Protocol

SafeBoosC

Safeguarding the brain of our smallest children

– an investigator-initiated randomised, blinded, multinational, phase II feasibility clinical trial on near-infrared spectroscopy monitoring combined with defined treatment guidelines versus standard monitoring and treatment as usual in premature infants

Protocol number:	Protocol SafeBoosC
Trial phase	Phase II
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Study site:	Multicenter; international

Summary

Background

25,000 infants are born extremely preterm every year in Europe. This group of infants carries a high risk of death and subsequent cerebral impairment for the infant, especially in the first 72 hours of life. Mortality is about 20%, and about 25% of survivors live with either cerebral palsy or low intelligence quotient. Preventative measures are keys to reducing mortality and morbidity in this population. There is evidence that the cerebral oxygenation time spent out of range (time with hypoxia or hyperoxia) is associated with poor outcome in infants. Near-infrared spectroscopy (NIRS) has been used to monitor tissue oxygenation since the mid-1980s, and quantification of oxygenation in a percentage from 0 to 100% has been possible for 10 years. Still, there are no clinical trials and thus no solid evidence of the clinical utility of NIRS in preterm infants. Thus, research on the benefits and harms of cerebral monitoring using NIRS as a part of clinical management of premature infants is much needed.

Objectives

The primary objective of the SafeBoosC trial is to examine if it is possible to stabilise the cerebral oxygenation of extremely preterm infants during the first 72 hours of life through the application of cerebral NIRS oximetry and implementation of a set of defined clinical treatment guidelines. We hypothesise that by using the specified treatment guidelines to respond to cerebral monitoring readings outside the target range, we would reduce the burden of hypo- and hyperoxia and consequently reduce brain injury.

Trial design

This is an investigator-initiated randomised, blinded, multinational, phase II feasibility clinical trial involving preterm infants from 12 European countries.

Inclusion and exclusion criteria

The inclusion criteria are: neonates born more than 12 weeks preterm (gestational age up to 27 weeks and 6 days); decision to conduct full life support; parental informed consent; and cerebral NIRS oximeter placed within 3 hours after birth.

Sample size

A 50% reduction of the time with hypoxia or hyperoxia in the experimental group compared to the control group, a standard deviation on the time spent outside the therapeutic range of 83.2 %hours, with a type I error (alpha) of 5% and a type II error of 0.05 (power of 95%) requires inclusion of 75 preterm infants in the experimental group and 75 preterm infants in the control group.

Intervention

The premature infants will be randomised into one of two groups (experimental or control). Common is that both groups will have a cerebral oximeter monitoring device placed within three hours after birth. In the *experimental group*, the cerebral oxygenation reading is visible, and the

infant will be treated accordingly using a defined treatment guideline. In the *control group*, the cerebral oxygenation reading is NOT visible, and the infant will be treated as usual.

Trial duration

Monitoring by cerebral oximeter will be started as soon as possible and within 3 hours after birth and the intervention will last for 72 hours. Thereafter, each neonate will be followed up at term date (approximately three months after brith) and at 24 months after term date.

Outcome measures

The primary outcome is the burden of hypo- and hyperoxia in %hours during the first 72 hours after birth. The secondary outcomes are brain activity on an amplitude-integrated electroencephalogram (aEEG), blood biomarkers (brain fatty acid binding protein (BFABP), neuroketal, and S100β), serious adverse reactions (SARs), severe brain injury, and all cause mortality at term date (approximately three months after birth). The exploratory outcomes are burden of hypoxia, burden of hyperoxia, neonatal morbidities, brain injury score on magnetic resonance imaging (MRI), number of therapies implemented during the intervention, physiological variables (mean blood pressure (BP), pulse oximeter oxygen saturation (SpO2), and partial pressure of carbon dioxide (pCO2)), and psychomotor impairment according to neurodevelopmental scales at 24 months after term date.

Safety

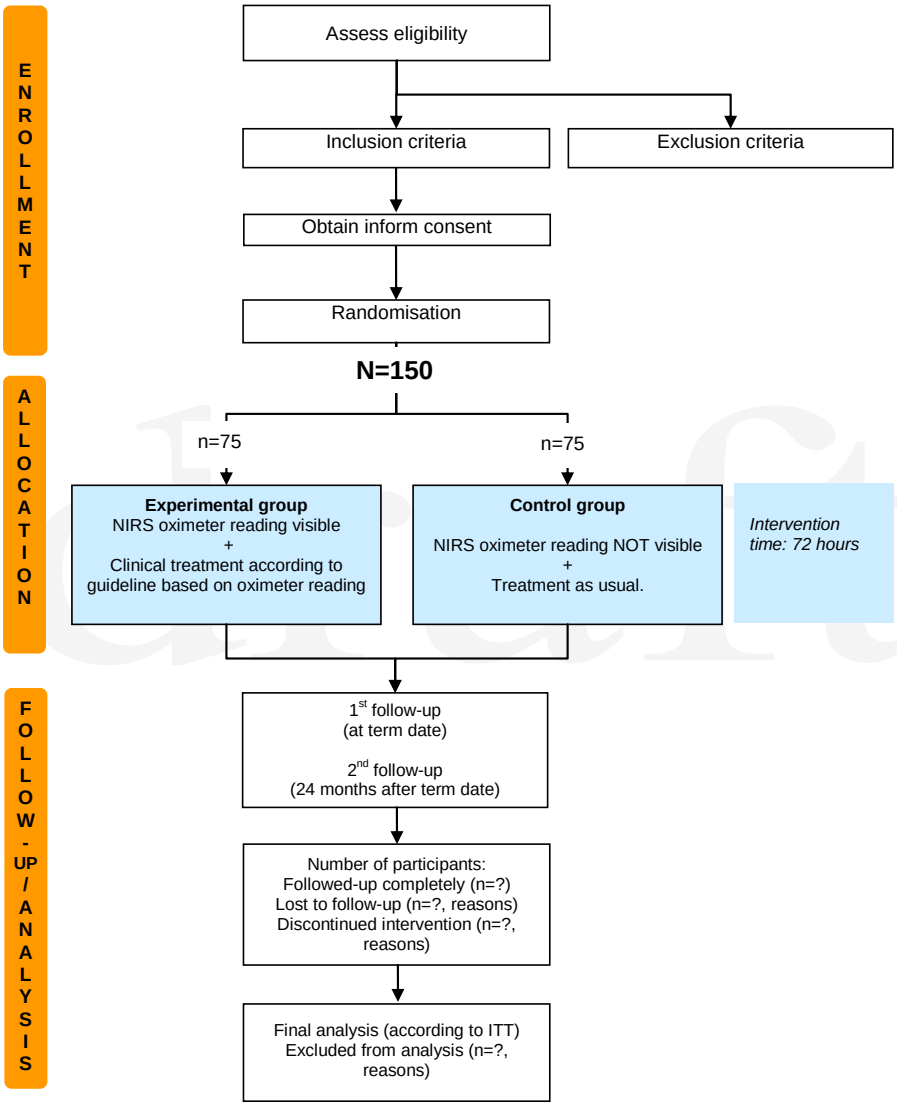
Predefined serious adverse reactions and suspected unexpected serious adverse reactions (SUSARs) will be recorded and reported to the appropriate competent authorities and ethics committees.

Ethical considerations

The approval from the relevant ethics committees will be sought. Parental informed consent will be obtained prior to randomisation. The trial will be conducted in compliance with the guidelines of the current Helsinki Declaration and the International Conference on Harmonisation good clinical practice guidelines (ICH GCP). Procedures will be established to prevent and/or minimise risk of complication for participants, such as complications related to the device and the treatment guideline includes only interventions that are commonly used during intensive care in this population.

SafeBoosC trial flow chart

SafeBoosC-Phase II trial



Source: adapted from the CONSORT Statement, 2010

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List of abbreviations

AE	Adverse events
aEEG	Amplitude-integrated electroencephalogram
AR	Adverse Reaction
ASQ	Ages & stages questionnaires
AUC	Area under the curve
BFABP	Brain fatty acid binding protein
BP	Blood pressure
BPD	Broncio pulmonary dysplasia
BSID-III	Baleysscale of infant development, third version
cPVL	Cystic Periventricular Leucomalacia
CRF	Case record form
CTU	Clinical Trial Unit
CUS	Cerebral Ultrasound
EAR	Expected adverse reaction
eCRF	electronic Case Record Form
EEG	Electroencephalogram
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immune assay
ESAR	Expected Serious Adverse Reaction
GA	Gestational age
GCP	Good clinical practice
HB	Haemoglobin
ICH	International Conference on Harmonization
ITT	Intention-to-treat
IVH	Intraventricular haemorrhage
LLT	Lowest level term
MAR	Missing at random
MedDRA	Medical Dictionary for Regulatory Activities – a medical coding system
MI	Multiple imputation
MMRM	Mixed model with repeated measures
MNAR	Missing not at random

MRI	Magnetic resonance imaging
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
NIRS	Near-infrared spectroscopy
pCO2	Partial pressure of carbon dioxide
PDA	Patent ductus arteriosus
PPV	Positive predictive value
PT	Preferred term
PV-IVH	Periventricular-intraventricular haemorrhage
PVL	Periventricular leucomalacia
RCT	Randomised clinical trial
ROP	Retinopathy of prematurity
rStO2	Regional cerebral tissue oxygen saturation
S100β	Acidic calcium binding protein found in the nervous system
SAR	Serious adverse reactions
SAE	Serious Adverse Event
SafeBoosC	Safeguarding the brain of our smallest children
SD	Standard deviation
SOC	System organ class
SpO2	Pulse oximeter oxygen saturation
SUSAR	Suspected unexpected serious adverse reaction
Term date	Is defined as gestational age of 40 weeks. The term date will be approximately three months after birth for this extremely preterm population.
UAR	Unexpected Adverse Reaction
3D-MRI	Three-dimensional magnetic resonance imaging

1. Introduction and background

1.1 The population and condition

Infants born more than 12 weeks preterm (extremely preterm) carry a high risk of death or long-term cerebral impairment. Currently, mortality is about 20%, and about 25% live with either cerebral palsy or low intelligence quotient (1). Every year 25,000 extremely preterm infants are born in Europe. Psychomotor impairment is the major cause of reduced quality of life and increased costs of medical care, rehabilitation and special education in this population. Because of the long life-expectancy of children, this is an important problem.

Unfortunately, prevention has not been successful; the rate of extremely preterm birth is stable or even increasing. Although there are risk factors, such as multiple pregnancy, and previous preterm birth, most extremely preterm births occur in otherwise normal and healthy women.

1.2 Pathophysiology

1.2.1 The transition from foetal to infant circulation

The transition from foetal to neonatal life is a particular problem in the extremely preterm infant. In foetal life, blood circulation includes only minimal perfusion of the lungs due to a large right-to-left shunt through the foramen ovale to the left side of the heart and through the arterial ductus from the pulmonary artery to the descending aorta. At birth, increased oxygenation of the body results in a systemic vasoconstriction and increasing arterial blood pressure. This may become a problem since the immature myocardium is intolerant to increased afterload. Also, as the lung function improves, the resistance of the pulmonary vessels drops to the effect of causing left-to-right shunting over the arterial duct, which increases the need for left ventricular output. The immature myocardium's ability to increase stroke volume is limited due to poor diastolic function. The increased afterload and possible left-to-right shunting may cause low systemic blood flow (2)

1.2.2 Cerebral autoregulation

Autoregulation is the ability to keep the organ blood flow constant despite fluctuations in perfusion pressure. It is accomplished by regulation of the arterial tone so that low perfusion pressure results in vasodilation and high perfusion results in vasoconstriction. On the systemic level, organs such as the brain, heart, and adrenals are vital and autoregulation maintains normal organ blood flow when systemic blood flow is low, while non-vital organs (e.g., skin and kidney) vasoconstricts to direct the circulating blood to the vital organs.

Cerebral autoregulation has limited capacity and is thought to be particularly fragile in the immature brain (3). Pressure passive flow is a state where the blood flow follows blood pressure. It is hypothesized that the fluctuations in flow that this entails is a potential cause of cerebral haemorrhages in premature infants. It is a problem that, at the current state, the identification of the threshold systemic blood pressure below which cerebral blood flow begins to fall is not possible (4).

1.2.3 The vulnerable brain

All the organs are immature when an infant is born more than 12 weeks before term. The immaturity and functional limitations of the lungs, heart, intestine, kidneys, liver, and endocrine system all contributes to the acute problems of extremely preterm birth. The brain is special, however, in the sense that brain damage results in death or in neuropsychological deficits such as cerebral palsy, cognitive deficit, attention deficit disorder, and major psychiatric disorder. These damages result in long-term consequences for children after extremely preterm birth.

The most easily identifiable type of brain damage in extremely preterm birth is periventricular-intraventricular brain haemorrhage (PV-IVH). Its severity varies: in the mildest form, the haemorrhage is limited to the subependymal germinal matrix – possibly with a small intraventricular clot. The most severe form is a large periventricular haemorrhagic infarction primarily located in the central white matter in one or both hemispheres. This predicts a high probability of death or cerebral palsy and may result in hydrocephalus (5). Hydrocephalus needing surgical treatment carries a poor neurodevelopmental prognosis. Periventricular leucomalacia (PVL) is a non-haemorrhagic white matter damage. In the mildest form, the condition is non-cystic and predicts poor psychomotor development. The most severe form of PVL is when the condition becomes cystic (cPVL) 2-5 weeks after the damage is induced and is a strong predictor of cerebral palsy (6).

1.2.4 Mechanisms of brain damage in preterm infants

The mechanisms of the brain damage in preterm infants are complex. Some of the mechanisms are evoked before birth or even before the start of delivery such as a foetal inflammatory response induced by infection ascending to the foetal membranes. Also, late effects such as insufficient nutrition and poor growth during the first months of life may play a role.

The days after birth, however, are likely to be of particular importance. This is the period of change from a state of low oxygen pressure ('Mount Everest in-utero') to a state of 'normoxaemia'. Moreover the circulatory adaption to birth is as described problematic in the preterm infant. Thus fluctuations in systemic blood flow are common during the first days of life.

The following postnatal factors have been shown or are thought to be associated with brain injury: respiratory distress syndrome (7), hypocapnia due to inadvertent hyperventilation (8), low blood pressure (9), perturbations in arterial and venous pressure (10), and also low cerebral blood flow (11). Clinical and experimental evidence is suggesting that hyperoxygenation is dangerous due to lack of a developed antioxidant defence system (12).

The most likely common mechanism for these associations, is disturbance of cerebral circulation and impairment of cerebral autoregulation which is probably an important contributor to this.

1.3 Current clinical management

Current standard of care of the extremely premature infants during their first 72 hours involves a number of different parallel interventions:

- *Respiratory support:* continuous positive airway pressure or mechanical ventilation is almost universal and surfactant is usually administered within the first 24 hours.
- *Haemodynamic support:* Before diagnosing a patent ductus arteriosus (PDA), prophylactic use of indomethacin can be used. Either indomethacin or ibuprofen can be used for the closure of a PDA. Fluid boluses, inotropics, or vasopressors are used to treat hypotension, although the level or targeted blood pressure is controversial (13).
- *Fluid balance/nutrition:* Close observation of hourly and daily estimations of in- and output, scheduled fluid administration, and blood sugar monitoring. Most infants initially receive full parental nutrition and will slowly be introduced to breast milk.
- *Monitoring*: Invasive/non-invasive blood pressure monitoring, continuous pulse oximetry, transcutaneous partial pressure of carbon dioxide (pCO_2), and electrocardiographic monitoring with frequent measurements of arterial blood gases, electrolytes and temperature.

Treatment of the extremely premature infants has certainly improved over the last three decades despite great areas of unknown territory. However, the treatment of hypotension, the optimal arterial oxygen content, the optimal pCO_2 level, and many other possible interventions are dealt with on more or less loose grounds. And while still more comprehensive monitoring is implemented in the intensive care of premature infants, an end-organ monitoring with sufficient high time resolution to guide evidence-based treatment interventions is lacking. Near infrared spectroscopy has the potential to become that monitor of the brain.

1.4 Assessment of brain injury and neurodevelpmental deficit

1.4.1 Ultrasound

Cerebral ultrasound (CUS) is a standard tool for diagnosing conditions such as haemorrhage and hypoxic-ischaemic lesions. Furthermore, signs of brain atrophy at term equivalent age are associated with neurodevelopmental outcome in preterm infants(14). The pooled probability for a normal neuromotor outcome of a normal ultrasound was 94% (95% confidence interval (CI) 92% to 86%) and 82% (95% CI 79% to 85%) for a normal cognitive outcome. Additionally, for IVH Grade I-II, the probability of abnormal outcome was 9%, and for IVH Grade III 26% (95% CI 13% to 45%) (15). Parenchymal haemorrhagic infarction predicted an abnormal neurodevelopmental outcome with an increased risk positive predictive value (PPV) of 47% (95% CI 31% to 64%) (16). Cystic PVL was predictive of cerebral palsy with a PPV of 77% (95% CI 59% to 89%) (16). Cerebellar haemorrhage predicted abnormal outcome with a PPV of 71% (95% CI 42% to 90%) (17)

1.4.2 aEEG

Amplitude-integrated electroencephalography (aEEG) is a technique were the EEG signal from bilaterally placed electrodes is recorded and amplified. The signal is then passed through an asymmetrical band pass filter, which minimises noise and artefacts before it is displayed bedside. The aEEG is widely used in term infants with asphyxia. It has shown good predictive

value (18) and is suitable for detection of seizure activity (19), cerebral haemorrhages (20) as well as the effects of variation in pCO2 and blood sugar in the first day of life in extremely preterm infants (21).

1.4.3 MRI

Three-dimensional magnetic resonance imaging (3D-MRI) of the newborn brain is helpful in identifying extra information about brain injury and maturation, which cannot always be visualised on cranial ultrasound. Furthermore, when using advanced post-processing techniques, it is possible to measure the volume of the total brain volume and of various brain structures, which might be helpful in identifying non-visual brain injury or differences. Differences in cortical folding and other brain maturation measurements can be precisely measured in a quantitative way to assess cerebral growth and maturation as well as in relation to sulci formation that has been described to be related to cognitive outcome. Thus, 3D-MRI provides us valuable information about brain growth/development and neurodevelopmental outcome.

1.4.4 Biomarkers

Earlier research demonstrates that several neuro-biomarkers are released into body fluids in response to perinatal asphyxia or hypoxic-ischaemic brain injury. Some of them have also been correlated to severity of hypoxic ischaemia and long-term neurological outcome in neonates within the first day of life. Moreover, the course of these biological markers could correlate with the process of hypoxic ischaemic injury and thus have a diagnostic value for brain injury in preterm neonates with hypoxic ischaemic injury. Serum can be analysed for the rise in the following chemical biomarkers: brain fatty acid binding protein (BFABP), neuroketal, and S100β.

- *Brain fatty acid binding protein (BFABP)* is a 15 kD protein, which is specific for brain tissue. It is released from asterocytes after mechanical damage, ischaemia and oxidative brain damage. BFABP is determined by means of enzyme linked immune assay (ELISA) with BFABP specific monoclonal capture antibodies and polyclonal detection antibody in plasma/serum as well as urine. Due to its low concentrations, 100 µl is needed for a BFABP ELISA.

- *Neuroketal* is formed in the brain via the neuroprostane pathway during oxidation of docohexaenoic acid. Neuroketals rapidly adduct to lysine, and these crosslinks induce neurodegenerative disease. Neuroketal thus contributes to the injurious effects of oxidative pathologies in the brain. Determination of neuroketals is performed by competitive enzyme immunoassay in 100 µl of plasma, serum or urine.

- *S100β* is a putative marker for brain damage. S100 β is an acidic calcium-binding protein found in the nervous system of vertebrates. It is a dimer of α and β subunits. As such, S100β, which consists of two β subunits, is present in high concentration in glial and Schwann cells. It leaks from the cerebrospinal fluid into blood after cerebral damage and is a sensitive indicator of brain injury and increased permeability of the blood brain

barrier. The determination of S-100β concentrations in serum will be performed with an ELISA. It can be detected in 50 µl samples of serum, heparin plasma or urine..

1.4.5 Neurological developmental assessment tool

Neurodevelopmental outcome should be evaluated by standardised tests which are psychometric measures designed to inventory an infant's skills against a 'normal' population within a specific assessment. The standardisation is designed to reduce measurement error by precluding subjective interpretation of the child's responses. Bayley Scales of Infant Development (BSID) are such a standardised tests (22). BSID was revised and re-standardized in 1993 to form the BSID-II and in 2005 to form the BSD-III and is widely used worldwide as outcome measure in clinical and research practice. BSID-III provides a multi-domain assessment of children aged 1 to 42 months. It reveals five major developmental domains: cognitive, language, motor, adaptive behaviour, and social-emotional. The scale also includes a parent report form, the Adaptive Behaviour Questionnaires that provides scores based on parents' perceptions of the child's level of function. In this trial, the BSID-III will be performed at 24 months after term date, using the cognitive, language, and motor domains.

The Ages & Stages Questionnaire (ASQ) is a parental survey that comprises questionnaires for children age 4 months to 5 years. It consists of 6 questions in each of 5 developmental domains covering motor as well as mental development. The agreement between ASQ and with concurrent assessment by BSID-III is good and ASQ can be a substitute for the BSID-III in cases or at centres where the BSID-III is not possible (23).

1.5 Cerebral oximetry monitoring

Near-infrared spectroscopy (NIRS) is a non-invasive technology that has been utilised to assess the adequacy of peripheral and cerebral oxygenation in the preterm infant (24). Near-infrared light penetrates deep into the tissue, and through spectroscopy, it is possible to monitor tissue oxygenation. NIRS uses the relative transparency of human tissue to light in the near-infrared region of the spectrum. The oxygen-dependent absorption of light by haemoglobin enables the calculation of relative changes in the oxygenated and deoxygenated haemoglobin (25). The NIRS has been used in newborns since 1985 (26) and it is particularly suitable for the neonatal population due to their thin scalp and skull. Newer generations of oximeters provide an absolute value of tissue oxygenation ($rStO_2$). This is most often done by spatially resolved spectroscopy. The assumption behind is that light propagates in a diffusional manner in a highly scattering media such as human tissue, and that the light attenuation by scattering is constant with light distances over 3 centimetres. The $rStO_2$ can then be expressed as (k x O2HB)/(k x O2HB + k x HHB), where k is the scattering component which cancels out (27). Examples of some of the commercialised NIRS devices are INVOS® System, NIRO Series, and Casmed Foresight.

NIRS is based on the same principles as the widely used pulse oximetry, but where pulse oximetry uses the pulsating signal and thereby selectively measures arterial blood, NIRS measures the light attenuation of the tissue as a whole. This means that venous blood contributes more to the attenuation than arterial blood simply because venous blood has a

greater volume. The ratio of venous:arterial contribution is generally considered to be 75:25, although this has been found to differ between and within infants (28). It is thus not surprising that cerebral tissue oxygenation has shown a fair correlation with the saturation in cerebral venous blood drawn from the jugular bulb (29). The Bland-Altman limit of agreement is ± 15-20% (30,31). It has to be remembered that $rStO_2$ is volume weighted, whereas jugular bulb saturation is flow weighted. Good agreement cannot be expected. It could be that during conditions where the microcirculation is compromised such as sepsis, $rStO_2$ may give a better picture of the cerebral oxygen balance than jugular bulb saturation (32).

Different NIRS devices differ in absolute values and in dynamic ranges (33).There is no 'gold-standard' for tissue oximetry. Therefore, comparison of devices can be done in a standardised setup in the forearm of healthy adults. Reproducibility is assessed by re-setting of the sensors during steady state and a test of dynamic range is done by arm exercise and subsequent arterial occlusion by a cuff. Four devices have been compared in a preliminary study:

- NIRO 200 NX;
- NIRO 300;
- INVOS 5100c; and
- OxyPrem (a prototype).

The reproducibility of the NIRO 200 NX, NIRO 300, and the INVOS 5100c was similar (within-subject standard deviation 4.35%, 4.10% and 5.46%, respectively) (unpublished data). In addition, these devices showed similar absolute oxygenation ($rStO_2$) values (63% to 70%) and similar dynamic range. The OxyPrem showed significantly better reproducibility, but a somewhat lower absolute oxygenation ($rStO_2$) value (60%) and dynamic range only two-thirds of the other three instruments.

Based on these results it has been decided that both NIRO 200 NX and INVOS 5100c are eligible for use in the SafeBoosC trial. Other candidate NIRS device for use in the SafeBoosC trial will be tested by the same procedure and should be within five percentage points in mean values and dynamic range in comparison with these instruments, and have a reproducibility of 6% or better.

Each participating centre is required to provide their own NIRS device(s) for the SafeBoosC trial. If (all of) the provided NIRS device(s) have a CE-mark that indicates use for this SafeBoosC trial population, the trial does not need approval by the competent authority (medicines agency) of the participating country. However, if one of the NIRS devices, used in a given centre, does not have a CE-mark that indicates use for this SafeBoosC trial population, the trial must be approved by the relevant competent authority in the participating country.

1.6 Regional oxygenation saturation in preterm infants

A study conducted by McNeil et al, 2011, characterises the baseline cerebral $rStO_2$ in 12 stable preterm infants (GA 29-34 weeks) during the first weeks of life to be between 66-83% (34). In the study conducted by Zhou et al., 2009, where they enrolled a total of 223 full-term newborns, the 'normal' cerebral $rStO_2$ was determined to be between 62 ± 2% and cerebral hypoxia was

defined as $rStO_2$ less than 58% (35). Derived from data, collected over time, from about 390 preterm babies (born at GA<32wks) during the first 3 days of life, van Bel et al. (unpublished data) concluded the $rStO_2$ baseline target range in this population to be 55-85% (±2SD) with the mean value of 71%.

1.7 Clinical data

There is a variety of observational studies that document, that cerebral oximetry in preterm infants gives meaningful physiological data. Wolf and Greisen systematically reviewed 36 studies in neonates that all contribute to an understanding of oxygen delivery-consumption balance in this population (25). Based on their study, however, we are able to conclude that evidence of the clinical benefits and harms of cerebral oximetry in preterm infants exist.

We systematically searched in four medical literature databases on March 22, 2011: Cochrane Library, MEDLINE, EMBASE, and Science Citation Index Expanded. Using the keywords 'near-infrared spectroscopy', 'NIRS', 'oximetry', 'preterm', 'infant', and 'newborn', the searches yielded a total 267 hits. The search found no published randomised clinical trials (RCTs) focusing on the effect of cerebral monitoring in preterm infants in combination of clinical treatment guidelines based on the $rStO_2$ produced by the NIRS oximeter.

A search on ClinicalTrials.gov (www.ClinicialTrials.gov) on March 30, 2011 using the keyword 'NIRS' and age limited from birth to 17 years identifies 36 studies. Of the 36 studies, only three studies include premature infants:

- NCT01255189 – an ongoing study that will explore the effect of positional changes on cerebral oxygenation meassurements in preterm infants. It will not treat the infants according to oximeter readings.
- NCT01251068 - an ongoing study that will explore the effect of blood transfusion on cerebral and muscle oxygenation in anaemic preterm infants. The NIRS reading will not be used in decision making, but is the primary outcome.
- NCT01258517 - an ongoing study to determine the optimal and the most safe surfactant administration technique with regard to cerebral oxygenation in low birth weight infants.

Hence, none of these studies investigate the possible effects of oximeter cerebral monitoring.

The evidence of cerebral oximetry in adults is also limited. To date, there is only one published systematic review of evidence of clinical utility of cerebral oximetry in adults during coronary surgery concluding that, with data from 47 trials including more than 5,000 participants, the methodological quality of the trials was low and therefore clinical benefits and harms are uncertain (36).

1.8 Trial rationale

There is accumulating evidence that hypoxia and hyperoxia is associated with the risk of brain injury and death in prematurely born infants. Thus, the monitoring of cerebral oxygen saturation levels in the first hours after birth and subsequent treatment according to pre-specified

guidelines has a great potential for prevention. Despite this, there are no randomised clinical trials on the benefit and harms of NIRS in preterm infants. Yet, the technology is increasingly implemented in clinical care. To obtain evidence-based knowledge on the benefits and harms of cerebral monitoring using NIRS as a part of clinical management of premature infants, a large-scale RCT is needed.

2. Trial objective and hypothesis

The objectives of this phase II trial are to:

- examine whether it is possible to reduce the burden of cerebral hypoxia and hyperoxia through the application of NIRS and the implementation of a set of defined clinical treatment guidelines.
- evaluate the feasibility of a large-scale randomised clinical trial of complex interventions in European neonatal units;
- explore the relationships between cerebral hypo- and hyperoxia, clinical interventions, and markers of brain injury in extremely preterm infants.

We hypothesise that by using a specified treatment guideline to cerebral monitoring readings outside the target range of 55-85% we would reduce the burden of cerebral hypo- and hyperoxia in order to reduce brain injury in preterm infants.

3. Trial design

This is an investigator-initiated randomised, blinded, multinational, phase II feasibility clinical trial that will enrol 150 preterm infants from European countries (Figure 1).

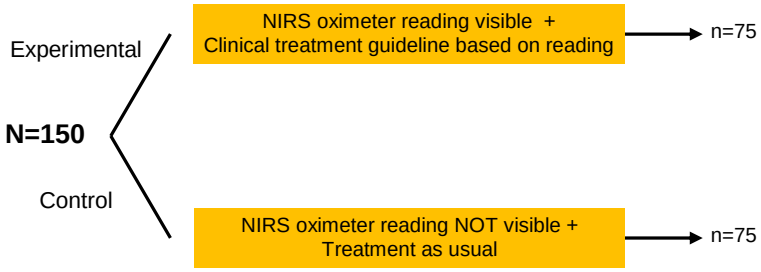

Figure 1: Trial design

3.1 Randomisation

Participants will be randomised into either the experimental group or the control group. The allocation sequence will be computer-generated with a varying block size, and is kept concealed for all investigators. The ratio of allocation is 1:1. The randomisation will be stratified by the variable gestational age (low gestational age (<26 weeks) vs. high gestational age (≥26 weeks)). Randomisation will be centralised at the Copenhagen Trial Unit and web-based.

Singleton infants will be randomised individually. Multiple birth infants will be randomised as a 'pair' or a 'group', i.e., all siblings will be allocated to the same treatment group. Randomisation of multiple birth infants will count as 'one randomisation' in the total sample size of 150 infants. In centres, where only one or two cerebral monitoring devices are available, it may not be possible to include infants from multiple births.

3.2 Trial interventions

The premature infants will be randomised into one of the two groups. Common is that both the experimental and the control group will have a cerebral NIRS oximeter monitoring device placed within three hours after birth.

- *Experimental:* The cerebral oxygenation reading is visible, and the infant will be treated according to a defined treatment guideline (see appendix A).
- *Control:* The cerebral oxygenation reading is NOT visible, and the infant will be treated as usual.

3.3 Duration

Cerebral monitoring will start before three hours of age and the intervention will last for 72 hours, as these are the most critical. Each neonate will be followed up at the term date and 24 months after the term date (Figure 2).

Trial timeline

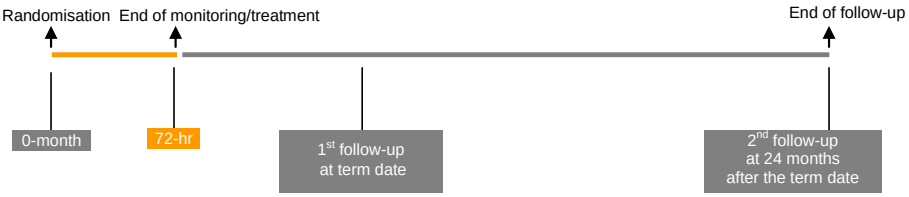

Figure 2: SafeBoosC Trial timeline

3.4 Blinding

Due to the nature of the intervention, the intervention cannot be blinded for the clinical staff and the parents. However, blinding will be used in all other aspects of the trial. Firstly, the allocation

sequence will be concealed for the investigators and all other trial personnel. Secondly, outcomes will be assessed by an outcome assessment committee, blinded to the allocation of the participants. Thirdly, the statistical data analyses will performed with the two intervention groups concealed as, e.g., X and Y. Finally, two conclusions of the results of the trial will be drawn – one presuming X is the experimental group and Y the control, and one assuming the opposite. Hereafter, the blinding will be broken.

4. Participants

4.1 Inclusion criteria

Neonates meeting the following criteria will be included:

- Neonates born more than 12 weeks preterm (gestational age up to 27 weeks and 6 days).
- Decision to conduct full life support.
- Cerebral NIRS oximeter placed within 3 hours after birth.
- Obtained parental signed written informed consent.

4.2 Exclusion criteria

Neonates meeting the following criteria will be excluded:

- A clinical decision not to provide life support.
- Not possible the place the cerebral NIRS oximeter within 3 hours after birth.
- Lack of parental signed written informed consent.

4.3 Participant discontinuation and withdrawal

The participant's parents are free to withdraw the participant from the investigation or from the SafeBoosC trial entirely at any time, and this will not have any consequences for the participant's further treatment. When possible, the parents will be asked if they will allow the participants to remain in the trial for the remaining parts, e.g., the follow-up assessments, and inclusion of their already collected data in a database, a registry, and/or a publication.

4.4 Recruitment feasibility

Neonates are expected to be included from 12 NICUs in 12 European countries. The units have estimated the likely rate of recruitment between 10 and 40 infants per year. The total is about 145-310 infants per year. The trial should therefore have good chance to be able to recruit patients within a year.

5. Interventions

5.1 Common to both groups

Both groups will have a cerebral NIRS oximeter placed within three hours after birth. Cerebral oximeters meeting the following validity and reliability criteria will be allowed in the trial: precision better than 6%, accuracy as well as dynamic range within 5% points, sensor type appropriate for the oximeters meeting the aforementioned criteria. In centres that have more than one NIRS device it shall be decide which device(s) that will used before the randomisation.

5.2 Experimental group

In the experimental group, the reading of the cerebral oxygenation will be visible. A clinical treatment guideline recommending adjustments of respiratory and cardiovascular support will be followed to keep cerebral oxygenation within defined target range. The treatment guideline is described in Appendix A and will be used for all participants allocated to the experimental group regardless of countries where the treatment is given.

5.3 Control group

In the control group, the reading of the cerebral oxygenation will NOT be visible. The infants will be given the best standard treatment – 'treatment us usual'.

A box will be used to cover the cerebral NIRS oximeter used in the control group to ensure that the reading of the oximeter is not visible to the physician, . This box will be locked with the key only accessible to the authorised personnel who is not involved in the trial and who will also check the correct recording of these data. These data will not be communicated to the clinical personnel or other parties.

5.4 Concomitant medication/treatment

There will be no per-protocol concomitant medication or treatment.

6. Outcome measures

The outcome measures are listed below. Please see more details in table 1 and in appendices B-E.

6.1 Primary

The primary outcome is:

- Burden of hypo- and hyperoxia in %hours during the first 72 hours after birth.

6.2 Secondary

The secondary outcomes are:

- Brain activities on aEEG as assessed by the interburst interval.
- Blood biomarkers (brain fatty acid binding protein, neuroketal, and S100β).
- Serious adverse reactions.
- Severe brain injury by cerebral ultrasound.
- All-cause mortality.

6.3 Exploratory

The exploratory outcomes are:

- Burden of hypoxia.
- Burden of hyperoxia.
- Neonatal morbidities (necrotizing enterocolitis (NEC) stage 2-3, bronchopulmonary dysplasia (BPD) defined as oxygen requirement at 36 weeks, and retinopathy of prematurity (ROP) stage 3+ and above).
- Mild brain injury by cerebral ultrasound.
- aEEG band power and EEG patterns.
- Brain injury score on MRI.
- Number of therapies implemented during the intervention (mechanical ventilation, volume substitution, blood transfusion, inotrope, vasopressor, ductal closure).
- Physiological parameters (mean BP, SpO_2, and pCO_2)
- BSID-III (cognitive score, verbal score, and motor score).
- ASQ (score between 0-60 from 5 domains).

6.4 Outcome assessment tools

The outcome assessment tools used in the trials are the aEEG, CUS, biomarkers, MRI, and the psychomotor scales (BSID-III and ASQ). Table 1 lists the outcome measures to be assessed and the time points of the assessments.

6.5 Outcome assessment committee

The reading and interpretation of the outcomes will be centralised and the assessor will be blinded of the participants' intervention group (see table 1).

7. Data collection and trial assessment schedule

7.1 Case record form

Trial data will be collected using electronic case record form (eCRF). The CRFs will be created in collaboration between the Data Manager at the Copenhagen Trial Unit and the sponsor using an electroninc data capture system (see section 11.1).

7.2 Trial assessment schedule

Trial data will be collected according to the time points presented in the trial schedule in Table 2 and entered in the eCRF.

Table 1: Assessment tools and outcome measures

Tools	Outcomes	Time points*	Centralised reading
Cerebral NIRS oximeter	• Burden of hypoxia • Burden of hyperoxia	• During the 72 hours intervention period	Department of Neonatology, Rigshospitalet, Denmark
aEEG/EEG (Appendix C)	• Interburst interval (IBI) • Power in delta band • Power in theta band • Power in alpha band • Power in beta band	• 64 ± 8 hours after randomisation	Uppsala University, Sweden
Biomarkers (Appendix B)	• BFBP • Neuroketal • S100β	• At birth (cord blood) • 6 hours after randomisation • 64 hours ± 8 after randomisation	Haemoscan B.V., The Netherlands
Investigator's assessment and medical records	• Serious adverse reactions	• During the 72 hour intervention period	Not centralised
CUS (Appendix E)	• Parenchymal haemorrhagic infarction • IVH • Cerebellar haemorrhage • cPVL • Cerebral atrophy • Post-haemorrhagic hydrocephalus	During the intervention and follow-up period, according to these assessment points: • At 1-4 days after birth • At 7 days after birth • At term date	Laboratory for Neuroimmunology and Developmental Origins of Disease, The Netherlands
Investigator's assessment and medical records	• All-cause mortality	• Term date • 24 months after term date	Not centralised
Investigator's assessment and medical records	Neonatal morbidities: • NEC stage 2-3 • ROP stage 3+ and above	• Term date	Not centralised
Investigator's assessment and medical records	Neonatal morbidities: • Oxygen requirement	• At 36 weeks	Not centralised
MRI (Appendix D)	• Volumetric • Cortical folding with formation of the sulci • Diffusion tensor imaging	• At term date ± 2 weeks (approximately three months after brith)	Laboratory for Neuroimmunology and Developmental Origins of Disease The Netherlands
Investigator's assessment and medical records	Therapies implemented: • Mechanical ventilation • Volume substitution • Blood transfusion • Inotrope • Vasopressor • Ductal closure	• During the 72 hour intervention period	Not centralised
CRF	Physiological parameters • Mean BP • Mean SpO_2 • Mean pCO_2	• During the 72 hour intervention period	Not centralised
BSID-III	• Cognitive score • Verbal score • Motor score	• 24 months after term date	Not centralised
ASQ	• Communication • Gross motor • Fine motor • Problem solving • Personal-social	• 24 months after term date	Not centralised

* Each time point for assessment is indicated with a margin, e.g., the assessment at 64 hours after birth is indicated with a margin of ± 8 hours. This means that every effort should be made to conduct the assessment within this time slot. However, if it is not possible to comply with the time margin, the assessment should still be conducted, and the time should be noted in the CRF as a deviation from the protocol.

Table 2: Trial schedule

Visit Number	0	1		2				3	4	5
Visit description	Screening	Randomisation		Intervention period (Cerebral monitoring ± visibility and treatment according to guideline or 'us usual')				Follow-up		
Visit code	V0	V1a	V1b	V2a	V2b	V2c	V2d	V3	V4	V5
Time period	0-hour	0-hour after birth	3 hours after birth	3 hours after birth	6 hours after birth	24 hours after birth	64 hours after birth	7 days after birth	Term date	24 months after term date
Informed consent[1]										
In- and exclusion criteria										
Maternal history										
Maternal antenatal concomitant medication										
Infant concomitant medication										
Placement of cerebral NIRS oximeter[2]										
Cord blood (biomarker)										
Blood/urine (biomarker)[3]										
Serious adverse reaction										
aEEG[4]										
CUS[5]										
MRI										
BSID-III										
ASQ										
All-cause mortality										

Key:
1= can be obtained before birth
2= between 0-3 hours after birth
3= between 3-12 hours after birth
4= +/- 8 hours after birth
5= 1-4 days after birth, 7 days after birth, and at term date or before discharge

8. Assessment of safety

8.1 Adverse events and reactions

8.1.1 Definitions

Adverse events (AE): any undesirable event occurring to a participant during a clinical trial, whether or not considered related to the trial intervention.

Serious adverse event (SAE): any adverse event that results in death; is life-threatening, requires prolongation of existing hospitalisation, result in persistent or significant disability or incapacity, or requires intervention to prevent permanent impairment or damage.

Adverse reactions (AR): all untoward and unintended responses related to the intervention (cerebral oximeter and/or the subsequent treatment).

Expected adverse reactions (EAR): adverse reactions, we expect to be related to the intervention (cerebral NIRS oximeter and/or the subsequent treatment) are:

- Local skin reactions (irritating or allergic rashes, burns, etc.).
- Reactions related to the manipulation of the patient during positing and re-positing of the cerebral NIRS oximeter sensors.
- Accidental displacements of the endotracheal tube or extubation.
- Accidental displacement of venous or arterial catheters, and severe hypoxia or bradycardia otherwise unexplained.
- Other expected adverse reactions encompass reactions to any interventions directed at improving respiratory status, cardiovascular status, oxygen transport, and blood glucose level (see appendix A).

Serious adverse reactions (SAR): any adverse reaction that results in death, is life-threatening, requires prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or requires intervention to prevent permanent impairment or damage.

Expected serious adverse reactions (ESAR): any of the expected adverse reactions (listed above) that results in death, is life-threatening, requires prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or requires intervention to prevent permanent impairment or damage.

Suspected unexpected serious adverse reaction (SUSAR): an adverse reaction which is both serious and unexpected, i.e., not listed above (in expected adverse reactions).

Table 3: Classification of causality of adverse reactions

Certain	Event or laboratory test abnormality, with plausible time relationship to the SafeBoosC trial intervention; cannot be explained by disease or other treatments; response to withdrawal plausible (pharmacologically, pathologically); event definitive pharmacologically or phenomenologically (i.e., an objective and specific medical disorder or a recognised pharmacological phenomenon); or rechallenge satisfactory, if necessary.
Probable / likely	Event or laboratory test abnormality, with reasonable time relationship to the SafeBoosC trial intervention; unlikely to be attributed to disease or other treatment; response to withdrawal clinically reasonable; rechallenge not required.
Possible	Event or laboratory test abnormality, with reasonable time relationship to the SafeBoosC trial intervention; could also be explained by disease or other treatments; information on drug withdrawal may be lacking or unclear.
Unlikely	Event or laboratory test abnormality, with a time to the SafeBoosC trial intervention that makes a relationship improbable (but not impossible); disease or other drugs provide plausible explanations.
Conditional / unclassified	Event or laboratory test abnormality; more data for proper assessment needed; or additional data under examination.
Unassessable / unclassifiable	Report suggesting an adverse reaction; cannot be judged because information is insufficient or contradictory; data cannot be supplemented or verified.

8.1.2 Registration and reporting of adverse events and reactions

An overview of the classification of reactions can be in table 3. An overview of the recording and reporting can be found in table 4.

The preterm patient population is a very seriously ill group. Most adverse events may be of a serious nature with or without NIRS intervention. The mortality is high and mortality will thus be analysed as a secondary outcome measure. Both intervention groups are expected to have a very high proportion of serious adverse events. It is therefore not meaningful to register and report all of these. However, the neonatal morbidities, that are believed to be the most important predictors for late development and health, are recorded as an outcome measure (necrotising enterocolitis stage 2-3, oxygen requirement, and retinopathy of prematurity stage 3+ and above, as well as severe intraventricular haemorrhage and periventricular leucomalacia).

Adverse reactions with a ‘certain’ or ‘probable/likely’ relation to the intervention will be recorded (see table 3). Adverse reactions with a ‘possible’, ‘unlikely’, ‘conditional/unclassified’ or ‘unassessable/unclassifiable’ relation will not be recorded.

All serious adverse reactions (related to the cerebral oximeter and/or the subsequent treatment) will be registered and analysed as a secondary outcome measure. Mortality and other serious adverse reactions will therefore not be reported during the trial to the regulatory authorities.

The trial site investigators will report all SARs and SUSARs to the sponsor through the eCRF within 24 hours of knowledge. The sponsor will review all SUSARs. If the judgement of sponsor differs from the investigator, both assessments of the SUSAR shall be included in the SUSAR

report to the authorities. The sponsor will immediately inform all investigators of any SUSAR as soon as s/he is made aware of the event. The sponsor will submit an expedited report of all SUSARs to the drug agencies via EudraVigilance within 7 days for fatal and life-threatening events and 15 days for all others. The sponsor will submit an annual report safety report to the authorities according to the guideline stated in the European Directive 2001/20/EC.

Figure 3: Classification of adverse events and reactions

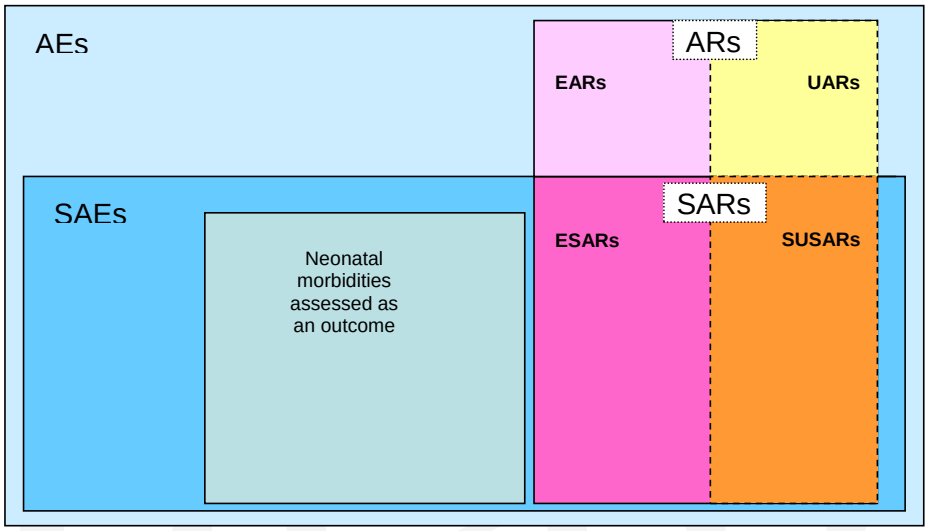

Table 4: Recording and reporting of events and reactions

Type of event	Recorded in the trial	Reported as an outcome measure	Reported to the authorities
AEs	-	-	-
SAEs	-	-	-
EARs*	yes	-	-
UARs*	yes	-	-
Neonatal morbidities	yes	yes	-
ESARs*	yes	yes	-
SUSARs*	yes	yes	yes
*Adverse reactions with a 'certain' or 'probable/likely' relation to the intervention			

8.2 Cerebral NIRS monitoring device

To ensure safe application of the device, investigators and the involved staff will be trained on how to place the device (see section 9.2). To minimise skin irritation related to the device, the oximeter will be moved to different location every 4-6 hours.

8.3 Data Monitoring and Safety Committee

An independent Data Monitoring and Safety Committee (DMSC) is established to monitor the any cases of SUSARs with 'certain' or 'probably/likely' relationship with the cerebral NIRS oximeter or the application of the treatment guideline. The members of the DSMC are: Jan Miletin, Cuno Uiterwaal, and Heike Rabe. The charter will be written prior to any analysis.

9. Ethical Considerations

As there are no randomised clinical trials (RCT) on the benefit and harms of NIRS in preterm infants, we find it ethically suitable that the control group receives 'treatment as usual'. All interventions proposed in the treatment guideline are commonly used in this patient group. There is clinical equipoise, which means that there is genuine uncertainty over whether the cerebral oximeter and subsequent treatment will be beneficial or may even harmful to the participants. To obtain evidence-based knowledge on the benefit and harms of cerebral monitoring using NIRS as a part of clinical management of premature infants, a large-scale RCT is needed. The SafeBoosC phase II trial serves as a feasibility trial for such a large phase III trial.

The SafeBoosC trial will only start the randomisation of participants after the approval from the relevant ethics committees and competent authorities has been received and parental inform consent is obtained. All parents will receive written and oral information about the trial before they are asked for their written consent. They will only enrol their newborn in the trial by their own free will and can cancel their consent for participation at any time. If a parent wishes to cancel the participation, the patient will be treated according to the respective hospital's standard procedures. The trial will be conducted in compliance with the guidelines of the current Helsinki Declaration and the International Conference on Harmonization of Good Clinical Practice Guidelines (ICH 2011, Declaration of Helsinki 2011).

9.1 Informed consent procedure

Parents of potential participants will be invited to enrol their preterm newborn before delivery and given the Parent Information Sheet (Appendix F) on the trial.

9.2 Risk of complication for participants

The following procedure will be implemented to prevent and/or minimise risk of complication for participants.

Related to devices

All investigators are highly experienced in cerebral NIRS oximetry and will use devices that are routinely used in their units. Correct reading of the monitor (mainly to prevent false reading which may lead to wrong treatment) is critical. The investigators will train local staff as appropriate and trained staff will closely supervise all patients during the intervention.

Related to treatment guideline

All investigators have been involved in the development of the guideline and have approved the final version. The investigators will train local staff as appropriate and will be available for consultation as needed.

9.3 Benefit for participants

This trial is designed with the anticipation that benefits justify the risks for all participants. The participants in the both the groups will receive careful attention from qualified physicians and hospital staff during the trial and a closer follow-up after hospital discharge.

10. Statistical plan and data analysis

10.1 Sample size estimation

An unpublished dataset of cerebral NIRS oximetry in 23 extremely preterm infants monitored from the first hours after birth for 72 hours by the INVOS 4100 NIRS device in Utrecht during the period January 2004 until January 2008 was used to estimate the sample size (unpublished data). The cerebral saturation was recorded in 5 second-values. The data was censored by the proprietary Signal Base software. The recording was reliable 79% of the time, ranging from 48% to 97%. The time spent with a cerebral saturation below 55% and above 85% was calculated and the burden of hypo- and hyperoxia was calculated by summing the percentage deviation over time area under the curve – AUC). The burden of hypo- and hyperoxia was corrected for the loss of recording time and expressed as %hours per 72 hours.

There was a relatively high correlation between time spent in hypoxia and the burden of hypoxia. Overall, hyperoxia was only about 10% of hypoxia. There was a trend towards inverse correlation between hypoxia and hyperoxia. The distribution of AUC outside the range was skewed towards the right. The mean was 76.0%hours ± SD of 83.2. After a log-transformation, the distribution was not significantly different from normal. The mean was 1.64 ± SD of 0.50 (Figure 4).

As a result, the statistical distribution has to be normalised by logarithmic transformation. Reducing the duration of time with hypoxia or hyperoxia by 0.30 (corresponding to a 50%

reduction of the time with hypoxia or hyperoxia) with a type I error (alpha) of 5% and a type II error of 0.05 (power of 95%) requires randomisation of a total of 150 preterm infants (or 'pair' or 'groups of infants' in case of multiple births): 75 preterm infants in the experimental group and 75 preterm infants in the control group.

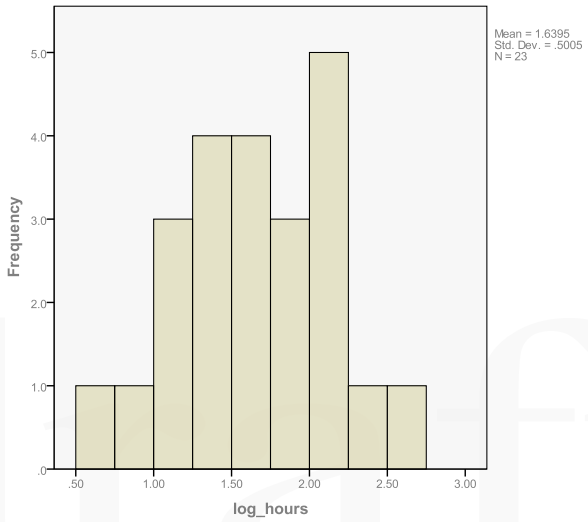

Figure 4: The distribution of the primary outcome burden of hypo-and hyperoxia expressed as %hours and transformed logarithmically

10.2 Data analysis and statistical methods

All analyses will be performed blinded with the two intervention groups concealed as, e.g., X and Y. All analyses are intention-to-treat analyses. Table 5 shows each class of outcome, its category and priority, the mathematical type of measure, value of optimistically imputed missing value, value of pessimistically imputed missing value, time at which or period during which it was measured and type of statistical methodology applied to measure the effect of the intervention. The following describes the statistical models that will be applied and how missing values and multiplicity issues are dealt with.

10.2.1 Statistical models

Depending on the specific type of outcome measure, one of the four types of regression analyses will be applied. The indicator variable I (1 if the experimental group is coded A and 0 if it is coded B) is included as a co-variate and the outcome measure as the dependent variable.

- *Type 1* includes a continuous outcome measure. The general linear univariate model is used. If the assumptions of this model (normally distributed residuals, variance homogeneity) are not fulfilled with reasonable approximation either directly or after suitable transformation of the dependent variable, a non-parametric test is used (Mann Whitney).

- *Type 2* includes a binary outcome measure and logistic regression analysis is used.

- *Type 3* includes an ordinal quantity (three categories) as outcome measure. The proportional odds model will be used if the score test for the proportional odds assumption is not significant ($p > 0.05$). If it is, the applicability of the linear by linear model (37) is examined graphically (38). If this model does not work, a Chi-square test of independence will be used thus ignoring the ordinal nature of the data.

- *For type 4 (mixed-model analyses),* each biochemical outcome measure (y) measured at 6 and 72 hours after birth is viewed as a linear function of time (t). The basic model is $y = a + b*x + c*t + d*x*t$ assuming no effect of the intervention. X is the baseline marker value in cord blood. This is compared to the model $y = e*I + a + b*x + c*t + d*x*t + f*I*t$ which includes the impact of the intervention on the intercept and slope of the linear function using a likelihood ratio test. I is the intervention indicator and a, b, c, d, e and f are the coefficients of the regression equation. If the test is significant, each of the coefficients d and e are tested for significant difference from 0 (i.e. it is tested if the intervention influences the intercept and/or the slope of the linear function). A mixed-model with repeated measures (MMRM) is used (39). Applying Akaike's criterion it is decided whether a compound symmetric covariance matrix or an unstructured one should be used.

All of the above analyses will be repeated with an indicator of the centre where the participant is treated is included. Included are also the protocol specified binary stratification variable and the interaction between this and the intervention indicator. Major discrepancies between results of this analysis and the above not including the stratification variable and the centre indicator will be discussed.

Except for the mixed-model analysis, the above analyses must be 'complete-case' analyses (participants with missing values are excluded). The results of the MMRM analysis (following imputation of missing co-variate values, see below) and for the rest of the analyses the results based on multiple imputations (see below) are the official results. However, if less than 5% of an outcome measure is missing, only a complete case analysis will be done and the result will be the official one.

10.2.2 Dealing with missing values

Using the MMRM secures that missing values will not create any bias as long as the values are missing at random (MAR), a rather mild restriction implying that the probability of missing data on Y is unrelated to the value of Y, after controlling for other variables in the analysis. However,

if co-variate values are missing, such as baseline values in cord blood, these values are first imputed using the method of multiple imputations (MI) and then the MMRM analysis is done. When the MMRM model is not used, the method of MI will be used to impute missing values, thus securing that missing values will not create any bias as long as the MAR condition is fulfilled.

In the MI analysis, the model variables and additional variables significantly related to the variables with missing values and/or the missingness of these variables will be used. The method to be used is the fully conditional specification method of SPSS (version 17.0). This is an iterative Markov chain Monte Carlo method that can be used when the pattern of missing data is arbitrary (monotone or non-monotone). The default number of iterations will be used initially and then increased if the Markov chain has not converged. Prior to the MI, the distributions of the continuous variables are inspected to see if serious deviations from the normal distribution that need transformations are present. Constraints are set to restrict the range of imputed values of continuous variables so that they are plausible. Ten imputed data sets are produced.

To assess the potential impact of values missing not at random (MNAR), two sensitivity analyses will be done.

1. *Analysis 1.* Parameter estimates obtained by complete case analysis (in the MMRM analysis only participants without missing values are included) are compared to those obtained by MI or MMRM analysis where the latter includes all participants. In addition, the results obtained by MMRM directly and by MMRM preceded by MI of outcome measures are compared.

2. *Analysis 2.* Let BEST be the intervention group in which during the experiment a specified outcome measure (X) is beneficially and significantly changed as compared to the other intervention group. Two types of values are used for imputation of missing values of X, an optimistic one (x-op) supporting the findings of the complete case analysis and a pessimistic one (x-pes) not supporting the findings. Four analyses are done. In the first, all missing values are imputed by x-op, in the second by x-pes, in the third all missing values in group BEST are imputed by x-op while those in the other group are imputed by x-pes and in the fourth analysis all missing values in group BEST are imputed by x-pes while those in the other group are imputed by x-op (worst case analysis). Table 3 (columns 4 and 5) shows the values chosen for x-op and x-pes respectively.

10.2.3 Dealing with multiplicity

The null hypotheses corresponding to the outcome measures are gathered into two families (see table 5) according to priority and the gate keeping method of Dmitrienko et al., (40). Parallel gate keeping will be used to adjust the observed p values. The originally computed p values and the corresponding adjusted ones obtained using the gate keeping method will both be reported and the problems arising from the fact that the sample size calculation is based on the primary outcome measure only will be discussed.

Table 5: Statistical measurements

Statistical type of analysis (family)[a]	Category of outcome (priority/No)[b]	Mathematical type of outcome measure	Values imputed for sensitivity analysis		Times of measurements				
			X-op	X-pes	During intervention	Measured at 6 and 72 hours	Measured at 72 hours	Measured at term date (approx. three months after birth)	Measured at 24 months after term date
1 (1)	Hypoxia or hyperoxia burden(P/1)	Continuous	Min[c]	Max[d]	yes				
1 (2)	aEEG(S/1)	Continuous	n.a.	n.a.			Yes		
4 (2)	Biomarkers(S/3)	Continuous	Max[d]	Min[c]		yes			
1	Hypoxia-burden (E/1)	Continuous	Min[c]	Max[d]	Yes				
1	Hyperoxia-burden (E/1)	Continuous	Min[c]	Max[d]	yes				
2	Severe adverse reactions(E/1)	Binary	0	1	yes				
2	Mortality(E/1)	Binary	0	1				yes	
2	Morbidities(E/5)	Binary	0	1				yes	
3	Brain injury score(E/1)	Categorical	0	3				yes	
2	Therapy(E/6)	Binary	0	1	yes				
1	Physiological quantities(E/3)	Continuous	Max[d]	Min[c]	yes				
2	Mortality between 3 month and 24 months (E/1)	Binary	0	1					yes
1	BSID-III (E/3)	Continuous	Max[d]	Min[c]					yes

a) Family of null hypotheses.
b) P stands for primary outcome. S for secondary outcome and E for outcome subject to explorative hypothesis generating analysis. No stands for number of this category of outcome.
c) The minimum value in the distribution of the whole material.
d) The maximum value in the distribution of the whole material.

11. Data management

11.1 Data handling and archiving

Source data will be registered in patient's medical records or on specially devised work forms. A common web-based electronic case report form (eCRF) will be devised to enable a central database. Data entry into the central database plus medical records and work forms is the responsibility of the investigator. Data will be stored in accordance with guidelines issued by the Danish Data Protection Agency, with which the trial will also be registered. After the establishment of a 'clean file', the database will be locked, data will be send for statistical analysis at the Copenhagen Trial Unit. The trial database will hereafter be kept according to the respective national laws. After end of trial, the data will be archived for five years according to good clinical practice guideline.

After completion of statistical data analysis, data will be pseudo-anonymised and deposited at the Danish Data Archive. Also, data will be made available for other researchers by uploading a completely anonymised dataset onto www.clinicaltrials.gov.

There will no biobank set up for blood or urine samples. All project related data and samples that may be related to the individual patient shall be destroyed at once or upon completion of analyses of trial data.

The investigator(s) permits trial-related monitoring, audits, regulatory inspection(s) by providing direct access to the source data and other relevant documents. Trial data will be handled according to regulations of data protection agency in the respective countries.

11.2 Medical coding

Adverse reactions (ARs) will be coded using MedDRA coding system by lowest level term (LLT), preterm term (PT), and system organ class (SOC). Concomitant medications will not be coded.

11.3 Monitoring

We will perform GCP-monitoring restricted to the following issues:

- All patients for documented informed consent.
- All patients for status on primary outcome, adverse events and mortality at term date follow-up.
- 24 patients randomly chosen (about two from each site) for documented delivery or non-delivery of the intervention compared to source data being patients' hospital records. This corresponds to 16% of the total sample.

Two visits will be performed:

- Initiation visit at all sites.
- Follow-up visit after the last patients' have completed 'term date' follow-up.

The co-ordinating centre will continuously monitor that all electronic case record forms (eCRFs) are fulfilled according to the protocol. The GCP-unit at Copenhagen University Hospital will perform the monitoring on the Danish site.

12. Quality assurance

The trial will be carried out in accordance with the Helsinki Declaration and ICH GCP guidelines as well as national laws and regulations.

12.1 Trial monitoring

The trial will be monitored according to the ICH GCP. The monitoring activities will be in accordance with the agreed monitoring plan and in compliance with existing regulations to monitor the consistency between the eCRFs and source data.

12.2 Device quality control

Each site is responsible for adhering to the quality control measures described in the oximeter manufacturer's users guidelines.

13. Trial and funding timeframe

Trial stages	Timeframe
Protocol development	Spring 2011
Protocol finalised (first draft)	May 31, 2011
Site selection	Ongoing – depend on finance
Recruitment phase	May 2012 – May 2013
Assessment phase	Last participant recruited (Primary outcome May 2013)
Final analysis	2013
Publication	Early 2014 on primary and secondary outcomes

Funding	Timeframe
START	December 2010, awarded 183,848 DKK
Danish Strategic Research Council	Dec, 2011

14. Legal aspects

14.1 Finance

The SafeBoosC project has received 183,848.00 DKK from the Danish Council for Strategic Research (DSF) under the Preparation of International Application (START) theme in December 2010. An application to the DSF seeking for 10,320,640.00 DKK under the Strategic Research in Individuals, Disease and Society theme, and was invited for phase II application Sept. 2011. The decision will be rendered in December 2011.

14.2 Participant insurance

The participants will be insured in accordance with existing legislation of their respective country.

14.3 Publication plan

The trial will be registered on ClinicalTrials.gov prior to the randomisation of the first participant. Attempts will be sought to publish all results, positive, neutral as well as negative, in a peer-reviewed international journals. Authorship will be determined according to the International Committee of Medical Journal Editors. Attempts will be made to publish a list with contributions in all publications.

15. Appendices

15.1 Appendix A: Treatment guidelines and justifications

SafeBoosC Clinical Guidelines

Assessment of cerebral oxygen saturation
Regional cerebral tissue oxygen saturation ($rStO_2$) is a composite measure of tissue oxygen saturation across arterial, capillary and venous beds and reflects a balance between cerebral oxygen delivery (CDO_2) and cerebral metabolic rate ($CMRO_2$). In preterm infants, the $CMRO_2$ is unlikely to vary much and a change in rStO2 largely reflects changes in CDO_2. The factors which influence CDO_2 are arterial oxygen saturation (SaO_2), haemoglobin concentration ([Hb]), and cerebral blood flow (CBF).

Establishment of monitoring of cerebral oxygenation
As soon as possible or before 3 hours of age

Period of monitoring of cerebral oxygenation
72 hours

Instruction
Document the intervention chosen and any changes in $rStO_2$ following intervention.

Recommendation for clinical interventions
The rStO2 target normal range is 55% to 85%. If the $rStO_2$ is out of normal range and there is no reason to believe that it will normalise without intervention, consider one of the interventions (identified in '•') listed below and reassess 30 to 60 minutes after the intervention. Generally, only one intervention should be chosen at a time. All the interventions proposed here are commonly used in this patient group. After each intervention, the level of evidence for each intervention (I-III) and recommendation (A-E) are given. For further explanation, see below.

$rStO_2$ < 55%
Aim of intervention: A low $rStO_2$ reflects a low CDO_2. The interventions should be directed to increasing SaO_2, [Hb], and/or CBF.

Assess respiratory status:
SaO2 low in normal range, consider:
- Increase FiO_2 (II-1/A) (41)
- Increase mean airway pressure (III/B) (42-44)

PCO2 low in normal range, consider:
- Decrease minute ventilation (II/A) (45-48)

Assess cardiovascular status:
Blood pressure low in normal range, consider:
- Vasopressor-inotropes (I/B) (49,50)
- Fluid bolus (normal saline) (I/C) (51,52)
- Decrease mean airway pressure (III/B) (44,53-55)

Poor systemic circulation, consider if:
Echocardiography shows low cardiac output and low SVC flow
- Inotropes (I/B) (52,56-60)
- Fluid bolus (normal saline) (I/C) (51,52)
- Decrease mean airway pressure (III/B) (44,53-55)
- Reduce vasopressor (III/ B) (61)

Echocardiography not available but has at least 2 of the following signs:
Lactate > 3.5 mmol/l
CRT > 3 seconds
Urine output < 1 ml/kg/hour
consider:

- Inotropes (I/B) (49,50)
- Fluid bolus (normal saline) (I/C) (51,52)
- Decrease mean airway pressure (III/B) (44,53-55)
- Reduce vasopressor (III/B) (61)

Patent ductus arteriosus, consider:

- Medical treatment (II-2/B) (53,54,62,63)

Assess oxygen transport:
Haemoglobin low in the normal range, consider:
Red blood cell transfusion (I/B) (45,64-66)

rStO2 > 85%
Aim of intervention: A high $rStO_2$ reflects impaired oxygen utilisation and/or disturbed cerebral autoregulation (hyperaemia) and interventions should be directed at identifying and treating the underlying cause.

Assess respiratory status:
SaO2 high in normal range, consider:
Decrease FiO2 (II-2/A) (67-71)
Decrease mean airway pressure (III/B) (42,43)

PCO2 high in normal range, consider:
Increase minute ventilation (II/A) (45-48)

Assess blood glucose level:
Blood glucose < 2.5 mmol/l, consider to:
Increase glucose intake (III/A) (72,73)

Level of evidence and recommendation of intervention
The level of evidence (Table 1) and recommendation for a given intervention (in brackets and Table 2)) were graded according to the U.S. Preventive Services Task Force system (74).

Table 1: Hierarchy of research design and level of evidence

Level of evidence	Type of study
I	Evidence obtained from at least one properly randomized controlled trial
II-1	Evidence obtained from well-designed controlled trials without randomization
II-2	Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one centre or research group
II-3	Evidence obtained from multiple time series with or without intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence
III	Opinions of respected authorities, based on clinical experience, descriptive studies and case reports, or reports of expert committees

Table 2: Recommendation grid

	Net benefit			
Quality of evidence	substantial	moderate	small	zero/negative
Good	A	B	C	D
Fair	B	B	C	D
Poor	E	E	E	E
Standard recommendation language	A= Strongly recommended (good evidence that the intervention improves important health outcomes and benefits substantially outweigh harms). B= Recommended (at least fair evidence that the intervention improves important health outcomes and benefits substantially outweigh harms). C= No recommendation for or against routine provision of the intervention (fair evidence that the service can improve health outcomes but the balance of the benefits and harms is too close to justify a general recommendation). D= Recommends against routinely providing the intervention (at least fair evidence that the service is ineffective or that harms outweigh benefits). E= Insufficient to recommend for or against routinely providing the intervention (evidence that the intervention is effective is lacking, of poor quality, or conflicting and the balance of benefits and harms cannot be determined).			

Abbreviations

SVC	*Superior vena cava*
CRT	*Capillary refill time*
FiO_2	*Fraction of inspired oxygen*
$rStO_2$	*Regional cerebral tissue oxygen saturation*
SaO_2	*Saturation of Oxygen (arterial blood)*
PCO_2	*Partial Pressure of Carbon Dioxide*
CDO_2	*Cerebral oxygen delivery*
$CMRO_2$	*Cerebral metabolic rate*
[Hb]	*Haemoglobin concentration*
CBF	*Cerebral blood flow*

15.2 Appendix B: Procedure for assessment of chemical biomarkers

OUTCOME MEASURES
Outcome measure 1: S100β
Outcome measure 2: BFABP
Outcome measure 3: Neuroketal

MEDIUM
Blood and Urine
One millilitre (ml) of blood will be collected in heparin. Commercially available tubes containing lithium or sodium heparin are available to collect 2 ml of blood. These tubes can be used without vacuum and filled with 1 ml from a syringe. The higher final heparin concentration is no problem. Blood must be kept cold after collection on ice or in the fridge at 4ºC until it is centrifuged (1200xg for 12 minutes, or 13000 rpm in a microcentrifuge) to obtain platelet poor plasma. As much as possible plasma is then collected, transferred to small screw-cap tubes and stored frozen (80 ºC for prolonged storage or 20 ºC for < 1 month). If possible, 1 ml of urine can be collected and stored frozen without further treatment. Two country letters, inclusion (randomisation number), Plasma or Urine, time points (urine samples may extent to 4 or 5). Example: DK 5 P 3 = Rigshospitalet patient 5, plasma collected after 72 hrs. The principal investigator keeps record of the patient identity linked to the patient number.

TIME OF COLLECTION
Blood:
- 0-hour (cord blood) – if possible,
- 6 hours after birth, and
- 72 hours after birth

Urine (if possible) - special instruction (e.g., can be +/-2 hours of specified time points):
- 6 hours after brith, and
- 72 hours after birth.

METHOD OF ASSESSMENT OR SCORING
The determination of S100β concentrations in serum will be performed with an enzyme linked immune assay (ELISA). It can be detected in serum or heparin plasma and urine in 50 µl samples. EDTA or citrate plasma cannot be used for measurement of S100β. Brain fatty acid binding protein BFABP is determined by means of ELISA with BFABP specific monoclonal capture antibodies and polyclonal detection antibody in plasma/serum as well as urine. 100 µl is needed for a BFABP ELISA, due to its low concentrations. Determination of neuroketal is performed by competitive enzyme immunoassay in 100 µl of plasma/serum or urine.

15.3 Appendix C: Procedures for assessment of aEEG/EEG

OUTCOME MEASURES

Outcome measure 1: Interburst interval (IBI)
Outcome measure 2: Power in delta band
Outcome measure 3: Power in theta band
Outcome measure 4: Power in alpha band
Outcome measure 5: Power in beta band
Outcome measure 6: Background pattern
Outcome measure 7: Sleep wake cycling
Outcome measure 8: Seizures

MEDIUM
Raw EEG-data

TIME OF COLLECTION
Single-channel EEG is applied for at least 120 minutes at 48-72 hours after birth. This should be at least 3 hours after administration of bolus morphine, if possible. Hydrogel electrodes or needle electrodes may be used. Electrodes are placed at the P3 and P4 positions according to the international 10–20 system; a frontal reference electrode is also applied.

METHOD OF ASSESSMENT OR SCORING
The EEG is divided in 10-min. epochs. The quality of all EEG epochs is visually assessed without knowledge of patient group. Epochs showing artifacts that could affect the quantitative analysis are discarded.

IBIs are measured by using an automated algorithm and averaged in each 10-minute epoch. The IBI detector algorithm is based on a non-linear energy operator that reflects both amplitude and frequency content of the EEG. Power spectral analysis is performed using fast Fourier transformation with a time base of 10 seconds. For every epoch, total and relative (%) band power is calculated within the following frequency bands: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8 –13 Hz), and beta (13–30 Hz).

If more than one 10 min. epoch is analysed, the simple average of all measures is used for further analysis

15.4 Appendix D: Procedures for MRI examination

OUTCOME MEASURES
Outcome measure 1: Volumetric measurements:
Outcome measure 2: Cortical folding with formation of the sulci:
Outcome measure 3: Diffusion tensor imaging:

MEDIUM
X-ray (MRI scan)

TIME OF COLLECTION
At term date (approx. 3 months after birth) ± 2 weeks

METHOD OF ASSESSMENT OR SCORING
The MRI protocol consists of anatomical scans (T1 3D weighted image and T2 weighted image), DWI and DTI. The T1 and T2 weighted images are performed in coronal slices and the T1-3D weighted image at around 100-110 slices. The DTI images are performed in axial slices covering the whole brain. The focus is on the following features: brain tissue volumes, including myelinisation, cortical folding with formation of the sulci, in relation to DWI/DTI.

Volumetric measurements: The segmentation is performed on the MR images of individual patients. The segmented tissue types include: unmyelinated and myelinated white matter, cortical and central gray matter (basal ganglia and thalamus), cerebro-spinal fluid around the brain, ventricles, cerebellum and brainstem. This segmentation method was developed for neonates (75). Quantitative measurements of the cerebral tissue volumes are performed by the knn-approach segmentation method which is based on the probability of each tissue classes from signal intensities in T1- and T2-weighted images and the anatomic location. Thereafter, the volume of each tissue is quantified.

Cortical folding with formation of the sulci: For cortical folding, the inner cortical surface is segmented and reconstructed in 3D from the T2-weighted images. The surface is detected between the developing cortex and white matter zone. The global area of this inner cortical surface is then computed. The segmentation of the inner surface is used as it highlights the cortical sulcation pattern more precisely than the outer surface, particularly where sulci are still not deeply folded. Finally, the local surface curvature is estimated from the mesh local geometry: positive curvatures correspond to the gyri top, and negative curvatures to the folds bottom. So the 3D image is then created. The sulci are defined as connected components of negative curvature and labelled manually according to post-mortem and MRI atlases and prenatal images. To characterize the sulci maturation according to gestational age, their sulci areas is calculated. Thereafter, for each infant, the sulcation index can be computed, defined as the ratio between the areas of sulci and the surface of the total cortex. This surface increases with the age-related brain growth. The sulcation index, thus, characterizes the proportion of sulci according to the whole brain size, and is a measure of maturation of the cortex. A rough measurement of apparent cortical thickness is estimated according to the ratio between cortical volume and surface.

Diffusion tensor imaging: Diffusion tensor imaging (DTI) scans are obtained to perform analyses of the diffusion coefficients on the brain averages, as well as on individual patients. It visualizes the movement of fluid in the brain, and can be used to identify white matter tracts. We will study the maturation of white matter fiber tracks in relation to cortical development.

15.5 Appendix E: Procedure for assessment of cranial ultrasound

OUTCOME MEASURES
Outcome measure 1: Parenchymal haemorrhagic infarction / inhomogeneous
Outcome measure 2: IVH Grade III
Outcome measure 3: Cerebellar haemorrhage
Outcome measure 4: Posthaemorrhagic hydrocephalus
Outcome measure 5: cPVL
Outcome measure 6: Cerebral atrophy

MEDIUM
Sonogram

TIME OF COLLECTION
- Early scans (day 1-4): presence of parenchymal haemorrhagic infarction / inhomogeneous increased echogenicity in the periventricular white matter and/or IVH Grade III, presence of cerebellar haemorrhage
- Late scan (day 7): presence of parenchymal haemorrhagic infarction / inhomogeneous increased echogenicity in the periventricular white matter and / or IVH Grade III, presence of cerebellar haemorrhage, posthaemorrhagic hydrocephalus
- Term or before discharge scan: cystic PVL according to classification of De Vries, presence of cerebral atrophy based on serial CUS. , normal CUS, presence of cerebellar haemorrhage on CUS

METHOD OF ASSESSMENT OR SCORING
The assessment classification sequences are:
1. Normal brain scan
- No cysts
- No ventricular dilatation
- No enlargement of extra-cerebral spaces
- Normal cortical grey matter

2. Mild brain injury:
- Grade 1-2 IVH (including GLH)
- Persistent pathologic non-decreasing inhomogeneous flaring at day 7
- Thinning of the corpus callosum
- Ventriculomegaly, ventricular index < p97

3. Severe brain injury will be defined as
- Intraventricular haemorrhage with ventricular dilatation (ventricular index > p97)
- Posthaemorrhagic ventricular dilatation
- Parenchymal haemorrhagic infarction
- Local cystic lesions (unilateral)
- Cystic periventricular leukomalacia (bilateral)

15.6 Appendix F: Parental Information Sheet

Information for parents who may have a baby born extremely preterm

Dear Parent

Your doctor may have explained to you that your baby may be delivered much too early and may need intensive care. We understand that this may be a very stressful time for you and that it can be hard to take in a lot of information at this time. This leaflet gives you information about a clinical trial called SafeBoosC that we are conducting to help improve the care of babies in the neonatal unit. We give this leaflet to all parents in your situation. In case a doctor or nurse ask you about joining the SafeBoosC trial later on, you will already know something about the trial.

What are we trying to find out?

SafeBoosC is a European trial trying to find out if knowing the oxygenation of the brain may be beneficial. Most extremely preterm babies need treatments to help their breathing and many babies need treatments to improve their blood circulation. It is possible that the doctor may adjust this treatment to reduce the time the brain is exposed to too little or too much oxygen. If the SafeBoosC trial suggests more benefit than harm, we plan to launch a large trial with approximately 4,000 babies.

How can we know the oxygenation of the brain?

We use an instrument called a near-infrared oximeter. It has as thin cable to a sensor (a soft patch measuring 3 x 5 cm). The sensor is put on the head of the baby and held in place by a bandage. The sensor uses near-infrared light. The light goes some centimetres into the brain and measures the colour of the red blood cells as it changes with oxygenation. The oximeter does that every 5 seconds and the value of oxygenation is shown on the oximeter at all times.

How will this change the treatment of your baby?

If the monitor shows a value outside the normal range the doctor may try to adjust some part of the treatments that your baby receives. We have worked out a specific list of treatments and the ways that they may be adjusted. These treatments are all used in routine clinical practice in order to normalise respiration, blood circulation, blood transport of gasses (oxygen, carbondioxide), and blood sugar concentration.

How will the research be done?

SafeBoosC is a randomised clinical trial. Many treatments are tested by randomised clinical trials, as it is the most reliable way to find out if they are effective.

A randomised clinical trial means that, for half of the babies entered into the SafeBoosC trial, the doctor will know the oxygenation of the brain. The other half will also have the sensor put on the head and the oximeter will record the oxygenation, but the doctor will not see the result. A randomised clinical trial means that the decision about whether the oxygenation will be known will be determined by chance, like the toss of a coin. We need 150 babies from 12 hospitals in Europe to get an impression of the benefits and harms of knowing the oxygenation of the brain combined with the list of treatment options.

Are there risks?

Near-infrared light is difficult to see but can go some centimetres into the body. The heat is less than that on a normal summer day and thus, it has no risks to the brain. There is, however, a small risk of skin burn which can be further minimised by moving the placement the sensor every 4 to 6 hours. The nurse will do this very carefully in order not to disturb the child.

We hope that the adjustments of treatment will be effective to reduce the time of exposure of the brain to too little or too much oxygen. We hope that this is beneficial to the baby. But there may be unforeseen risks to some of the adjustments of treatment. All unexpected adverse events will be reported as a part of the research.

What would being part of the trial involve?

You may be asked by the doctor or nurse to consider joining the SafeBoosC trial if your baby is expected to be delivered more than 12 weeks before the term date.

If you agree to your baby taking part in the trial, he or she will have the sensor put on the head within three hours after birth for 72 hours. A dedicated person will take care of the near-infrared oximeter and make sure that the result is available or not available to the doctor as decided by randomisation.

Up to three blood and urine samples will be taken . This is a minimal amount of blood and the samples will only be taken if the baby has a catheter in a blood vessel or the urinary bladder that may be used for the sampling.

On the third day of life 3 electrodes will be put on the head and the spontaneous brain electrical activity (EEG) will be recorded for three hours.

All other observations, monitoring, and treatments will be done as usual in the neonatal department. In particular, ultrasound scanning of the brain will be done several times as is the normal routine.

We will record details about your baby and the treatments from the hospital notes. After discharge to the home, we will follow up the baby's progress and note any changes.

At the time of normal time of birth of your baby (approximately after three months), you may be asked to have your baby examined by magnetic resonance imaging to help the doctor predict the future of his or her development.

24 months after the original term date (approximately, after 27 months), you will be asked to have the development of you baby assessed.

Any information that we collect in the trial will be kept completely confidential and in a secure place. Only people involved in the trial will have access to it. Additionally, we ask you to sign a consent form, in order to grant the authorities access to your baby's data in case of an audit or an inspection.

Does my baby have to take part in SafeBoosC?
It is your decision whether or not you want your baby to take part in SafeBoosC. Please remember that if you decide to enrol your baby in the SafeBoosC trial, you can change your mind later without having to give a reason. Your decision to withdraw your baby from the trial will not affect your baby's care in any way.

If you decide that you do not want your baby to join SafeBoosC, he or she will still be given the highest level of care and attention by staff.

Will we be told the results of SafeBoosC if we join?
Yes. If you decide that you would like your baby to join SafeBoosC we will keep in touch with you to tell you the results of the trial when they become available (in year 2013-2014), if you wish so.

Thank you
Thank you for taking the time to read this leaflet. If you would like more information about SafeBoosC, please ask your doctor or nurse.

Contact Information:
SafeBoosC Trial
Sponsor: Prof. Gorm Greisen
Address:
Telephone:

15.7 Appendix G: Informed consent form

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