fraction of inspired oxygen (FiO_2), end tidal carbon dioxide [$EtCO_2$], spontaneous breathing rate, total breathing rate, positive end-expiratory pressure [PEEP], set inspiratory pressure [Pinsp], level of pressure support [PS] above PEEP)

The endpoints, see Section 6.12 above, will be presented for each treatment group and each time-point (every 2-hour) using descriptive statistics.

7.11.4 Frequency and type of AnaConDa-S device deficiencies during isoflurane sedation

The proportion of patients with at least one reported AnaConDa-S device deficiency will be presented for the isoflurane group using descriptive statistics.

Furthermore, the number of occurrences of AnaConDa-S device deficiencies will be presented in a frequency table showing the number AnaConDa-S device deficiencies reports of each type (3 possible types: Malfunction (fracture or loosening), Use error (Implant not sterile or similar), Inadequate labelling (see identity)) for the isoflurane group. Also, number of deficiency of device with respect to Identity (label unclear or missing, Safety (fracture implant or similar), Quality (broken package or similar), Performance of the device (implant failure and removed), Durability or Reliability will be presented as well as action taken and what the device deficiency resulted in.

7.12 Additional Analyses

Conditional of the U.S. Food and Drug Administration (FDA) approval of the study, the complete set of statistical analyses might 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.

The analyses will be performed to align with the US Food and Drug Administration definition of a paediatric population to support a future US registration. This population should be only slightly smaller than FAS, see Table 1 and Table 2 above, and have the same significance level, i.e. claims of the study regarding primary endpoint results could be based on this population. Further, it is expected to be minimal loss of power, in the analysis of the 3-16 years old population, compared to the 3-17 years population (FAS), due to that it is expected that the 3-16 years population will be close to 90 patients, i.e. the estimated minimum size of FAS which will yield close to 80 % power given the assumptions stated in Section 5.1 above.

7.13 Changes to Planned Analysis

The protocol states in Section 13.5.3.3, in the definition of “Failure”, that failure time will be connected with a 60-minute (±30 minutes of time of COMFORT-B assessment). In order to use the mid-point of the intervals the failure time will be connected with a 120-minute (±60 minutes of time of COMFORT-B assessment) instead.

The protocol stated in Section 7.2.2 as one of the secondary safety objectives:

“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.”

This was revised and neurological symptoms was removed from the objective and respective endpoint. Per protocol section 6.3 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. While a broader scope for these events were used in the data collection, including for example stroke or meningitis, a more specific definition is applied in analyses. The definition referred to in CSP section 12.2.7.3 and SAP section 7.10.3.1.3 was chosen for purposes of comparison with the publications referred to. Accordingly, the definition used for analysis is essentially a clarification of planned analyses rather than a change to the same. The broader scope will nevertheless be presented separately in summary overviews.

The protocol stated in Section 7.3.2 as one of the secondary safety endpoints:
“Need for additional inotropic/vasopressor agent and change in VIS score during study treatment period compared to baseline.”
To clarify that the endpoint evaluation will be based on the change in VIS score the text was revised to: “Need for additional inotropic/vasopressor agent defined as change in VIS score during study treatment period compared to baseline.”

8 Derived Variables

8.1 Primary endpoint

For information on endpoint derivations, see sections 7.9.1.1.3, 7.9.1.1.7, and 7.9.1.1.8 above.

8.2 Secondary efficacy endpoints

8.2.1 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

All endpoints will be derived based on the period from first blinded COMFORT-B assessment to end of IMP administration, where end of IMP administration is defined as when the study treatment is stopped or reduced significantly (>25%) to prepare for extubation. If the first blinded COMFORT-B assessment at +2 hours is missing for any reason then the planned timepoint (+2 hours after initiating study sedative treatment) will be used as the starting point.

Three endpoints are defined:

i) Dose of opiates (µg/kg/h, i.e. total dose/sedation time)

All opiate infusions will be included, as well as all bolus doses, i.e. also opiates given during medical procedures as described in Section 7.7.1.2 and 7.7.1.3 will be considered in this endpoint.
Analyses will be based on Fentanyl IV equivalent dose derived according to the following procedure:

a) Define Opioids using SDG Analgesia producing opioids
b) Derive ug/kg (or mg/kg depending on decimals needed) per Opioid by route using the reference above.
c) Derive Fentanyl IV equivalents using the below conversion factors in Table 8.

Table 8 Conversions for Fentanyl IV (mg) equivalent dose

Preferred name	Route	Conversion factor
Methadone	Nasogastric/Oral 0-20 mg	Methadone dose (mg)/75
	Nasogastric/Oral 21-50 mg	Methadone dose (mg)/37.5
	Nasogastric/Oral >50	Methadone dose (mg)/30
Morphine	Oral	Morphine oral dose (mg)/300
Morphine	IV	Morphine IV dose (mg)/100
Nalbuphine	IV	Nalbuphine IV dose (mg)/100

Piritramide (mg)	IV	Piritramide IV dose (mg)/150
Remifentanil	IV	Remifentanil IV dose (mg)*1
Sufentanil	IV	Sufentanil IV dose (mg)*10
Tramadol	Oral	Tramadol oral dose (mg)/1500

Opiate dose (total dose/sedation time) will be calculated for the period prior and post 24 hours, and for the total study treatment period. In addition, it will also be calculated for each 4-hour interval.
To calculate the dose of opiates for the different periods the following will be applied:
Dose of opiates:

1. Accumulate ug/kg (boluses and infusion) per time period
2. Exclude ug/kg taken during procedure outside the ICU per time period
3. Include entire ug/kg from boluses as taken within a time period
4. Exclude time during procedure outside the ICU from hours per time period
5. Divide by hours per time period

Calculate hours per time periods:

- Baseline = [Randomization] – [2 hours prior to randomization]
- Total = [EoT/Start of Wake-up] – [2 hours after start of study drug]
- ≤24 h = [min(EoT/Start of Wake-up; 24h)] – [2 hours after start of study drug]
- >24 h = [EoT/Start of Wake-up] – [24 hours after start of study drug]
  etc.
- ≤4 h = [min(EoT/Start of Wake-up; 4h)] – [2 hours after start of study drug]
- >4 to ≤8 h = [8 hours after start of study drug] – [4 hours after start of study drug]
  etc.

ii) Dose of study drug

*Isoflurane dose will be measured as the infusion rate (mL/h), as the end-tidal concentration (Vol %), MAC and by minute volume (mL/L)
*Midazolam (mg/kg/h).

Isoflurane and midazolam dose will be calculated by 4 hours, by 12 hours, by 24 hours, and for the total study treatment period.

Dose will be derived from total dose including bolus doses during the periods as defined above. To calculate the dose of study drug for the different periods the following will be applied:
<u>Dose of study drug (mL/h for Isoflurane, mg/kg/h for Midazolam)</u>

1. Accumulate mL and mg/kg (boluses and infusion) per time period
2. Exclude mL and mg/kg taken during time off study drug per time period

3. Include entire mL and mg/kg from boluses as taken within a time period
4. Exclude time off study drug from hours per time period
5. Divide by hours per time period

Calculate hours per time periods:

- Total = [EoT/Start of Wake-up] – [2 hours after start of study drug]
- ≤24 h = [min(EoT/Start of Wake-up; 24h)] – [2 hours after start of study drug]
- >24 h = [EoT/Start of Wake-up] – [24 hours after start of study drug]
  etc.
- ≤4 h = [min(EoT/Start of Wake-up; 4h)] – [2 hours after start of study drug]
- >4 to ≤8 h = [8 hours after start of study drug] – [4 hours after start of study drug]
  etc.

For isoflurane, in addition to the infusion rate, dose will be divided with minute volume (mL/L), and the end-tidal concentration (Vol %) as well as MAC will also be presented.

Calculation of isoflurane dose by minute volume, over the course of the sedation, will use the rule that collected ventilator parameters are assumed to describe the settings for the succeeding period until next registered reading of ventilator parameters. Ventilator parameters including minute volume and total breathing rate (breaths per minute) are collected every 2 hours for isoflurane patients. Isoflurane infusion rate will be divided by the minute volume corresponding to the time period. In general, for a time period >X to ≤ Y the mean of the minute volume measurements at X and up to the measurement prior to Y will be calculated. If all measurements at X and up to the measurement prior to Y are missing, then use the mean minute volume of the measurement prior to X and the measurement at the Y time points. If the last measurement within the time period is missing then use LOCF.

Mean end-tidal concentrations (%) will be calculated using the same approach as for mean minute volume.

MAC hours:
Isoflurane MAC hours will be calculated as the patients mean end-tidal concentration divided by MAC for the relevant age-group.

$$\mathrm{MAC}_{age} = \mathrm{MAC}_{40} \times 10^{-0.00269(\mathrm{age}-40)}$$

Where MAC_{40} is 1.17

See Nickalls R.W.D, Mapleson W.W Age-related iso MAC charts for isoflurane, sevoflurane and desflurane in man **(7)**, LeDez KM, Lerman J. The minimum alveolar concentration (MAC) of isoflurane in preterm neonates **(8).**

iii) Doses of other analgesics

No derivations needed.

8.2.2 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

Patients with less than 8 hours on study treatment from first blinded COMFORT-B will be excluded from analysis of these endpoints.

All endpoints will be derived based on the period from first blinded COMFORT-B assessment to 4 hours, and the last 4 hours of study treatment before end of IMP administration, where end of IMP administration is defined as when the study treatment is stopped or reduced significantly (>25%) to prepare for extubation, respectively. A variable will be created taking the value 1 or 2, where period=1 corresponds to the first 4 hours, and period=2 corresponds to the last 4 hours.

Three endpoints are defined:

i) Dose of opiates (µg/kg/h, i.e. total dose/sedation time)

All opiate infusions during each of these two periods will be included, as well as all bolus doses, i.e. also opiates given during medical procedures as described in 10.3.2 and 10.3.3 will be considered in this endpoint. In addition, the change in dose of opiates will be calculated between period 2 and period 1. Furthermore, the time between period 2 and period 1 will be calculated by taking difference between the time-point in the middle of period 2 minus the time-point in the middle of period 1.

Opiate dose (total dose/sedation time) will be calculated for the first 4 hours, and for the last 4 hours, respectively.

During the first 4 hours, the total dose of opiates during that period will be calculated as described in Section 8.2.1. The procedure will be similar for the last 4 hours.

ii) Dose of study drug

*Isoflurane dose will be measured as the infusion rate (mL/h), as the end-tidal concentration (Vol %), MAC and by minute volume (ml/L).
*Midazolam (mg/kg/h).

Dose will be derived from total dose including bolus doses during these periods for each target sedation depth.

For isoflurane, in addition to the infusion rate, dose will be divided with minute volume (mL/L), and the end-tidal concentration (Vol %) and MAC hours will also be presented.

Calculation of isoflurane dose by minute volume, during these periods, will be calculated as described in Section 8.2.1.

iii) Doses of other analgesics

No derivations needed.

8.2.3 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-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)

Three endpoints are defined:

i) Rescue propofol dose (mg/kg/h, i.e. total dose/sedation time)

Rescue propofol dose, defined as bolus propofol doses (total dose/sedation time), will be calculated by 12 hour periods, and by 24 hour periods, and for the total study treatment period using the same approach as for calculation of opiate dose (see Section 8.2.1)..

ii) Rescue ketamine/es-ketamine (mg/kg/h, i.e. total dose/sedation time) (es-ketamine converted to ketamine-equivalents)

The dose of es-ketamine (mg/kg) will be converted to ketamine-equivalent dose by multiplying es-ketamine dose by 2.

Rescue ketamine-equivalent dose, defined as bolus ketamine/es-ketamine doses (total dose/sedation time), will be calculated by 12 hour periods, and by 24 hour periods, and for the total study treatment period using the same approach as for calculation of opiate dose (see Section 8.2.1).

iii) Dose of α2-adrenergic agonists (µg/kg/h, i.e. total dose/sedation time)

Intravenous α2-adrenergic agonists infusions (µg/kg/h), including bolus infusions (µg/kg), will be calculated by 12 hour periods, and by 24 hour periods, and for the total study treatment period separately for Clonidine and Dexmedetomidine using the same approach as for calculation of opiate dose (see Section 8.2.1).

8.2.4 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

The number of doses of rescue sedation (as per the definition in 7.7.1.1) in the time period will be calculated as the number of recordings of rescue sedation.

8.3 Secondary safety endpoints

8.3.1 Time from end of study drug administration to extubation if study drug is terminated for extubation

Time (h) will be calculated from end of study drug administration to extubation, if study drug is terminated for extubation, for the patients with endo-tracheal tube. That is, this endpoint will only be evaluated in the subgroup of patients where the study drug is terminated for extubation, and only for patients with endo-tracheal tube, i.e. tracheostomy patients will be excluded from this analysis. The end of study drug administration 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.

In the analysis, extubation will be regarded as an event. If the wake-up for extubation is terminated and the patient remains intubated, the time to extubation will be censored at the time-point when the wake-up is terminated.

8.3.2 Proportion of observations with spontaneous breathing efforts during study treatment

For each patient, during the study treatment period from the first blinded COMFORT-B assessment to end of study treatment period, the proportion of observations made every 2-hour where the patient has either “Both controlled and spontaneous breaths” or “Spontaneous with support” on the eCRF variable “Ventilator mode” will be calculated.

8.3.3 Need for additional inotropic/vasopressor agent defined as change in VIS score during study treatment period compared to baseline

The following endpoint is defined:

ii) Change in VIS score during study treatment period compared to baseline

For each 24 hours period (prior and post 24 hours) individual median VIS score, based on observed data will be derived.
The following formula will be used (See McIntosh et al. Validation of the Vasoactive-Inotropic Score in Pediatric Sepsis **(6)**):

VIS = dopamine dose (µg/kg/min) +
dobutamine dose (µg/kg/min) +
100 x epinephrine dose (µg/kg/min) +
10 x milrinone dose (µg/kg/min) +
10,000 x vasopressin dose (U/kg/min) +
100 x norepinephrine dose (µg/kg/min)

For VIS scoring, no use of a listed drug should be entered as 0 in the formula for calculating VIS score.
Test results reported in other units will be converted from their original units to the standardised units specified in table above before analysis, using appropriate conversion factors. Data listings will include test results in both original and standardised units.

The change from baseline will be calculated for the follow-up time-points after baseline. It is defined as a continuous endpoint.

To calculate the dose of the different medications for the different periods the following will be applied:
Dose of medication for calculation of VIS score:

1. Accumulate ug/kg/min or U/kg/min per time period
2. Exclude dose taken during procedure outside the ICU per time period
3. Include entire ug/kg from boluses as taken within a time period
4. Exclude time during procedure outside the ICU from hours per time period
5. Divide by minutes per time period

Calculate hours per time periods:

- Baseline = [Randomization] – [2 hours prior to randomization]
- Total = [EoT/Start of Wake-up] – [2 hours after start of study drug]
- ≤24 h = [min(EoT/Start of Wake-up; 24h)] – [2 hours after start of study drug]
- >24 h = [EoT/Start of Wake-up] – [24 hours after start of study drug]

8.3.4 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

The following endpoint is defined:

i) Presence of withdrawal symptoms as assessed using the SOS-PD scale every 8 hours until the end of the 48-hour post study treatment monitoring or ICU discharge, whichever comes first

The subgroup of patients fulfilling the criteria for evaluation of withdrawal symptoms, i.e. patients exposed to more than a total of 96 hours sedation (including pre-study sedation period), will be included in the evaluation of this endpoint. Each of these patients will be classified as showing presence of withdrawal symptoms (Yes/No), applying the rule that values ≥ 4 on the SOS-PD scale equals presence of withdrawal symptoms.

8.3.5 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

The following endpoint is defined:

i) Presence of delirium as assessed using the SOS-PD scale every 8 hours until the end of the 48-hour post study treatment monitoring or ICU discharge, whichever comes first

The subgroup of patients fulfilling the criteria for evaluation of delirium, i.e. patients admitted to the ICU for at least 48 hours (including period prior to study enrolment), will be included in the evaluation of this endpoint. Each of these patients will be classified as showing presence of delirium (Yes/No), applying the rule that presence of delirium exists if

i. part 1b is positive (i.e. ticked 'Yes'), OR
ii. the score of step 2 is ≥ 4, OR
iii. symptoms of hallucination are observed

8.3.6 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)

The following endpoints are defined:

i) Presence of psychomotor dysfunction during study sedation period and/or in the 48 hours after discontinuation of study treatment

Each patient will be classified as showing presence of psychomotor dysfunction (Yes/No) based on manual review of any reported adverse events with LLT or PT corresponding to the definition in 7.10.3.1.3 or reporting of SOS-PD symptoms or adverse events listed in Table 9.

Table 9. Psychomotor dysfunction event SOS-PD items and AE PT/LLT

SOS-PD Symptoms	MedDRA 23.0 LLT	MedDRA 23.0 PT
Tremors	Tremor	Tremor
Motor disturbance	Psychomotor skills impaired	Psychomotor skills impaired
Muscle tension	Muscle tension	Muscle tightness
Attentiveness	Attentiveness decreased	Disturbance in attention
Purposeful acting	Choreiform movement	Chorea
Hallucinations	Hallucination	Hallucination

ii) Duration of study treatment period (h).

The duration of study treatment period is calculated from start of IMP to the time when the Investigator stops IMP administration or reduces it significantly (>25%), with the aim to extubate the patient.

iii) Cumulative study treatment dose for each study treatment (in mg/kg for midazolam and in MAC hours for isoflurane) during the study treatment period up to psychomotor event use definition for cumulative dose mg/kg for Midazolam and MAC hours for Isoflurane as defined in Section 8.2.1 but using the time up to the psychomotor event.

8.3.7 30 days/hospital mortality

The objective is to compare the 30 days/hospital mortality in isoflurane- vs midazolam-treated patients.

The following endpoints are defined:

i) Mortality up to the day 30 visit

Survival time will be counted from the day of randomisation. Death will be regarded as an event. If the patient is alive at day 30 the survival time will be censored at day 30. Deaths registered after day 30 will be disregarded and survival time will be censored at day 30.

ii) Hospital mortality up to the day 30 visit

Survival time will be counted from the day of randomisation. In the analysis, death at hospital only will be regarded as an event. If a patient dies outside the hospital, the survival time will be censored at the day the patient left the hospital the last time. If the patient is alive at day 30 the survival time will be censored at day 30. Deaths at hospital registered after day 30 will be disregarded and survival time will be censored at day 30. Patients without the event but withdrawing or completing the study prior to day 30 will be censored at the day of completion/withdrawal.

8.3.8 Ventilator-free days at 30 days from start of study treatment period

i) Definitions of ventilator days and ventilator-free days

Day is considered 24 hours, counted from midnight to midnight. Day when starting ventilator is a ventilator-day. Day when patient has ventilator at midnight and is extubated before 12 noon is a ventilator-free day. Day when patient has ventilator at midnight and extubated after 12 noon is a ventilator-day. Day when patient is intubated is a ventilator-day, irrespective of when intubation occurred. A ventilator-free day is defined as a period of 24 hours from midnight to midnight in which the patient at no time-point (exceptions above) has an endotracheal tube or tracheostomy and is connected to the ventilator.

Patient's total number of ventilator days and ventilator-free days from day of start of study treatment period up to day 30 will be derived based on these definitions.

When calculating the number of ventilator-free days up to day 30, deceased will render the days up to day 30 to be classified as ventilator days.

8.3.9 Time in intensive care unit/hospital at day 30 from start of study treatment period

i) Definitions of ICU-days and ICU-free days

Day is considered 24 hours, counted from midnight to midnight. Day when admitted is an ICU-day. Day when patient is at ICU at midnight and discharged before 12 noon is an ICU -free day. Day when patient is at ICU at midnight discharged after 12 noon is an ICU-day.

Patient's total number of ICU-days and ICU-free days from day of start of study treatment period up to day 30 will be derived based on these definitions.

When calculating the number of ICU-days up to day 30, deceased will render the days up to day 30 to be classified as ICU-days.

ii) Definitions of hospital days and hospital-free days

Day is considered 24 hours, counted from midnight to midnight. Day when admitted is a hospital day. Day when patient is at hospital at midnight and discharged before 12 noon is a hospital-free day. Day when patient is in hospital at midnight and discharged after 12 noon is a hospital-day.

Patient's total number of hospital days and hospital-free days from day of start of study treatment period up to day 30 will be derived based on these definitions.

When calculating the number of hospital days up to day 30, deceased will render the days up to day 30 to be classified as hospital days.

8.3.10 Days alive and not in the ICU at day 30 from start of study treatment period

i) Definitions of ICU-days and ICU-free days

Day is considered 24 hours, counted from midnight to midnight. Day when admitted is an ICU-day. Day when patient is at ICU at midnight and discharged before 12 noon is an ICU -free day. Day when patient is at ICU at midnight discharged after 12 noon is an ICU-day.

Patient's total number of ICU-days and ICU-free days from day of start of study treatment period up to day 30 will be derived based on these definitions.

When calculating the number of ICU-free days up to day 30, deceased will render the days up to day 30 to be classified as ICU-days.

8.3.11 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

See Section 7.10.3 for details on which data that will be presented. In Sections 7.10.3.1.1 and 7.10.3.1.2 further details are given on which adverse events are considered as common adverse events and which are considered as sedation-related adverse events.

8.3.12 Frequency and intensity of adverse events from start of study treatment to day 30

See Section 7.10.3 for details on which data that will be presented.

8.3.13 Changes in vital signs, blood gases, body temperature and urinary output from baseline to end of study treatment

i) Changes in vital signs from baseline

See Section 7.10.6 for details on which data that will be presented.

ii) Changes in blood gases from baseline

See Section 6.14 for details on which data that will be presented.

iii) Changes in body temperature from baseline

See Section 6.11 for details on which data that will be presented.

iv) Changes in urinary output from baseline

See Section 6.10 for details on which data that will be presented.

8.3.14 Changes in clinical chemistry and haematology parameters from baseline up to the 48-hour post-study treatment monitoring

i) Changes in clinical chemistry and haematology parameters from baseline

See Sections 6.13 and 7.10.4 for details on which data that will be presented.

8.4 Exploratory endpoints

8.4.1 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

The mean and median dose MAC value (unit-less) for isoflurane will be calculated as the patients end-tidal concentration divided by the MAC (%) for the relevant age-group.
Mean and median dose of midazolam required for achieving the target level of sedation will be calculated as stated in Section 8.2.1, over time, and by age group.

8.4.2 Number of study drug bolus doses given per 24 hours during midazolam and isoflurane sedation of mechanically ventilated patients

Number of study drug bolus doses will be calculated for the period prior and post 24 hours, and for the total study treatment period.

8.4.3 Ventilator parameters (ventilation mode, tidal volume, minute volume, fraction of inspired oxygen (FiO_2), end tidal carbon dioxide [$EtCO_2$], spontaneous breathing rate, total breathing

rate, positive end-expiratory pressure [PEEP], set inspiratory pressure [Pinsp], level of pressure support [PS] above PEEP)

See Section 6.12 for details on which data that will be presented.

8.4.4 Frequency and type of AnaConDa-S device deficiencies during isoflurane sedation

The following endpoint is defined:

i) Occurrence of AnaConDa-S device deficiencies

Each patient in the isoflurane group will be classified with respect to occurrence of AnaConDa-S device deficiencies (Yes/No).

Taking into consideration that one patient can report more than one AnaConDa-S device deficiency, the total number of occurrences per patient will be calculated.

ii) Type of AnaConDa-S device deficiencies

Each report of AnaConDa-S device deficiency will be connected to a device deficiency category (3 possible types: Malfunction (fracture or loosening), Use error (Implant not sterile or similar), Inadequate labelling (see identity)).

Taking into consideration that one patient can report more than one AnaConDa-S device deficiency, the total number of occurrences of AnaConDa-S device deficiencies per type will be calculated per patient.

8.5 Disposition of Patients

A screening failure is defined as a screened but not randomised patient.

8.6 Demographics and Baseline Characteristics

8.6.1 Age

Age will be computed as the integer part of the time in years between the date of birth and the day of randomisation, using the SAS function yrdif() with the basis parameter set to ‘age’. For patients for whom only the year of birth is collected, age will be computed as the difference between the year of the day of randomisation and the year of birth.

8.6.2 Body Mass Index

N/A.

8.7 Change from Baseline

Change from baseline will be computed as the difference between a post-baseline value and the corresponding baseline value.

8.8 Time to Event

The time to the defined event in days will be computed as the time in days from the defined start date to the date of event or censoring.

8.9 Compliance

Not applicable. Treatment in this study is continuous.

8.10 Duration

See definition of duration endpoints in the respective endpoint sections.

8.11 Exposure

Efficacy and exploratory endpoints, see Sections 7.9.2.1, 7.9.2.2, and 7.11.1, will reflect exposure to study drugs. The duration of the study treatment period will also be calculated as defined in Section 8.3.6.

8.12 End of study treatment

End of study treatment and end of sedation will be defined as the time when the Investigator stops IMP administration or reduces it significantly (>25%), with the aim to extubate the patient.

9 References

Information on references given as name of first author and year in this document, can be found in the CSP.

1. EMA Committee for proprietary medicinal products (CPMP) Points to consider on switching between superiority and non-inferiority. July 2000.
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10 Signoff

We have read this SAP for the SED002 study and confirm that, to the best of our knowledge, the statistical analyses to be performed in this study are accurately described.

Sedana Medical AB: Pontus Larsson, Senior Biometrics Manager

SIGNATURE AND DATE

LINK Medical SAP Author: Malin Schollin, Sr Biostatistician

SIGNATURE AND DATE

Verification

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Initiator

Intility eSign (Ie)
esign@intility.no

Signing parties

Malin Schollin (MS)
Malin.Schollin@linkmedical.eu
761636014

Signed 2023-03-08 15:48:07 CET (+0100)

Pontus Larsson (PL)
pontus.larsson@sedanamedical.com

Signed 2023-03-08 17:05:00 CET (+0100)

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