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XENON AND COOLING THERAPY IN BABIES AT HIGH RISK OF BRAIN INJURY FOLLOWING POOR CONDITION AT BIRTH: A RANDOMISED OUTCOME STUDY (COOLXENON3 STUDY)

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Protocol Summary

Title	**XENON AND COOLING THERAPY IN BABIES AT HIGH RISK OF BRAIN INJURY FOLLOWING POOR CONDITION AT BIRTH: RANDOMISED PILOT OUTCOMES STUDY (COOLXENON3 STUDY)**
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Study Centres **1. Primary Site – Bristol** **2. Second Site - London**	1. St Michael's Hospital Southwell Street Bristol BS2 8EG 2. Hammersmith Hospital / Imperial Neonatal Service Du Cane Road, London W12 0HS
Test Article	Xenon Neonatal Breathing Device
Objectives	Primary Objective: This is a randomised two group pilot outcomes study comparing Bayley III neurodevelopmental outcome scores between; i) the established treatment of cooling the body down to 33.5 °C for 3 days; to ii) Xenon inhalation for 18 hours in combination with whole body cooling to 33.5 °C for 3 days This will be applied in term newborn babies who are at moderate or high risk of brain injury following poor condition around the time of birth. Secondary Objectives: Potential outcome predictors are based on a combination of: 1) Time in hours after birth when background activity as recorded by amplitude integrated EEG (aEEG: from single channel EEG)[1] and onset of sleep wave cycling (SWC). 2) Cerebral MRI lesions in T1W and T2W sequences[2] and MRI biomarkers including absolute thalamic N-acetyl aspartate (NAA) quantification, lactate/NAA peak-area ratio from proton spectroscopy[3] and whole brain white matter water-diffusion fractional anisotropy analysed with tract-based spatial statistics[4] from two MR images performed around 48 hours of age and around7 days of life. 3) Peak value of Lactate Dehydrogenase within the first 72h of life[5], was a good predictor in cooled infants. 4) Age in hours when plasma lactate has fallen to 5mmol/l 5) Resistance Index (RI).[6] Tertiary Objectives:

	We will monitor a number of variables before/during and after the period of Xenon delivery. This group of physiological and biochemical parameters includes those recorded during both our CoolXenon1 and CoolXenon2 studies. Parameters for which data will be collected are: i. Gas exchange: Transcutaneous oxygen saturation, end-tidal carbon dioxide concentration, blood gas data (from arterial sample), mechanical ventilation requirements (rate, peak inspiratory pressure, PEEP settings). ii. Haemodynamic: Systolic, mean and diastolic arterial pressure, heart rate. iii. Any problems during weaning and extubation, such as post-extubation stridor, use of steroids. iv. Coagulopathy (INR >2, APTT> 50) v. Inotrope requirement, cardiac Troponin T vi. Hepatic impairment, (raised Alanine aminotransferase > 40, Alkaline phosphatase > 100, peak Lactate dehydrogenase within 72h of age) vii. Renal impairment, oliguria (urine output < 1 ml/kg/h after day 1 of life and raised creatinine > 100) viii. Infection (culture proven, need for antibiotics $\geq$ 5 days, raised CRP ~ within the first 24h and at 4 days of life of life ix. Estimation of sedation with validated sedation scale N-PASS and plasma morphine concentrations. x. Estimation of adrenocortical function during xenon inhalation and hypothermia using plasma, salivary and urine cortisol measurements and plasma adrenocorticotropic hormone. xi. Number of anticonvulsive drugs needed to treat seizures xii. Length of hospital stay xiii. Time to full oral feed by bottle or breast xiv. MRI assessment by T1 and T2 weighted images using the scoring system by Rutherford *et al*.[2] xv. In some infants when the 3Tesla MRI scanner is available assessment of brain metabolites using magnetic resonance spectroscopy (MRS) where the ratio Lactate/N-acetyl aspartate has been shown to be a good early biomarker for outcome.[3] xvi. Safety
Patient Population	Newborn infants >/=36 weeks gestation which satisfy all St Michael's Hospital standard inclusion criteria for cooling (i.e. at risk of brain injury following poor condition at birth) and which additionally satisfy the inclusion criteria of this study for the administration of Xenon by inhalation. Cooling should be started within 3h of age and Xenon within 5h.[7] Use of a shorter treatment delay in these entry criteria will optimise the possibility of finding an effect if there is one.

Population Size	52 babies in total
Structure	Prospective randomised comparative outcome study
Duration of Participation	Xenon administration period of 18 hours. Recruitment period up to two years from start of study. Short and long term monitoring until 18 months of age. Therefore total study duration 3.5 years.
Definition of End of study	The end of the trial as a whole will be when all recruited participants have completed the treatment and follow-up period ***and*** all data has been collected and analysed, with data queries answered.
Method of Assignment	Neonates meeting all entry criteria can be included.
Randomisation	Patients will be randomised between cooling (standard treatment) and Xenon+cooling groups on a 1:1 basis.
Statistical Analysis	This is a randomised pilot outcomes study. The statistician for this study is Professor Lars Walloe who has also advised on all the preliminary experimental Xenon work and the previous two clinical feasibility studies and a recent clinical evaluation of the Bayley examination which is used in this study.[8-11]
Adverse Events	Volunteered and Elicited

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Declarations and Signatures of Chief Investigator and Chair of Data Review Committee

I confirm that I have carefully read and understood this protocol and agree to conduct this clinical trial as outlined in this protocol, according to current Good Clinical Practice and local laws and requirements.

I have received and read all trial-related information provided to me.

The objectives and content of this protocol as well as the results derived from it will be treated confidentially and will not be made available to third parties without prior written authorisation from University Hospitals Bristol NHS Foundation Trust.

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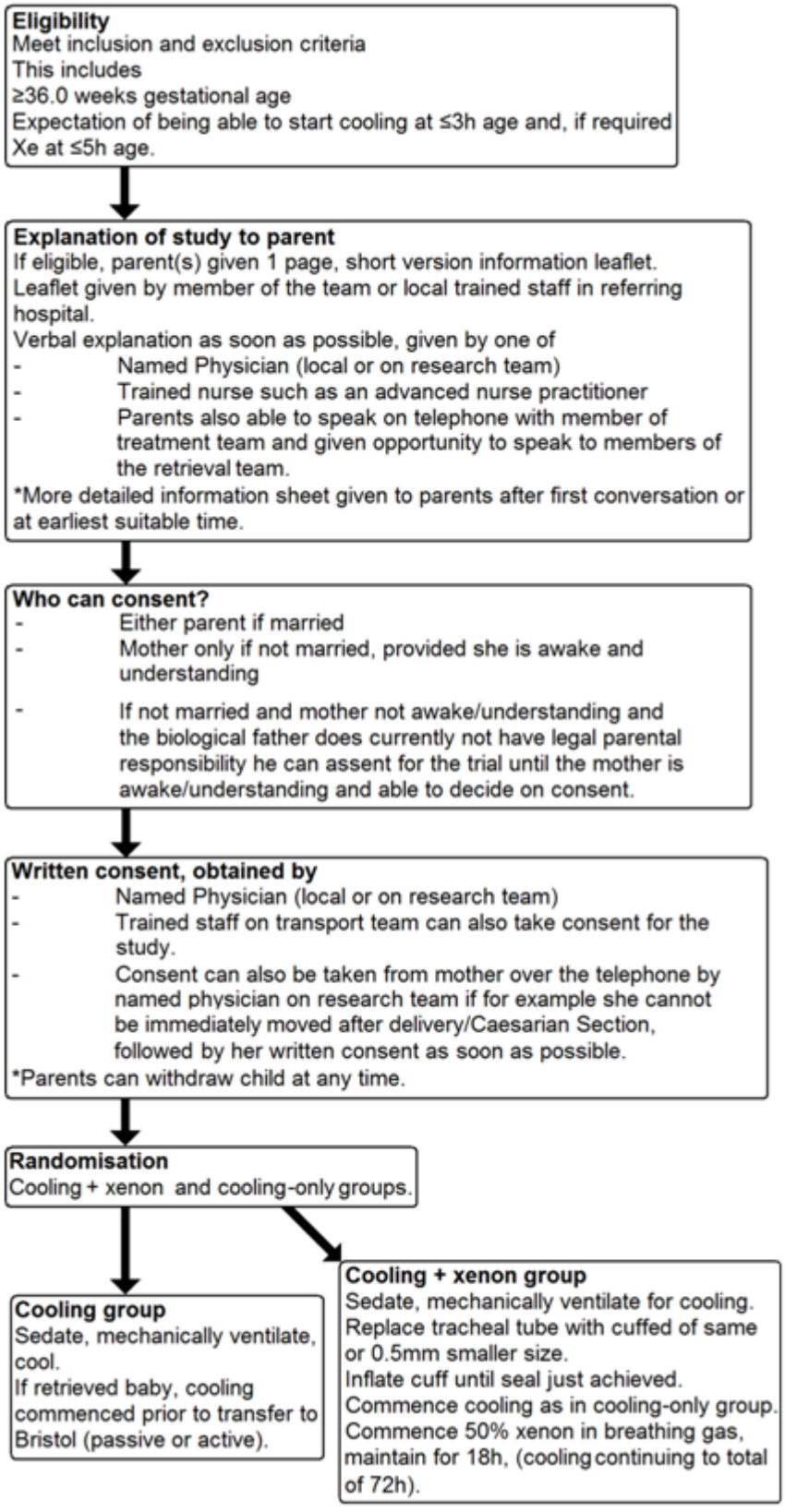
SCHEMATIC OF STUDY DESIGN



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LIST OF ABBREVIATIONS

AE	Adverse Event
aEEG	Amplitude integrated EEG
AEs	Adverse Events
ALT	Alanine Transaminase
AP	Alkaline Phosphatase
APTT	Activated Partial Thromboplastin Time
ARs	Adverse Reactions
AST	Aspartate Aminotransferase
BMI	Basal Metabolic Index
CF	Consent Form
CI	Chief Investigator
CRFs	Case Report Forms
CRO	Contract Research Organisation
CRP	C Reactive Protein
CT	Computerised Tomography
cTnT	increased cardiac Troponin T
DoB	Date of Birth
DQ	Developmental quotients
ECG	Electrocardiograph
EDD	Expected Date of Delivery
EEG	Electroencephalograph
EN	European Norm
$ETCO_2$	End Tidal carbon dioxide
EU MDD	European Union Medical Device Directives
FBC	Full Blood Count
FDA	Food and Drug Administration
FiO_2	Fraction Inspired Oxygen
GCP	Good Clinical Practice
Hb	Haemoglobin
Hct	Haematocrit
HI	hypoxic-ischaemic
HIE	Hypoxic-Ischaemic Encephalopathy
HT	Hypothermia
ICH	International Committee on Harmonisation
IDRG	Independent Data Review Group
INR	International Normalised Ratio
ISO	International Standards Organisation
LDH	Lactate Dehydrogenase
LMP	Last Menstrual Period
MHRA	Medicines and Healthcare products Regulatory Agency
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NHS	National Health Service
NICHD	National Institute of Child Health and Human Development

NICU	Neonatal Intensive Care Unit
NNT	Number Needed to Treat
NXBD	Neonatal Xenon Breathing Device
pCO_2	Partial pressure carbon dioxide
PEEP	Positive End Expiratory Pressure
PIS	Patient Information Sheet
PPV	Positive Predictive Value
QoL	Quality of Life
R&D	Research and Development
RBC	Red Blood Cells
REC	Research Ethics Committee
RI	Resistance Index
SAE	Serious Adverse Event
SaO_2	Oxygen saturation
SUSAR	Suspected Unexpected Serious Adverse Reaction
SWC	Sleep Wave Cycling
TT	Tracheal tube
UAR	Unexpected Adverse Reaction
WBC	White Blood Cells
Xe	Xenon
Xe-HT	Xenon-hypothermia

1.0 Overview

This is a prospective randomised pilot outcomes study to be undertaken at St Michael's Hospital, Bristol and Hammersmith Hospital, London. A short delay for recruitment is included to optimise the effectiveness of hypothermia (cooling, HT) and potentially Xenon. Cooled infants that are ≤3h old and start Xenon within ≤5h of age can be accepted into the study. In an experimental study, adding xenon with HT when both were commenced 5 hours after the insult did not provide any additional neuroprotection compared to HT alone[7]**;** however, 2h delayed Xenon when HT was started immediately was more effective than HT alone.[8] With the Xenon delivery system mounted in the ambulance we can start the Xe treatment in the local hospital and continue treatment during transport, thus starting Xe within 5h after birth. Since >90% of patients are retrievals from regional units within the network, the ability to reliably recruit to xenon within the 5h limit is now possible and has been demonstrated as one of the findings of the CoolXenon2 study. Preliminary data from the CoolXenon2 study show that the groups were well balanced with respect to severity of HI. Time to recovery of normal EEG trace is the best single early predictor of outcome[1] and is monitored in all infants. In CoolXenon2 we have found that this does not function as an early predictor as xenon suppresses the aEEG for the entire 18h period of xenon administration and for a short period afterwards. This means that analysed the traditional way, aEEG is not reliable as an outcome predictor during the whole of the Xenon administration periods and a few hours afterwards. Mortality was low, 7% in each group.

In CoolXenon 1 we demonstrated that it is technically feasible to deliver xenon in this patient group without adverse effects on physiology and in CoolXenon2 preliminary analyses we have found that i) Early recruitment losses due to the short birth to treatment-start time limits we have set were then successfully minimised by commencing xenon during retrieval, ii) As in CoolXenon1 there are no adverse physiological effects of giving xenon in this patient group and iii) one planned predictive early marker of outcome, aEEG, has not functioned as expected.

The study is a therefore a natural extension of, and includes lessons learned from, the previous studies we have undertaken (CoolXenon 1 and CoolXenon2) and examines the effect of inhaled Xenon gas in the treatment of newborn infants with hypoxic-ischaemic encephalopathy (HIE), in combination with cooling which is the standard treatment for this condition. The hypothesis is that the Xenon+cooling combination will produce better neuroprotection than the standard treatment, cooling-alone, as assessed at 18m using the established Bayley Scales of Infant Development, Xenon, a rare anaesthetic gas, is almost side-effect free and shows great promise as a method of enhancing the neuroprotection offered by cooling. It is, however, extremely expensive at £25-50/L.

Based on experience and clinical data from our previous trials, we now propose to study 52 full term infants with moderate or severe HIE who require mechanical ventilation and hypothermia for 72 hours. HT will be routinely applied if the standard existing criteria for this are met except recruitment will be earlier, ≤3h rather than 6h as in our standard institutional cooling protocol. We have recently shown that cooling ≤3h gives better outcome[12]: Time Is Brain: Starting Therapeutic Hypothermia within Three Hours after Birth Improves Motor Outcome in Asphyxiated Newborns. Thoresen M, Tooley J, Liu X, Jary S, Fleming P, Luyt K, Jain A, Cairns P, Harding D, Sabir H. *Neonatology*. 2013 Sep 12;**104**(3):228-233.

Candidates for the study will be randomly assigned to one of two groups: (i) the standard treatment for this condition, 72 hours of cooling, *i.e. the treatment they are already receiving*, or (ii) addition of 50% Xenon concentration in the breathing gas from the mechanical ventilator for 18 hours in combination with the existing cooling regime, with cooling continuing to 72 hours.

All efforts will be made to commence delivery of the Xenon and cooling as soon as possible after birth, with maximum delay of 5 hours for Xenon and 3 hours for cooling. In the event that a baby is retrieved from another local hospital to the study centre, an assigned research team member will accompany or meet the retrieval team and commence Xenon delivery at the local hospital or during the return transfer of the baby to Bristol. Xenon will be given in the breathing gas mixture via a special delivery system situated between the ventilator and airway of the infant. This device has been specifically developed to eliminate Xenon wastage,[9] without which this putative therapy would be completely unfeasible on cost grounds. It has recently been successfully used for Xenon delivery to a total of 28 babies with the same entry criteria (MHRA approved CoolXenon[10] and CooXenon2 studies)[11].

As in the CoolXenon2 study, an Independent Data Review Group (IDRG) will be convened. The IDRG will meet after the

recruitment of every 8th baby during enrolment of infants to the Xenon+cooling group and also review all serious adverse events occurring throughout the study.

2.0 Background and Introduction

2.1 Background

HIE is a major cause of severe cognitive and motor disability in term infants. Until 2005, there was no effective treatment for HIE. Since 1993 we have developed moderate HT as a neuroprotective treatment culminating in a successful clinical feasibility study in Bristol 1998-9 (10 infants). This experimental work led to the "CoolCap" trial[13] followed by the UK "TOBY" trial[14]. In both trials Bristol was a major recruiting centre♦.

The trials have shown that HT significantly reduced disability and cooling has now become standard treatment. As protection from clinically delayed HT (by 4.5h) is only partial, (absolute risk reduction 15%),[15] enhancing its effectiveness as well as starting earlier is paramount. More recent clinical trials have also shown effectiveness of HT.[16]

Since 2003 we have demonstrated that immediate Xenon and immediate hypothermia in combination, increase cerebral protection from 35% (HT alone) to 70% in newborn rats and pigs.[17,18] In 2011 we completed a human neonatal feasibility/safety study of 14 patients undergoing standard cooling therapy for moderate or severe perinatal asphyxia using a Xenon delivery system of the same design. A pilot study, comparing standard cooling to standard cooling plus Xenon, has also been successfully undertaken (CoolXenon2), examining technical ability to recruit with short birth to treatment delays, physiological effects and possible early predictors of outcome. There have been no technical problems due to the breathing system, use of the Xenon gas nor any adverse effects related to these in any of the human studies we have conducted to date. (Protocol CH/2011/3799. Xenon AND cooling therapy in babies at high risk of brain injury following poor condition at birth: RANDOMISED PILOT STUDY (COOLXENON2 STUDY). MHRA reference CI/2011/0061).

2.2 Review of Literature

Critical lack of oxygen delivery to the foetus during labour and delivery results in HIE in 1-3 per 1000 births at term. 60-70% of infants with moderate/severe HIE die or survive with serious disability such as cerebral palsy and/or learning difficulties. Some of the survivors are unable to walk, sit up, control head movement, feed or communicate. Until 2005 there was no treatment that had been shown to improve outcome in such infants.

Since 1993 Marianne Thoresen's group have taken moderate HT as a neuroprotective treatment through all the steps of therapeutic development starting with small (rat) [19,20] and large (pig) [21,22], animal models to a clinical feasibility study in Bristol (body or head cooling in 10 infants).[23]In collaboration with others our experimental work and clinical pilot led to the American funded "CoolCap" trial where Bristol was a major recruiting centre. The results were published in 2005 and showed a therapeutic benefit from hypothermia.[13] There are few cardiovascular effects of cooling and there may be some cardiac protection in addition[23,24]. This study was followed by the American National Institute of Child Health and Development body cooling trial which also showed reduced mortality and improved neurological outcome in the cooled group[25] We and colleagues in the UK (PI Denis Azzopardi) received MRC funding to carry out the largest of all the cooling trials, the "TOBY" trial recruiting a total of 325 infants. St. Michael's Hospital was a major centre in these trials and MT is one of the principal investigators and grant holders for the TOBY trial.[14] We have received further MRC funding to carry out long term follow up of this cohort which is on-going. Other trials in Europe[26]and Australia (ICE trial)[27] have also shown significant improvement in cooled infants.

In these 5 big trials[13,14,25-27], HT reduced death or disability from 66% to 51%. The number needed to treat (NNT) to prevent death or disability is 7.[16] Hypothermia has also been shown to be effective in adults after cardiac arrest and is being considered for other conditions where brain injury is the major problem (e.g. stroke, head trauma).

At the end of 2006, we finished recruiting to the TOBY trial and HT became standard of care in Bristol. Our local incidence of poor outcome in this group has been further reduced to 39%[1], and more recently as low as 34%, as compared to 48% for the cooled group in "TOBY"). Compared to our own historical controls using the same entry criteria where the incidence of poor outcome was 63% without active treatment, our Bristol cooling protocol has reduced the risk of a poor outcome by 38%. Thus HT improves outcome but does only partly abolish death or disability. There is clearly a need to

♦ Principal Investigator: Marianne Thoresen

explore other therapies that can be combined with HT to further improve outcome in this devastating condition.

For the past 8 years our research group has been investigating the rare anaesthetic noble gas Xenon, which is showing great promise as a neuroprotectant in HIE. Despite being considered "noble" because it is almost inert in conventional chemical reactions, Xenon is not completely inert in biological systems and has been known as an anaesthetic for decades. The group has built upon the initial work in cell cultures[28,29] by conducting both small and large animal studies of Xenon+cooling with positive results. In particular a suitable delivery and monitoring system capable of providing Xenon to the inhaled gas mixture of a mechanically ventilated infant in the neonatal intensive care unit has been developed and evaluated in a pig model of global HIE – replicating the clinical scenario in pigs of similar weight to human neonates. This has been developed by Dr John Dingley, Reader in Anaesthetics, a member of the Thoresen collaborative group and honorary consultant at University Hospital Bristol. The group has recently used this system in the first human neonatal feasibility/safety trial of Xenon + cooling in this group of neonates, which was carried out in Bristol over 12 months and successfully completed in September 2012 (including 18m follow-up) (See Appendix 4). The safety of Xenon has been further demonstrated by Sabir *et al*,[30] who showed there was no increased apoptosis after breathing Xenon for 24 hours, compared to a 10x increase when breathing isoflurane in a piglet model.

2.3 Rationale for the Study

We have undertaken a series of experiments since 2003 using Xenon with different dosages, durations and delays before treatment onset in our neonatal rat brain injury model and found that Xenon approximately doubles the neuroprotective effect in combination with hypothermia, compared to hypothermia alone[17,31,32]. Since 2006 we have investigated Xenon-hypothermia (Xe-HT) in our newborn piglet model of global hypoxia-ischemia.[18,33] With this model we have extensively examined the effect of Xe-HT, Xe alone, HT alone as compared to no treatment. We have examined the effect on the brain and all organs, in particular the heart, liver, kidney and lungs. We have also examined the biochemical changes in the blood throughout the treatment period in all the treatment groups.

In brief, we have not found any adverse effect of Xenon in the piglet model – and we can document:

- Improved neuroprotection compared to HT alone[18]
- Better cardiovascular stability (reduced need for inotropic support)[34]
- No extubation delay[18]
- No increase in oxygen requirements[18]

Our large animal trial of Xe-HT in piglets recently concluded with a very positive outcome supporting the result we found in rats, i.e. adding Xenon to HT doubles the neuroprotection in both animal models. Also we found in both studies that HT and Xenon had additive effects with no interaction.

A human neonatal feasibility study combining cooling (the established treatment) with Xenon inhalation via mechanical ventilation was completed by this group on April 5th 2011 (The CoolXenon study). This small scale safety study was a necessary requirement before a randomised clinical trial of cooling+Xenon that would examine neurological outcomes could be conducted. The findings were similar to those listed above and have been documented. The design was such that the Xenon duration increased from 2h to 18h, and 3 patients received the maximal duration of 18h Xenon which has been shown to be neuroprotective in the pig model. Increasing the duration had no adverse effect on the physiological parameters. Furthermore, the data from this study have confirmed that it is appropriate to start ventilation with Xe as early as 5h post-birth.

A second study (CoolXenon2) has also been successfully undertaken. This study initially was for 24 babies, 12 in each group. One main aim was to establish if we could recruit sufficient neonates within the 5h time limit from birth to Xenon. During this period, when Xe was started only after arrival in Bristol, we lost approximately 40% of potential recruits due to not being able to meet this ambitious time limit. After starting to run the Xe delivery system in the dedicated retrieval ambulance, we applied for an extension to the CoolXe2 study to evaluate the impact on recruitment this would have (8 additional patients, 4 per group) and from that point we have had no missed recruitment due to the 5h time interval.
The CoolXenon2 study revealed an unknown and unforeseen effect of Xenon; Xenon in itself depresses the aEEG trace during delivery which means it is unreliable as an early outcome predictor until the treatment has finished which will for most infants say ≥25h of age (5h old when starting xe +18h treatment + at least 1h recovery). We observed that Xenon depresses seizure activity *per se* (SPR abstract 2013, Appendix 4) and we observed that if Xenon was stopped very fast

seizures could occur on stopping Xenon. This means that there is a period where "time to recovery of normal trace" may or may not be affected by Xenon. These data illustrate the importance of examining a small dataset like CoolXenon2 when a new treatment is administered. This means that when analysed the traditional way, aEEG is not reliable as an outcome predictor during the whole of the Xenon administration periods and a few hours afterwards. Since we cannot quantify the aEEG response we do not have a validated early outcome predictor from which we can make a power calculation.

Therefore, in CoolXenon3 we will record all the same data as in CoolXenon2 and we will use Bayley III converted to Bayley II as appropriate.[11] The Bayley clinical neurodevelopmental examination at 18months of age is the primary outcome measure. In the standard treatment, all cooled babies currently undergo MRI brain assessment between 7-10 days of life to confirm the diagnosis of HIE by demonstrating the brain lesions consistent with hypoxia-ischaemia and to prognosticate long-term outcome for parents. In routine clinical practice, some of the cooled babies undergo MRI brain examination around 48 hours of life, when there are concerns of poor recovery of aEEG and abnormal neurology to estimate the severity of brain injury. Undertaking MRI brain assessment around 48 hours of age for all babies during cooling in both cooling and xenon+cooling arms of the study (after completion of xenon) will offer valuable data including early predictors of long-term outcome, assessing the severity of brain injury and progression of brain injury and early effect of xenon on brain injury. Our team is very experienced in looking after the cooled babies during transport and MRI examination. Other putative short-term predictors will continue to be recorded as secondary outcomes. With this enhanced dataset we will be able to validate any early predictors in infants cooled early with or without added Xenon.

There have been no technical problems due to the breathing system, use of the Xenon gas, nor any adverse effects related to these in any of the human studies we have conducted to date.

The safety of Xenon for the immature brain has been demonstrated by no increase in apoptosis[30] after 24 hours of breathing the gas in a neonatal piglet model; in comparison breathing isoflurane for the same duration resulted in a 10x increase in apoptotic cell death

3.0 Objectives

The aim of this study is to examine the potential neuroprotective effects of the cooling+xenon combination when compared to cooling alone.

3.1 Primary Objective

Our hypothesis is that the Bayley Scales of Infant and Toddler Development (Bayley III) scores at 18m of age will show a trend towards an enhanced neuroprotective effect of the Xenon-cooling combination over cooling alone.

3.2 Primary Endpoint

This is a randomised two group pilot outcomes study comparing Bayley scores between;

1) Xenon inhalation for 18 hours in combination with whole body cooling to 33.5 °C for 3 days, and
2) the established treatment of cooling the body down to 33.5 °C for 3 days.

This will be applied in term newborn babies who display signs indicating moderate or severe brain injury following poor condition at birth.

3.3 Secondary Endpoint

The following potential predictors of outcome will be used:

a. Time to recovery after birth of a normal aEEG[1]
b. MRI scan around 48hours and around day 7 of life, to assess lesions consistent with hypoxic-ischaemia,[2] absolute quantification of thalamic NAA and white matter fractional anisotropy using tract based spatial statistics.[3,4,35]
c. Peak LDH value within 72h of life[5,36]
d. Resistance Index (RI).

We will also collect data on:

e. Plasma glucose at birth (in cord blood)
f. Age in hours after birth when plasma lactate has fallen to 5mmol/L
g. Different inotropic drugs needed and the total duration in hours of any inotropic support

h. Number of anticonvulsants given
i. Amount and duration of sedation (e.g. plasma morphine level), stress level (plasma, salivary, urine cortisol level and adrenocorticotropic hormone)
j. Age at full oral feeds (breast or bottle or tube)
k. Neonatal hearing screening result before 14 days of life
l. Clinical examination at birth, at 7 days of age and at discharge including weight and head circumference

Neurodevelopmental follow up is a part of the routine follow up of infants with HIE, Bayley III examination at 18 months of age.

Disability will be defined as any of:

m. Bayley III Cognitive Composite score less than 85.[11]
n. Bayley III Motor Composite score less than 85 (Bayley scores will also be expressed as a Developmental Quotient (DQ) calculated from developmental age scores as this allows a larger range of scores for those functioning at a low level)[37]
o. Bilateral cortical visual impairments
p. Hearing loss needing amplification > 40dB.

In addition any Adverse Events will be recorded and criteria have been devised for stopping the study early if necessary. The IDRG will review data when 8, 16 and 24 infants have undergone Xenon treatment.

3.4 Tertiary Endpoint

A series of variables that are among the most relevant for evaluation of safety were examined closely in our previous studies. In both groups we will continue to routinely monitor and record the same set of physiological variables in the proposed study, for a subset of data for a longer time period until after rewarming.

- Endpoint 1 – defined as *Mechanical ventilation and gas exchange parameters*
  - The following will be measured before (from start cooling), during and after Xenon administration (for 12h or until 12h after end of cooling hours pending which variable):
    - transcutaneous oxygen saturation
    - end-tidal carbon dioxide concentration
    - blood gas data
    - mechanical ventilation requirements (rate, peak inspiratory pressure, inspiratory time, PEEP settings)
    - FiO_2
    - average percentage Xenon in inspired gas mixture (when applicable)
    - tracheal tube cuff pressure.
- Endpoint 2 – defined as *Haemodynamic parameters*
  - The following will be measured before, during and after Xenon administration:
    - systolic, mean and diastolic arterial pressures; and heart rate variability
- Endpoint 3 – defined as *Inotropic drug requirements*
  - The following will be recorded before, during and after Xenon administration until 12h after end of cooling:
    - drug, dose and duration
- Endpoint 4 – defined as *Marker of myocardial impairment/insult*
  - The following will be recorded before and after Xenon administration:
    - plasma levels Troponin T (marker of myocardial cell death) recorded before and after Xenon administration until 12h after end of cooling:
- Endpoint 5 – defined as timecourse and peak value of *Markers of hepatic impairment*
  - The following will be recorded before and after Xenon administration until 12h after end of cooling:
    - alanine aminotransferase , alkaline phosphatase and lactate dehydrogenase
- Endpoint 6 – defined as timecourse and peak value of *Markers of renal impairment*

- The following will be recorded before, during and after Xenon administration until 12h after end of cooling:
  - oliguria - defined as urine output < 1 ml/kg/h - from age > 24h
  - raised creatinine > 100 at > 24h of age.

- Endpoint 7 – defined as *Evidence of infection*
  - The following will be recorded:
    - culture proven
    - need for antibiotics for 5 or more days
    - raised CRP occurring within 24h or later.
- Endpoint 8 – defined as *Length of hospital stay including duration of stay in local hospital after discharge from St Michaels hospital*
  - This will be recorded
- Endpoint 9 – defined as *age when full oral feeding was achieved by breast or bottle or tube*
  - This will be recorded
- Endpoint 10 – defined as *Haematology measurements*
  - The following will be recorded before and after Xenon administration (routinely recorded 24 hrly):
    - Coagulopathy - INR > 2 and/or APTT> 50
    - FBC
- Endpoint 11 – defined as *Serum chemistry measurements*
  - The following will be recorded before and after Xenon administration
    - serum sodium, potassium, chloride, magnesium, phosphate, calcium, (urea, creatinine as above), glucose, (liver enzymes as above), bilirubin, albumin
    - drug levels may be analysed on clinical indication (Gentamicin levels, Phenobarbital levels)
- Endpoint 12 – defined as aEEG background pattern and *Evidence of seizures*
  - The following will be recorded before, during and after Xenon administration until 12h after end of cooling:
    - evidence from continuous aEEG/EEG recording
    - any anticonvulsant treatments given.

4.0 Study Design

This is a prospective randomised comparative clinical outcome study conducted in St Michael's Hospital, Bristol and Hammersmith Hospital, London.

4.1 Study design and procedures

Infants will be eligible for Xenon if the St Michael's hospital standard inclusion criteria for cooling are met and additional inclusion criteria for Xenon administration are met.

4.1.1 St Michael's hospital standard inclusion criteria for cooling

Standard Hypothermia Treatment Criteria for 72 hrs of cooling — all of criteria A, B and C.

A: *Infants ≥ 36.0 weeks gestation (estimated or clinical assessment) with at least ONE of the following:*

i. Apgar score of ≤5 at ten (10) minutes after birth
ii. Continued need for resuscitation, including tracheal or mask ventilation, at ten minutes after birth
iii. Acidosis defined as either umbilical cord pH or any arterial, venous or capillary pH within 60 minutes of birth less < 7.00
iv. Base deficit ≥16 mmol/L in umbilical cord blood sample or any blood sample within 60 minutes of birth (arterial or venous blood). If the infant meets criterion A then assess for neurological abnormality using criterion B and C (by trained personnel):

B*: Moderate or severe encephalopathy as evidenced by any of the following:*

i. Altered state of consciousness (reduced or absent responses or pathological irritability and hyper responsive and at least ONE or more of the following:
ii. Hypotonia
iii. Abnormal reflexes including oculomotor or pupillary abnormalities
iv. Absent or weak suck
v. Clinical seizures, as recorded by trained personnel

AND

C: At least 30 minutes duration of amplitude-integrated electroencephalography (aEEG) recording that shows abnormal background aEEG activity. The decision to cool is based on the worst 30 min section of the aEEG, not the best[38] or seizures (clinical or electrical) thus meeting ONE of the following:

i. Normal background with some (> 5 min) electrical seizure activity
ii. Moderately abnormal activity (upper margin of trace >10μV and lower margin <5μV)
iii. Suppressed activity (upper margin of trace <10μV and lower margin of trace <5μV)
iv. Definite seizure activity

4.1.2 Exclusion criteria for cooling in the CoolXenon3 study

a. Infants expected to be greater than 3 hours of age at the time of starting cooling treatment.
b. Futility. Where prognosis is considered to be hopeless e.g. no cardiac output for 20 minutes.

4.1.3 Additional inclusion criteria for Xenon

Before being considered for additional inhaled Xenon therapy via the breathing gas mixture, the infant would need to meet further additional entry criteria (all must be met):

i. Intubated, ventilated, sedated, being cooled.
ii. ≤5 hours old
iii. Any seizures under control.
iv. Weight > 2nd centile for gestational age
v. Stable cardiovascular parameters; Mean arterial pressure >40mmHg.
vi. Oxygen requirement via mechanical ventilator < 40%.
vii. Positive End Expiratory Pressure (PEEP) requirement ≤ 8cm H_2O
viii. Arterial/capillary/venous pCO_2 <7.0kPa (ideally arterial). Venous can be higher if peri-arrest and tissues have been very recently Ischaemic
ix. Postnatal age ≤5 hours
x. Absence of major congenital abnormalities, imperforate anus and in particular any bowel obstruction, congenital abnormalities suggestive of chromosomal anomaly or other syndromes that include brain dysgenesis. Congenital syndromes affecting the brain should be excluded when diagnosed.

4.2 Consent

Informed written consent will be obtained from a parent (only the mother can consent if parents are not married) after a full verbal and written explanation of the study by a named physician or trained nurse like an advanced nurse practitioner. Such staff will also meet with parents during the intervention period to ensure that they understand the study procedures and continue to consent to participate in the study. Information can be given by local trained staff and consent can also be taken over the phone with the mother if she is unable to be available for the consent process (e.g. cannot be moved shortly after Caesarean Section).

Approval for the study is being obtained from the Regional South West Research Ethics Committee covering St Michael's Hospital.

Most of the patients (61%) will be born outside the study hospital (Outborn) and brought to the hospital by a dedicated retrieval team and ambulance.

The local delivery hospitals will have medical staff (e.g. consultants, specialist registrars, advanced nurse practitioners) trained in consenting for the CoolXenon3 trial. A training log with signatures, a short CV and competency approval will be kept centrally.

Parents will receive a written information leaflet (1 page, short version) when the infant is eligible for cooling treatment. Staff will discuss CoolXenon3 with parents as soon as practically possible. Parents will be able to speak on the phone to a member of the treatment team if they need further information and help with the consent process. Parents will also be offered the opportunity to speak to members of the treatment team that are picking up the baby for transport.

Trained staff on the transport team can take consent for the study.

A more detailed information sheet regarding the trial will be given to parents after the first conversation or at the earliest suitable time.

4.2.1 Which of the parents can consent to letting the child enter the trial?

- Either parent if they are married.
- If the parents are not married, only the mother can consent - provided she is awake and understanding (e.g. not under the influence of anaesthetic drugs)

If the mother is not in a state to understand the situation and the biological father does currently not have legal parental responsibility he can assent for the trial until the mother is awake/understanding the child's condition and able to decide on consent.

A parent who is eligible to consent can, at any time and without a specific reason, withdraw the child from the study. In this situation, Xenon treatment will then be stopped as soon as possible and medically safe (within minutes).

4.3 Randomisation

The babies entered into the study will be randomised into two groups on a 1:1 basis. Randomisation will be via sealed opaque envelopes containing details of treatment allocation and [an] identifying adhesive label(s) to be attached to the CRF binder. Neonates meeting all the entry criteria can be included. In this study early randomisation before 5h of age is paramount. The maximum target delay before starting cooling is before 3h of age and for Xenon this value is 5h of age. These are maximum delay values however, so consent and randomisation need to take place as early as possible.

4.4 Clinical management

The neonates would be sedated, mechanically ventilated and cooled according to the protocol for cooling listed above, as this is standard treatment. In the case of retrieved babies, cooling is always started before transfer to Bristol (active or passive cooling). If not before, active cooling starts when the transport team arrives. Antibiotics, fluid management and sedative drugs used would be unmodified.

Neonates would be entered into the study according to the set criteria within a maximum of 5hrs of birth with respect to adding Xenon to the existing underlying standard cooling regime which has started within 3 hrs (passive or active).

4.4.1 Specific clinical management of cooling group

This group will be cooled according to the normal protocols of the hospital as this is standard treatment. In standard care, some of the cooled babies will undergo MRI brain assessment around 48 hours of life in addition to the routine MRI brain assessment around 7 days of life, to provide early information about severity of brain injury. In the study, all the babies will undergo MRI brain examination around 48 hours of age. At this time point the babies will be continuing the cooling therapy. Our team is experienced in looking after the cooled babies during transport and MRI examination with least discomfort to the babies. Moreover, all the babies will receive continuous morphine infusion to keep them comfortable during cooling, as part of their standard protocol.

4.4.2 Specific clinical management of Xenon+cooling group

After initial resuscitation and attainment of a stable state, and inclusion in the study, the tracheal tube would be replaced with a cuffed commercially available tracheal tube (Kimberley-Clark microcuff) of the same or 0.5mm smaller size as the existing one and the cuff inflated until leakage of gas around the cuff had ceased audibly and according to the ventilator monitor. This particular tube has a high volume, low pressure cuff and is specifically designed for long term use on the paediatric or neonatal intensive care unit. The cuff pressure will be monitored and recorded at the time of initial inflation, during Xenon administration every 2 hours and just prior to final deflation. If there are any post extubation adverse events such as stridor, or extubation fails secondary to vocal cord, nerve or subglottic damage, these will be recorded. In our

recent studies, of the 28 babies given Xenon, one developed post-extubation stridor requiring steroids♦.

1. The dose (fraction of Xenon in breathing gas) planned for each patient in the Xenon+cooling group will be 50%. It will be applied for 18 hours. It will be commenced as soon as is practicable but no later than 5h after birth once all the entry criteria / consent requirements have been met.
2. If a baby is being retrieved from another local hospital to the study centre (Bristol) using the existing Bristol-provided retrieval team and ambulance, AND they have been consented and randomised to receive Xenon AND if it is deemed probable that the delay between birth and the start of Xenon in Bristol would exceed an interval of 5 hours, then if logistically possible an assigned research team member will accompany or meet the retrieval team and commence Xenon delivery at the local hospital or during the return transfer of the baby to Bristol.
3. Full data sets will be acquired in two groups of 26 patients. The mortality rate in the patients referred for cooling to Bristol is 10.3%.
4. Cooling will be for 72 hours using the clinically chosen cooling device in both groups, as is current standard practice for cooling.
5. In standard care, some of the cooled babies will undergo MRI brain assessment around 48 hours of life in addition to the routine MRI brain assessment around 7 days of life, to provide early information about severity of brain injury. In the study, all babies will undergo MRI brain examination around 48 hours of life. At this time point, babies will have completed the xenon therapy and will continue with cooling therapy. Babies will be kept comfortable with continuous morphine infusion as per the standard local protocol. Our team is experienced in transporting cooled babies to the MRI and looking after the cooled babies during MR examination. This MRI will provide valuable data regarding the severity of brain injury, early effect of xenon on brain injury and determine the progression of brain injury with xenon+cooling compared with cooling alone.

4.5 Further information on the oxygen fraction in the breathing gas mixture

The Xenon we intend to use (LenoXe) is currently licensed for anaesthesia in adults. The LenoXe SmPC specifies that Xenon should be administered (for adult anaesthesia) with at least 30% oxygen in the breathing gas mixture.

It is our intention in this study to administer an oxygen fraction in the breathing gas to ensure adequate oxygenation of the blood of the neonate, but not to give an excess, as there is also a real risk of hyperoxic brain damage in this group of neonates if the oxygen fraction is too high. Consequently, in a neonate with normal lungs being sedated and ventilated for cooling rather than for any lung problems, the inspired oxygen fraction could be lower than 30% (air is 21% for example). The lowest fraction of oxygen in the breathing gas will always be used, sufficient to maintain an oxygen saturation on blood gas analysis or pulse oximetry of at least 96%, especially as hyperoxia is considered to be very harmful to the brain in infants who have just had a hypoxic-ischaemic insult.

The reason the LenoXe SmPc specifies 30% oxygen is following the normal practice of anaesthesia using other gases and vapours, because during anaesthesia in adults the ventilation/perfusion mismatch in the lungs becomes very slightly less efficient than in the awake state. This is due to changes in muscle tone, diaphragm position/shape and effects of gravity on distribution of blood flow through regions of the lung. Although humans are designed to breathe air (21% oxygen), a patient with normal lungs under anaesthesia would traditionally be given approximately 30% oxygen (more if there were any lung problems as clinically indicated).

It should be noted that this anaesthetic tradition to use a slightly higher inspired oxygen fraction than might strictly be required developed before it became commonplace and later mandatory to use pulse oximetry to continuously monitor oxygen saturation of the blood and end-expired carbon dioxide monitoring to continuously monitor the adequacy of ventilation (in terms of carbon dioxide removal from the blood) which became widespread in the late 1980's / early 1990's.

In the neonates of this study the situation is different as they have all recently suffered a hypoxic-ischaemic (HI) insult to the brain and other organs around birth. Ventilating them with a gas mixture containing an oxygen fraction even slightly higher than necessary is considered highly undesirable as in this situation it can actually worsen the subsequent brain

♦ In a recent very large study of cuffed tracheal tubes for anaesthesia in infants and neonates, the use of a cuffed tube did not increase risk of post-extubation stridor. In 2246 children, post-extubation stridor was noted in 4.4% of those with a cuffed tracheal tube and 4.7% of those with uncuffed tubes, with a much lower tube exchange rate in the cuffed tube group.

injury. This explains recent changes to resuscitation equipment for the newborn where the 100% oxygen supplies have been replaced with air/oxygen blenders. The new ILCOR Resuscitation Guidelines[39] state that term newborns should first be resuscitated in air, then with a higher concentration of oxygen if pulse oximetry or clinical signs suggest poor oxygenation.

It is worth noting that variations in ventilation/perfusion throughout the regions of a healthy neonatal lung are less affected by anaesthesia/sedation than in a (much larger) adult so the physiological (anaesthetic) justification for automatically using 30% oxygen is weaker in the neonate.

However the more relevant points with respect to this study are that although in anaesthesia practice it may be normal and quite harmless to set an inspired oxygen fraction slightly higher than strictly necessary, in neonates that have suffered a hypoxic-ischaemic insult to the brain and other organs around birth, it is considered ***highly undesirable*** to administer an inspired oxygen fraction that is any higher than the amount required for adequate oxygen delivery to the organs. We have recently shown experimentally that 30 min of 100% oxygen after hypoxia-ischaemia increased injury[40]. Consequently,

- we monitor *oxygen saturation of the blood* of the neonate using continuous pulse oximetry (with audible and visual alarms),
- we continuously monitor *adequacy of ventilation* with a neonatal end-expired carbon dioxide monitor which reflects the systemic arterial carbon dioxide content. While mandatory in adult intensive care practice this is not yet the case in neonatal practice due to unavailability of suitable monitors for neonates, a situation which is now changing.
- We also routinely monitor oxygen saturation of the blood periodically in ventilated neonates by analysing arterial blood samples using a blood gas analyser. In neonates that have experienced an HI insult around birth the attending physicians are not only checking that the oxygen content is not too low but also *they are checking it is not too high*. A slight elevation that would normally be acceptable is not acceptable in this particular group of babies and if too high they will actively request that the inspired oxygen fraction in the breathing gas be reduced.
- As a result of the above, *the inspired breathing gas might contain a theoretical maximum oxygen fraction of 40% as stated in the protocol if clinically required due to lung problems , but also this may for the above reasons be deliberately set as low as is required to maintain an oxygen saturation of >=96% if the lungs are normal.*

The desired inspired Xenon fraction is to be set at 50% as per the protocol. The “balance gas” in situations where a moderate oxygen fraction is being used will be nitrogen. This will simply be room air which although containing 21% oxygen is otherwise mainly composed of nitrogen.

4.6 Vigilance and monitoring

This will be ongoing during every case and include:

- Blood gases 2-4 hourly or as clinically indicated
- Cardiac echo /Doppler (where available)
- Blood Pressure
- Heart rate
- Haemodynamic Monitoring. Lidco Plus, Advantech, Ref HM 71-02, model PPC-150M. Lidco Ltd. 16 Orsman Road, London, N1 5QJ
- Oxygen saturation by pulse oximeter (SaO_2)
- Xe concentration and O_2 concentration in gas mixture
- End Tidal CO_2 ($ETCO_2$) (End expired carbon dioxide will be measured using a commercial medical monitor ($ETCO_2$ monitor) designed to monitor adequacy of ventilation in neonatal intensive care patients which displays the exhaled carbon dioxide as a value, but additionally as a waveform and uses a mainstream sensor in the breathing circuit)

4.6.1 Action to be taken with respect to typical problems in this patient group

1) Hypotension or inadequate cardiac output:
    - 10ml/kg 0.9% Saline (or occasionally 4% human albumin or blood) as required (x2)

- Dopamine infusion commenced (up to 20mcg/kg/min) followed by Dobutamine (up to 20mcg/kg/min), then Hydrocortisone up to 2.5mg/kg 6 hourly or other inotrope as indicated
- If not effective then Xe delivery will be stopped and conventional ventilation/gas mixture resumed.

2) Low SaO_2 or low oxygen partial pressure on blood gas analysis:
   - Increased oxygen fraction in breathing gas mixture as required up to maximum of 40%
   - If not effective, then Xe delivery stopped and conventional ventilation/gas mixture resumed. If reason for high FiO_2 is easily solved, e.g. improved by surfactant delivery or changed ventilation settings or clearing a blocked tube, then Xenon can be resumed.

3) High $ETCO_2$ or CO_2 partial pressure on blood gas analysis:
   - Increase peak pressure and/or breathing rate settings on mechanical ventilator
   - If not effective then Xe delivery stopped and conventional ventilation/gas mixture resumed. This is readily achieved by disconnecting the ventilator hoses from their point of attachment to the Xenon breathing system and connecting them directly to the tracheal tube of the neonate.

At the end of the prescribed period of Xe delivery the tracheal tube would be reconnected directly to the mechanical ventilator and normal ventilation and gas mixtures set as clinically appropriate. The cuff of the tracheal tube would be deflated. This tracheal tube would be replaced with an uncuffed tube at a later timepoint as clinically indicated or kept until the patient was extubated after rewarming. Cooling regimes last for 72hrs before slow rewarming commences (0.1-0.4°C per hour). The standard cooling protocol would continue for many more hours after cessation of the additional Xenon regime.

4.7 Risks/benefits and risk minimisation

4.7.1 Potential risks from Xenon

The known effects of Xenon in humans have all been derived from adult anaesthesia studies. Xenon is known to have minimal effects on the strength of the heart contractions or the blood pressure[41-44]. A slight slowing of pulse rate has been seen in some studies but this is often not statistically significant.

Xenon is analgesic and causes sedation. These might be considered useful properties in a sedated baby on an Intensive Care Unit.

Xenon is a dense gas. Erring on the side of caution we would not use it in this early study in any baby with airway narrowing or similar lung problems, the exclusion criteria reflect this. Note that the use in piglets weighing 1.5 kg and small airways did not display any ventilation/airway problems (Appendix 4). As with other anaesthetic gases it can diffuse into gas filled cavities in the body such as the bowel. This happens only slowly in adults, and more slowly than with other anaesthetic gases such as nitrous oxide, however for this reason it would not be used in any situation where the bowel was obstructed (our Xenon inclusion criteria reflect this).

In animal neonatal studies, there have been no side effects and we can document:

- Improved neuroprotection[18]
- Better cardiovascular stability[34]
- No extubation delay (delay coming off the mechanical ventilator)[18]
- No increase in oxygen requirements[18]

From studies using Xenon as an anaesthetic in adults, of which there are many, the experience is that Xenon has remarkably few side effects and in particular produces minimal haemodynamic disturbance compared to conventional anaesthetics[41-44].

As Xenon is almost chemically inert, it is eliminated from the body completely unchanged – it is eliminated from body tissues via the circulation and lungs at the end of the procedure. Xenon, although uncommonly used due to its high cost, does therefore have a license for use as an anaesthetic in adults in several countries, including the UK.

In 2010/2011 our group undertook and successfully completed the first human neonatal feasibility/safety study of Xenon+cooling, mainly 50% Xenon for periods of up to 18hrs. All survivors underwent a Bayley examination at 18 months of age. From this study, in terms of safety, we can document:

- It is feasible to give 50% Xenon for up to 18h in infants undergoing therapeutic hypothermia[10]

- Only 0.2-0.3L/hour Xenon was used with a closed loop Xenon breathing system
- A cuffed tube did not adversely affect ease of extubation
- Xenon is sedative and other sedation is needed when Xenon is discontinued
- No significant cardiovascular or respiratory changes before, during and after Xenon
- In one child with abnormal/immature lungs, Xenon did not increase FiO_2 requirement.

See Appendix 3

From the perspective of babies who may already be unwell, the main points to note therefore are:

i) Xenon is not broken down to any "by products" by the metabolism of the body as many other drugs are, as it is an almost chemically "inert" elemental gas.

ii) Xenon is very free of side-effects in the adult experience, which may be due to this "inert" nature, but perhaps most importantly:

- Xenon is eliminated from the body completely unchanged via the lungs within a few breaths. This means that if there is any problem, unlike the situation with any drug injected into a vein, Xenon is a *reversible treatment*, ie, it can be easily removed completely, rapidly and unchanged from the body at any time. *This ability to reverse application of Xenon and rapidly eliminate it unchanged from the patient in the event of any problem is, although unusual, a key factor from the perspective of safety.* When the Xenon is being administered, a special breathing circuit will be connected to the ventilator designed for this purpose. It will be operated by a member of the team who has documented experience running and troubleshooting with this equipment.

4.7.2 Potential risks from breathing system

We are using the same breathing circuit to deliver this gas as in previous MHRA approved studies; CoolXenon and CoolXenon2 (MHRA study references CI/2009/0043, CI/2011/0061 respectively). This has been developed over many years by a Consultant Anaesthetist who is a co-researcher on this team and world leader on this subject. Prior to use in these human studies, it has been tested extensively in laboratory neonatal pig simulations of the condition (see section 4.3.1).

To minimise risk of any technical problems:

a. The equipment meets all necessary international medical device safety and quality standards. It has been professionally constructed using medical grade materials.
b. The design is such that the Xenon system is placed between the standard mechanical ventilator and the baby. This means that if there is any technical problem, the ventilator can be immediately connected directly to the baby and the Xenon system removed in one simple intuitive operation.
c. The gas composition, in particular oxygen, of the circuit gas will be continuously measured, as will Xenon and the $ETCO_2$ (which reflects the arterial value).
d. The breathing system has an overpressure release valve in the event of any mishap.
e. Furthermore, if there were any problem due to the Xenon gas itself, reconnection directly to the ventilator would also allow all the Xenon to be breathed out from the baby within a few breaths, i.e. the Xenon treatment is rapidly reversible.
f. The Xenon will be delivered by trained personnel who have also been involved with laboratory trials using Xenon in animal models and previous MHRA approved clinical trials involving the device.
g. The possibility of interaction between the Xenon gas and the materials of the breathing circuit has been considered. As Xenon is chemically "inert" i.e. does not form chemical compounds except in the most extreme circumstances, there is no perceived risk.
h. A full risk assessment in accordance with standard medical device regulatory requirements has been undertaken and thoroughly documented.
i. The device meets all of the Essential Requirement of the EU Medical Device Directives, with the exception of those being evaluated in the study.

This design of breathing system has now been evaluated in two MHRA approved studies with this patient population. Summary data from the CoolXenon2 study:

	Cooling only (n=14)	Cooling + Xenon (n=14)
Birth weight, gram	3648 ± 503	3455 ± 394
Gestational age, weeks	40.7 ± 1.3	40.1 ± 1.3
Female, nr (%)	8 (57)	8 (57)
Outborn, nr (%)	9 (64)	8 (57)
Apgar 1 minute	2 (0-2.25)	1 (1-3.25)
Apgar 5 minutes	4 (3-5.25)	4 (2.5-6)
Apgar 10 minutes	6 (3.75-7)	5 (3-8.25)
Cord pH	7.03 ± 0.17	7.00 ± 0.19
Cord BE	-12.9 ± 5.2	-12.3 ± 8.7
Received anticonvulsants,	8 of 14	9 of 14
Received inotropes, %	71	57

Table 1: Summary preliminary data from CoolXenon2 study (BW, GA, Cord pH and BE are mean±SD apart, Apgar is median with IQR)

4.7.3 Use of cuffed tracheal tube

After onset of cooling and attainment of a stable state, the tracheal tube would be replaced with a commercially available cuffed tracheal tube (TT) (Kimberley-Clark microcuff) of the same or 0.5mm smaller size as the existing one.

To minimise the gas pressure in the cuff:

i) It would be inflated until leakage of gas around the cuff had just ceased audibly and also according to the ventilator monitor.

ii) This particular tube has a high volume, low pressure cuff and is specifically designed for long term use on the paediatric or neonatal intensive care unit. Publication of a large scale randomised multicentre trial of uncuffed versus modern cuffed tracheal tubes in small children (0-5yrs, n=2246) has recently taken place. The incidence of complications was no greater than that seen with uncuffed tubes. The conclusions were *"The use of cuffed TTs in small children provides a reliably sealed airway at cuff pressures of <or=20 cm H_2O, reduces the need for TT exchanges, and does not increase the risk for post-extubation stridor compared with uncuffed TTs"*[45].

iii) The cuff pressure will be monitored and recorded at the time of initial inflation, during Xenon administration every 2 hours and just prior to final deflation. It will also be minimised to typically 8-10 cm.H_2O pressure (maximum permitted cuff pressure in above quoted study was 20 cm.H_2O).

Any Adverse Events at or around the time of extubation, such as stridor or failure of extubation secondary to vocal cord, nerve or subglottic damage will be recorded. In this way any theoretical risk of post-extubation stridor will be minimised.

4.7.4 Experience with cuffed tube in neonates with Xenon

In 2010/2011 our group successfully undertook and completed the first human neonatal feasibility/safety study of Xenon+cooling, mainly 50% Xenon for periods of up to 18hrs. From this study, in terms of safety of the cuffed tube we can document:

- Babies were reintubated with a cuffed tube 0.5mm smaller than the uncuffed tube that had been in place previously. Cuff pressure was measured frequently during the period of Xenon delivery and the cuff was deflated post-Xenon delivery.
- The target cuff pressure was the lowest cuff pressure we could achieve without there being any gas leakage past the cuff. This was typically 11cm H_2O, - much lower than the maximum of 20 cm H_2O used in the above randomised tracheal tube study
- We observed stridor post-extubation in 1 of the 14 babies.

In the CoolXenon2 study we have observed stridor post-extubation in 2 of the 28 babies recruited to the study; one in the cooling only group and one in the cooling+Xenon group. These episodes of stridor were iatrogenic (due to head positioning and sedation effects from morphine) and not related to the cuffed endotracheal tube or Xenon. We aim to maintain the infants head in the midline and slightly extended. Infants are turned every 8 hours maintaining the midline position with slight extension.

4.7.5 Cleaning/disinfection

The entire circuit is single-use. Between patients, the entire breathing circuit and hoses will be replaced with a fresh item.

4.8 Potential benefit to research participants

The hypothesis is that babies enrolled into the study may derive additional neurological benefit from the Xenon/cooling combination exceeding that seen from cooling alone, following the pattern seen in all the *in-vivo* small and large animal studies performed so far.

4.9 Follow Up

4.9.1 Short term information and investigations (*before discharge from hospital*)

These are part of routine clinical practice.

1. Survival
2. Duration of hospitalisation
3. Time to full oral feeds, breast or bottle or tube
4. Hypoxic-ischaemic or other injury[2] and MRI biomarkers such as absolute NAA quantification in Thalamus and white matter fractional anisotropy using tract based spatial statistics[4] on MRI scan, which includes Magnetic Resonance Spectroscopy, undertaken around 48 hours and around day7 of life.
5. Intracranial or intraventricular haemorrhage (on MRI, CT or ultrasound)
6. Major venous thrombosis (on MRI or ultrasound)
7. Resistance Index (Doppler examination of cerebral blood flow velocities)
8. Amplitude integrated EEG (aEEG) continuously recorded until after rewarming. Assessment of time to normalisation of background activity, seizure occurrence and duration
9. Pulmonary hypertension needing treatment
10. Pulmonary haemorrhage
11. Pneumonia
12. Pulmonary air leak
13. Systemic hypotension needing inotropic support
14. Prolonged blood coagulation time assessed before Xenon and at ~24 hours and/or at clinically indicated timepoints
15. Cardiac arrhythmia (not temperature induced low Heart Rate)
16. Thrombocytopenia (platelets <100,000/ul)
17. Culture proven sepsis
18. Necrotising enterocolitis, medical, radiological or surgically proven
19. Plasma markers of global (increased lactate dehydrogenase (LDH)) or cardiac compromise (increased cardiac Troponin T (cTnT))
20. Renal failure treated with dialysis

4.9.2 Short term assessment neurological evaluation

1) Time to recovery after birth of a normal aEEG[1]
2) MRI scan undertaken around 48-hours of life and around day 7 of life[2,4,35,46]
3) Peak LDH value within 72h of life[5,36]
4) First plasma glucose after birth (usually from cord blood)
5) Age in hours after birth when plasma lactate has declined to <5mmol/l
6) Number and type of inotropic drugs needed during stay on the neonatal intensive care unit (typically the period from birth to 3-5 days of age) and the total duration in hours of any inotropic support.
7) Number of anticonvulsant drugs given during stay on the neonatal intensive care unit.
8) Amount and duration of sedation (e.g. plasma morphine levels) and stress levels (saliva, plasma and urine cortisol and Adrenocorticotropic hormone) while ventilated mechanically on the neonatal intensive care unit.
9) Age at full oral feeds (breast or bottle or tube)
10) Outcomes of neonatal hearing screening before 14 days of life
11) Clinical examination at birth, at 7 days of age and at discharge including weight and head circumference

4.9.3 Long term follow up (at 18 months)

Neurodevelopmental follow up is a part of the routine follow up of infants with Neonatal Encephalopathy. Bayley III examination at 18 months of age is used to compare the results from this study with our large local patient database and previous randomised controlled trials (CoolCap, Gluckman 2005; TOBY, Azzopardi 2009)[13,14] where Bayley II was also used: we were a main recruiting centre for these trials.

Disability will be defined as any of:

- Bayley III Cognitive and Language Composite score less than 85.
- Bayley III Motor Composite score less than 85.
    - Bayley III will also be expressed as developmental quotients (DQ) calculated from Developmental Age scores as this allows a larger range of score for those functioning at a low level[37]. In this study a Bayley III assessment[11] will be undertaken and data will be converted to Bayley II MDI and PDI for comparison with historical controls.
- Bilateral cortical visual impairments.
- Hearing loss needing amplification > 40 dB.

4.9.3.1 Additional follow up

We will do both short-term (14 days) and long-term (18 months survival) follow-up. In cooled babies we have shown that short-term predictors such as MRI at day 8[2], time to normal aEEG[1] and peak LDH before 72h[5] predict long-term outcome with a positive predictive value of 84%, 92% and 80% respectively. Absolute quantification of N-acetyl aspartate in Thalamus and white matter fractional anisotropy using tract based spatial statistics[4] predict long term outcome. In this study we will continue as a secondary outcome to examine these potential predictors for cooled babies receiving Xenon treatment.

4.10 Group size

Twenty-six babies will be recruited into each group (cooling+Xenon *vs.* cooling alone) at random. The cooling will be applied as standard treatment if cooling criteria are met. Addition of Xenon in combination with this underlying cooling will take place in the group randomised to receive cooling + Xenon.

4.11 Justification of Treatment / Procedure

Even with the best available treatment (hypothermia) infants with HIE have a considerable risk of death or disability of <40%. This study offers an additional treatment which has been shown in two animal species to improve (double) brain protection over and above the protection from cooling alone. Our large animal trial of Xenon-hypothermia in piglets conducted with a delivery system of the same design as proposed for this study concluded with a very positive outcome[18] supporting the result we found in rats, i.e. adding Xenon to HT doubles the neuroprotection[17,31].

This equipment has been successfully and safely used to deliver Xenon to a total of 32 human neonates to date in the MHRA approved studies, CI/2009/0043, CI/2011/0061; CooXenon1 and CoolXenon2 respectively.

5.0 Study Therapies

5.1 Test Article: Description

The neonatal Xenon breathing device (NXBD) is a gas delivery circuit with some similarities to an anaesthesia circuit that is temporarily placed between a standard mechanical ventilator and the tracheal tube of a neonate to deliver Xenon as a therapy in the breathing gas. It is designed for use in neonates who already require mechanical ventilation in this manner on a neonatal intensive care unit. Its purpose is to provide a gas mixture to the lungs that contains not only an appropriate oxygen concentration to maintain life, but also to provide a concentration of up to 50% Xenon in this mixture. The reason for this is that Xenon shows great promise as a neuroprotectant and has been shown to reduce neurological damage in both small and large laboratory *in-vivo* models of neonatal hypoxia-ischaemia (birth asphyxia for example). By re-circulating the exhaled gases after removal of carbon dioxide then, as the uptake of Xenon by a neonate is very low and the system does not "vent" any exhaled gas to the environment, it provides a very economical method of Xenon delivery. Such economy will be essential for any future clinical use of Xenon as this gas is both limited in availability and extremely expensive at approximately £20/litre. While the (minimal) Xenon uptake is replaced manually by the operator, the breathing device automatically adds oxygen as required to match metabolic uptake by the patient. This is not achieved

by complex sensors and electronics but by a straightforward mechanical mechanism for reliability. Furthermore, if Xenon replacement is stopped, the inspired oxygen concentration will tend to very slowly increase i.e. it will not fall as the patient consumes oxygen from the circuit – these are both inherent safety features. The most important and intuitive safety feature is that in the event of any problem, the mechanical ventilator patient connector can be disconnected from the breathing system and reconnected directly to the tracheal tube of the neonate, instantly restoring normal ventilation and also allowing any Xenon to very rapidly dissipate from the tissues of the neonate.

5.2 Test Article: Principles of Operation

If no fresh oxygen is supplied to a conventional anaesthesia circle system the patient will consume all oxygen present until a hypoxic mixture develops. Therefore in a closed circle system, fresh gases must be added at a rate exactly matching patient uptake of each gas. There are various ways to achieve this. In the simplest of closed underwater diving systems, the user will breathe pure oxygen around a circuit similar to an anaesthesia circle. In time, by metabolic oxygen consumption, the reservoir bag will completely collapse part-way through an inspired breath. At this point, by continued inspiratory effort, a demand valve on an attached oxygen cylinder will open, providing the missing volume of oxygen to complete the inspiration phase. If we now imagine this circle to contain a mixture of Xenon (slow patient uptake) and oxygen (metabolic uptake) a different situation develops. It can be envisaged that substitution of these two uptake volumes with pure oxygen at each end-inspiration would produce a slowly increasing inspired oxygen fraction (FiO_2).

We have incorporated these concepts into a ventilator driven closed circuit where a standard Neonatal Intensive Care Unit (NICU) ventilator, set to deliver 100% oxygen in each breath to the circuit, provides not only the motive power but also, via a special volume substitution valve, the required oxygen replenishment in place of the demand valve in the diving example.

When delivering a Xenon/oxygen mixture there would then be a background tendency for the FiO_2 to slowly increase while the inspired Xenon concentration decreases at a similar rate. This provides a degree of hypoxic mixture protection without use of oxygen sensor and computer software controlled electronic valves. In other words, adequate oxygen supply, the most critical function of any breathing system, is not entirely dependent on correct functioning of sensors, software and electronic dosing valves, as would be the case with an electronically controlled system.

Against this inherent safety feature of a very slowly increasing the FiO_2, and the minimal Xenon uptake of a neonate from the closed loop circle breathing system, Xenon can then be delivered to the circle in small volumes under manual control to offset this trend, replacing the minimal patient Xenon uptake.

The design of a breathing circuit to achieve these design aims is described in Figures 1a and 1b.

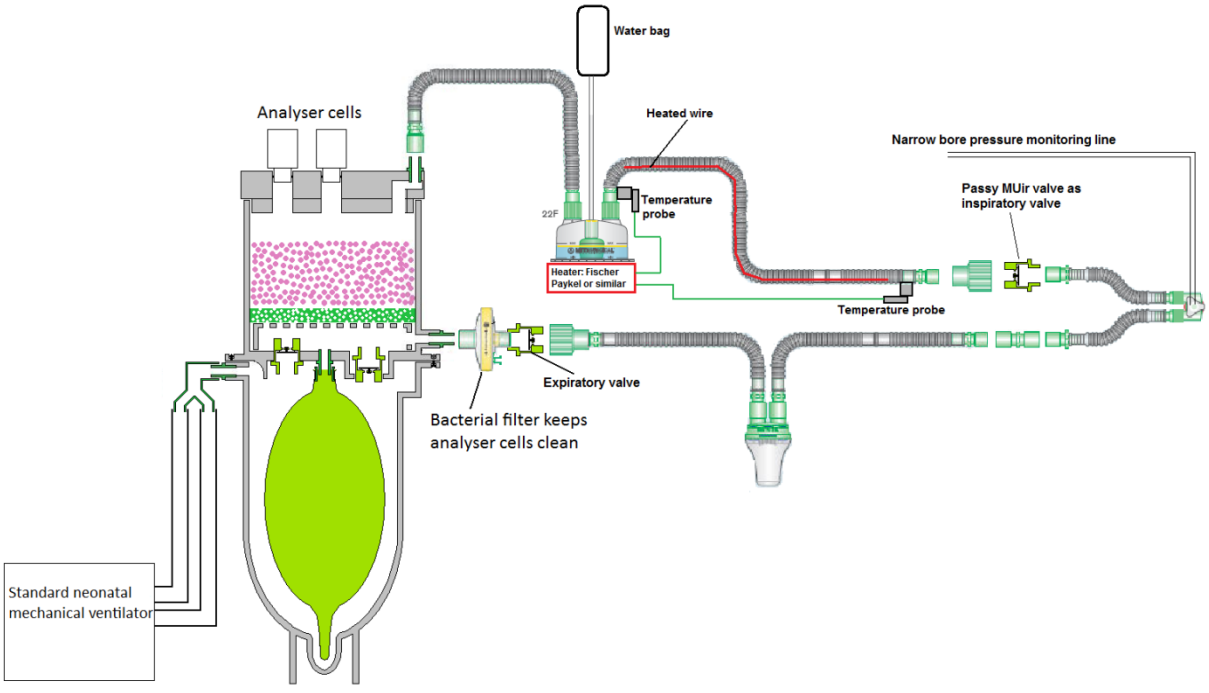

Figure 1a. Diagram of the breathing system

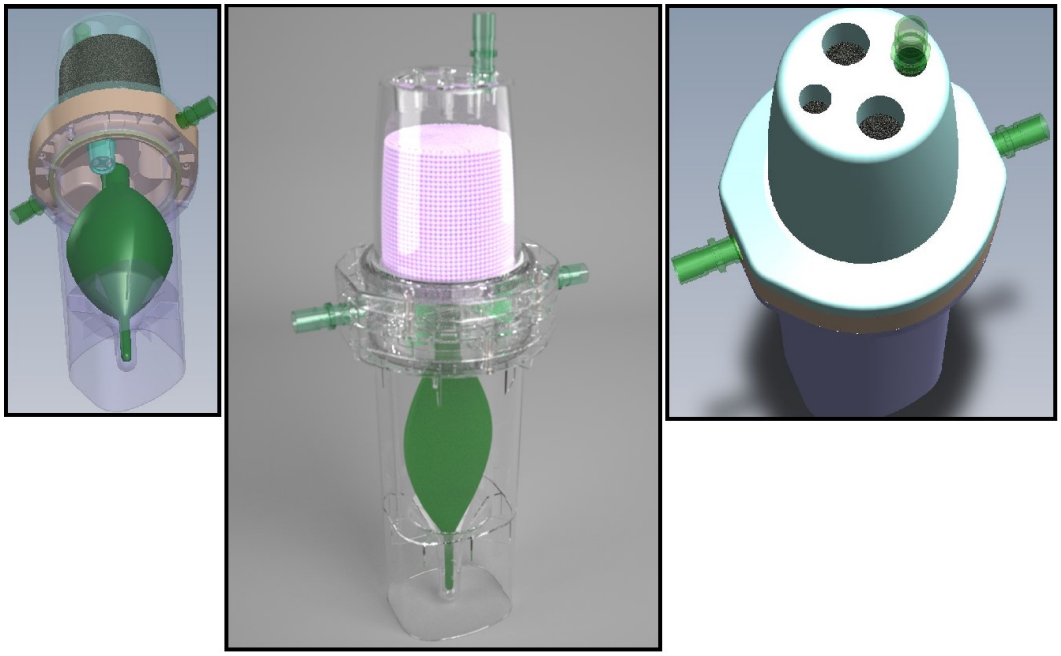

Figure 1b. Views of breathing system (without hoses)

The Xenon delivery system is designed to be fitted between a conventional NICU mechanical ventilator (SLE 2000, SLE, South Croydon, UK) and the tracheal tube. The latex-free bag in the lower chamber has a volume of 500ml, larger than a neonatal tidal volume, providing free space to accommodate added Xenon bolus volumes without overspill. The removable soda-lime canister has a volume of 510ml permitting long periods of ventilation without replacement.

In this design, a small aliquot of oxygen enters the closed circle upper part of the circuit at each end-inspiration from a lower bag-in-bottle chamber (where it is present as the driving gas from the ventilator) via a dedicated oxygen substitution valve. Modern NICU ventilators maintain a bias gas flow throughout the breathing cycle. At end-expiration this displaces any residual CO_2 from the "Y" connector reducing CO_2 rebreathing. However with a closed circuit, the absence of a bias flow combined with small neonatal tidal volumes means that an efficient self-closing, leak free, low opening pressure inspiratory valve was required to prevent rebreathing. A unidirectional valve performs this function. A similar valve design is used as the expiratory valve and as the oxygen substitution valve.

5.2.1 Gas delivery to breathing system

Oxygen consumed by the neonate from the circle is replaced automatically via the oxygen substitution valve as described above and in Figure 1. The slow-rising FiO_2 tendency (a condition of inherent safety against the development of accidental hypoxic mixtures) is then deliberately offset by occasional manual delivery of Xenon boluses to the circle, so maintaining the target Xenon concentration – the combination of closed circuit, low patient weight and low Xenon uptake making these Xenon doses very infrequent (<200ml Xe per hour from laboratory data).

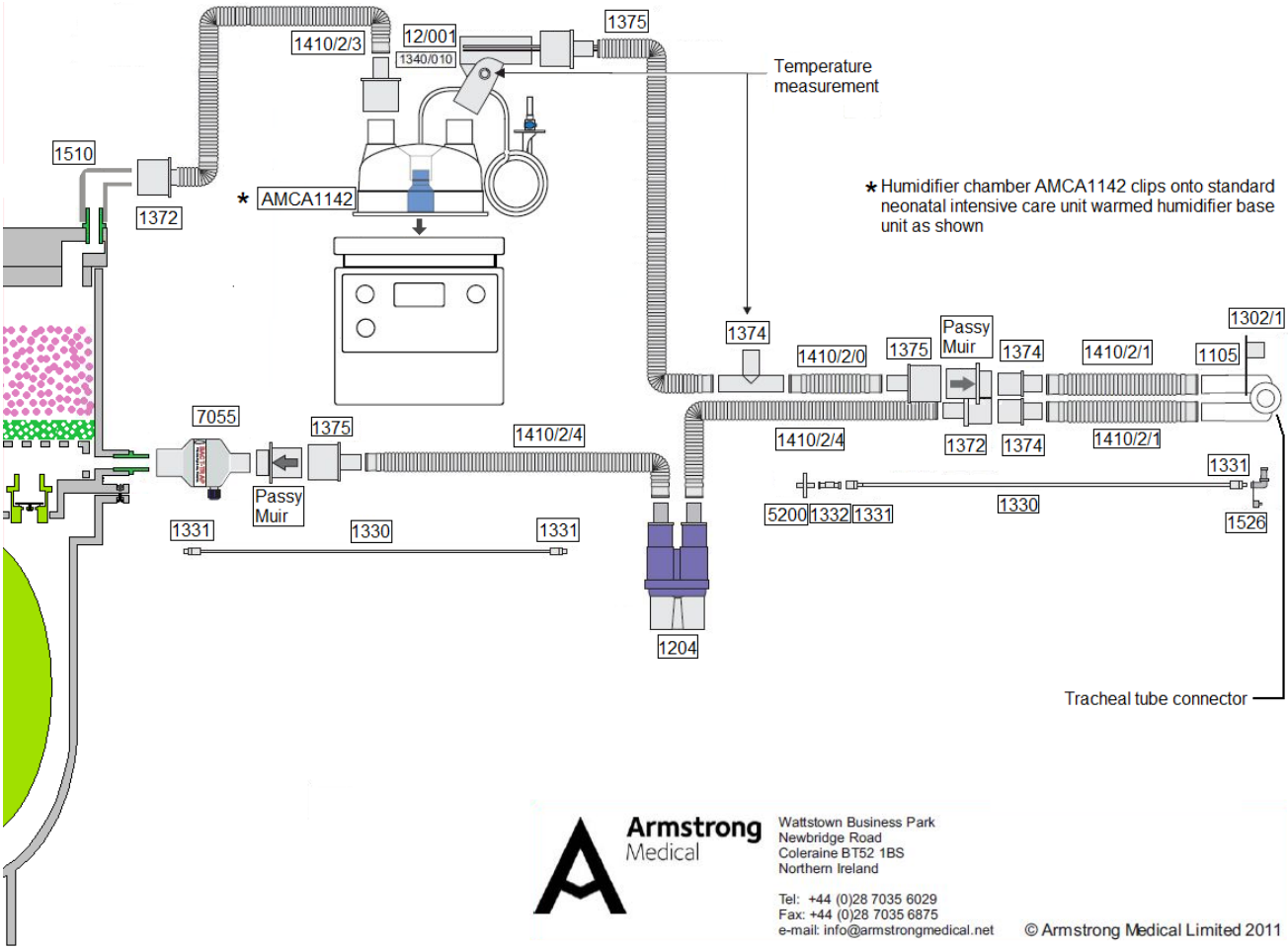

Figure 1c. Details of exact medical components used to construct the hoses of the breathing circuit, with part numbers. Hoses, humidification chamber and connectors all manufactured by Armstrong Medical Ltd, UK

Key to identified parts in Figure 1c.

1372	Connector
1410/2/3	Corrugated hose
AMCA1142	Water chamber to fit Fischer-Paykel humidifier base unit (an industry-standard humidification system for adult and neonatal ventilation systems).
12/001	Connector with port for Fischer-Paykel proximal temperature sensor and female socket to take power cable from Fischer-Paykel base unit for the heated wire system.
1375	Corrugated hose with heated wire.
1374	Connector with port to take distal temperature sensor of the Fischer-Paykel humidifier base unit.
1410/2/0	Corrugated hose link.
1375	Connector. Passy-Muir: Unidirectional valve
1374	Connector
1410/2/1	Corrugated hose link to tracheal tube connector.
1105	Tracheal tube connector.
1302/1	Cap for pressure monitoring port.
1526	Connector to fit pressure monitoring port 1302/1
1331	Male Luer fitting.
1330	Pressure monitoring hose.
1331	Male Luer fitting.
1332	Double female Luer connector.
5200	Filter
1410/2/1	Corrugated hose link from tracheal tube connector.
1372/1374	Hose connectors.
1410/2/4	Corrugated hose.
1204	Expiratory limb water trap.

1410/2/4	Corrugated hose.
1375	Connector. Passy-Muir: Unidirectional valve.
7055	Bacterial filter with side mounted Luer port, where fresh gas is delivered to the system.
1331/1330/1331	Hose for connection to 7055 Luer port.

We have previously delivered Xenon using high pressure Xenon cylinders with electronic valves actuated by both computer and manual control. After using servo control in the past we realised that due to the slow uptake of Xenon, manual addition of Xenon boluses would not be particularly onerous facilitating this more straightforward design devoid of any computer control systems.

The manual Xenon dosing mechanism is designed to function at ambient pressure for additional simplicity and safety, so eliminating any risk of the circle "flooding" with Xenon due to a cylinder/regulator/electronic malfunction (Figure 2).

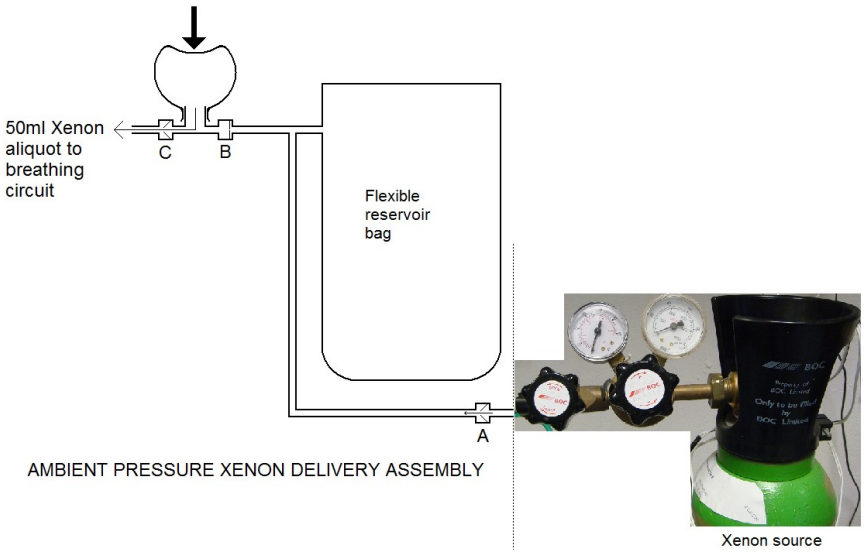

Figure 2.

A flexible bulb and valve arrangement allows manual addition of ~50ml Xenon boluses to the circle breathing system, from a previously filled reservoir bag of Xenon at ambient pressure. These are added to the expiratory limb of the circuit to allow maximum mixing with existing gases in the circle before entering the lungs. The small flexible bulb volume of 50ml was selected because the small bulb volume combined with its slow refill properties further aids even gas mixing, it being impossible to rapidly add a large Xenon bolus. Erring on the side of caution, our circuit was primed with oxygen. The Xenon/oxygen monitors are constantly observed as in the conduct of a conventional anaesthetic. Deviations in Xenon concentration of more than 2-4% from target are corrected by the operator adding Xenon to the circuit, each 50ml bolus typically producing a 2% increase in Xenon concentration. These corrections offset the inherent tendency of this circuit design to generate a very slowly increasing oxygen concentration (O_2 + minimal Xe consumptions being replaced with equal volume of oxygen via substitution valve).

The overall design aims were that this breathing system should (i) be driven by a standard NICU ventilator, (ii) have inherent hypoxic mixture prevention properties not dependent on electronics (iii) provide the user with the most intuitive treatment reversal method; reconnection of the neonate directly to the NICU ventilator. It performs all these functions.

5.3 Test Article: Clinical Development

A technical evaluation of the Xenon delivery system and the use of inhaled Xenon in an *in-vivo* laboratory model that replicated the human clinical scenario of; global hypoxic ischemic insult in a neonate followed by cooling (the standard treatment) and concurrent Xenon administration was published in 2010.[18] It describes the early clinical development and performance of the delivery system and is attached in full as Appendix A2.

5.3.1 Proof-of-Concept Pre-clinical Studies

Extensive pre-clinical work[18] has been undertaken. These data were provided to the UK Competent Authority during the review process for the CoolXenon1 and CoolXenon2 studies.

5.3.2 Proof-of-Concept and Pilot Clinical Studies

The Bristol group has undertaken two studies involving the use of inhaled Xenon. A clinical feasibility/safety study in 14 human neonates of inhaled Xenon in combination with cooling using this equipment design to deliver Xenon was successfully completed in March 2011. A further study has recruited 28 infants. In the CoolXenon1 or CoolXenon2 studies there were no significant physiological or biochemical changes or other problems attributable to the Xenon gas or the use of the NXBD.

5.4 Test Article: Regulatory Status

The NXBD is not CE marked in Europe and therefore any UK clinical study involving its use requires review by, and approval from, the UK Competent Authority, the Medicines and Healthcare products Regulatory Agency (MHRA). Ethical approval for the study will also be obtained from an appropriate Research Ethics Committee (REC) prior to commencement.

5.5 Test Article: Packaging and Labelling

The NXBD requires no packaging and will be clearly labelled in compliance with the requirements of the EU Medical Devices Directive. Devices used in any clinical trial will clearly be marked “Exclusively For Clinical Investigations”.

5.6 Test Article: Component Tracking

Use of the NXBD will be tracked and reconciled throughout and upon completion of the study. Any complications with the device or components of the device should be managed by contacting the study Sponsor via the following telephone numbers:

- Mary Perkins, R and D Dept Lvl 3, Education & Research Centre, Bristol BS2 8AE. Tel 0117 3420233 (office hours)
- Dr John Dingley Mobile: 07735 379471 (all other times)

5.7 Control/Comparator Therapy

This is a randomised comparative outcomes study. The control group will receive the standard treatment for this condition which is whole body cooling for 72h as it would be unethical to withhold the normal treatment from the control group. The treatment group (experimental group) will also receive an identical cooling regime but in addition will receive 50% Xenon within 5h of age in the breathing gas mixture via the NXBD breathing circuit for a period of 18h.

6.0 Patient Selection

Potential study participants will be identified via the existing identification system used at St Michaels for administration of their (standard) cooling regime for the region.

There is good evidence that the earlier cooling (now standard treatment) is started, the greater the ultimate benefit. Consequently neonates are often allowed to passively cool during retrieval to Bristol, where active cooling is then continued, in a bid to start the process as early as possible. This argument is also likely to be true for Xenon and there is *in-vivo* laboratory data to support this. Parents of potential participants in study therefore need to be approached as early as possible after birth if their baby meets entry criteria for the study.

Therefore they will first be approached by a trained medical member of the clinical team. This person will briefly explain the study to the parents of the neonate with the aid of the information sheet, and later a member of the team will obtain written consent where this has been agreed.

Informed written consent will be obtained from a parent (only the mother can consent if parents are not married) after a full verbal and written explanation of the study by a named physician or trained nurse such as an advanced nurse practitioner. Such staff will also meet with parents during the intervention period to ensure that they understand the study procedures and continue to consent to participate in the study. Information can be given by local trained staff and consent can also be taken over the phone with the mother if she is unable to be available for the consent process (e.g. cannot be moved shortly after Caesarean Section).

Approval for the study is being obtained from the Regional South West Research Ethics Committee covering St Michael’s Hospital.

Most of the patients will be born outside the study hospital (Outborn) and will constitute >90% of patients. They are brought to the hospital by a dedicated retrieval team and ambulance from Bristol. The local delivery hospitals will have

medical staff (e.g. consultants, specialist registrars, advanced nurse practitioners) trained in consenting for the CoolXenon3 trial. A training log with signatures, a short CV and competency approval will be kept centrally.

Parents will receive a written information leaflet (1 page, short version, Appendix 5) when the infant is eligible for cooling treatment. Staff will discuss CoolXenon3 with parents as soon as practically possible. Parents will be able to speak on the phone to a member of the treatment team if they need further information and help with the consent process. Parents will also be offered the opportunity to speak to members of the treatment team that are picking up the baby for transport.

Trained staff on the transport team can take consent for the study.

A more detailed information sheet regarding the trial will be given to parents after the first conversation or at the earliest suitable time.

6.1 Which of the parents can consent to letting the child enter the trial?

- Either parent if they are married
- If the parents are not married, only the mother can consent - provided she is awake and understanding (e.g. not under the influence of anaesthetic drugs)
- If the mother is not in a state to understand the situation (and the parents are living together but are not married) the father can consent on behalf of the mother. The consent process must be repeated with the mother as soon as she is able to understand the child's condition.

A parent who is eligible to consent can, at any time and without a specific reason, withdraw the child from the study. In this situation, Xenon treatment will then be stopped as soon as possible and medically safe (within minutes).

6.2 Inclusion Criteria

Patients meeting the following criteria will be considered eligible for the study:

Infants will be eligible for inclusion in this study if the St Michael's hospital standard inclusion criteria for cooling are met and additional inclusion criteria for Xenon administration are met.

6.2.1 St Michael's Hospital standard inclusion criteria for cooling

Standard Hypothermia Treatment Criteria for 72 hrs of cooling — all of criteria A, B and C.

A: *Infants ≥ 36.0 weeks gestation (estimated or clinical assessment) with at least ONE of the following:*

v. Apgar score of ≤5 at ten (10) minutes after birth
vi. Continued need for resuscitation, including tracheal or mask ventilation, at ten minutes after birth
vii. Acidosis defined as either umbilical cord pH or any arterial, venous or capillary pH within 60 minutes of birth less < 7.00
viii. Base deficit ≥16 mmol/L in umbilical cord blood sample or any blood sample within 60 minutes of birth (arterial or venous blood). If the infant meets criterion A then assess for neurological abnormality using criterion B and C (by trained personnel):

B*:* *Moderate or severe encephalopathy as evidenced by any of the following:*

vi. Altered state of consciousness (reduced or absent responses or pathological irritability and hyper responsive and at least ONE or more of the following:
vii. Hypotonia
viii. Abnormal reflexes including oculomotor or pupillary abnormalities
ix. Absent or weak suck
x. Clinical seizures, as recorded by trained personnel

AND

C: At least 30 minutes duration of amplitude-integrated electroencephalography (aEEG) recording that shows abnormal background aEEG activity. The decision to cool is based on the worst 30 min section of the aEEG, not the best[35] or seizures (clinical or electrical) thus meeting ONE of the following:

v. Normal background with some (> 5 min) electrical seizure activity
vi. Moderately abnormal activity (upper margin of trace >10μV and lower margin <5μV)
vii. Suppressed activity (upper margin of trace <10μV and lower margin of trace <5μV)

viii. Definite seizure activity

6.2.2 Additional inclusion criteria for Xenon

Before being considered for additional inhaled Xenon therapy via the breathing gas mixture, the infant would need to meet further additional entry criteria (all must be met): Before being considered for additional inhaled Xenon therapy via the breathing gas mixture, the infant would need to meet further additional entry criteria (all must be met):

i. Intubated, ventilated, sedated, being cooled.
ii. <5 hours old
iii. Any seizures under control.
iv. Weight > 2nd centile for gestational age
v. Stable cardiovascular parameters; Mean arterial pressure >40mmHg.
vi. Oxygen requirement via mechanical ventilator < 40%.
vii. Positive End Expiratory Pressure (PEEP) requirement ≤ 8cm H_2O
viii. Arterial/capillary/venous pCO_2 <7.0kPa (ideally arterial). Venous can be higher if peri-arrest and tissues have been very recently Ischaemic
ix. Postnatal age <5 hours
x. Absence of major congenital abnormalities, imperforate anus and in particular any bowel obstruction, congenital abnormalities suggestive of chromosomal anomaly or other syndromes that include brain dysgenesis. Congenital syndromes affecting the brain should be excluded when diagnosed.

6.3 Exclusion Criteria

6.3.1 Exclusion criteria for cooling

a. Infants expected to be greater than 3 hours of age at the time of starting cooling treatment.
b. Futility. Where prognosis is considered to be hopeless e.g. no cardiac output for 20 minutes.

6.3.2 Additional exclusion criteria for Xenon

i. Failure to meet any of the additional inclusion criteria for Xenon listed in section 5.1.
ii. Patients meeting the following criteria are NOT eligible for the study:
iii. Presence of any of:
   a. Major congenital abnormalities
   b. imperforate anus and in particular any bowel obstruction
   c. congenital abnormalities suggestive of chromosomal anomaly
   d. other syndromes that include brain dysgenesis. (Neonates with congenital syndromes affecting the brain should be excluded when diagnosed).

6.4 Prohibited Therapies

Not Applicable.

7.0 Subject Assignment to Treatment Group

The babies entered into the study will be randomised into groups; Xenon+cooling or cooling (standard treatment) alone. Neonates meeting all the entry criteria can be included.

Immediately after enrolment, the patient will be assigned a study ID number, chronologically, from a specified series on an enrolment log specific to the Investigational Site. The Subject's study number and date of enrolment will be entered onto the enrolment log in the Study Binder.

The babies entered into the study will be randomised into two groups on a 1:1 basis. Randomisation will be via sealed opaque envelopes containing details of treatment allocation and [an] identifying adhesive label(s) to be attached to the CRF binder. Neonates meeting all the entry criteria can be included.

8.0 Methods and Procedures

8.1 Informed Consent

Informed written consent will be obtained from a parent (only the mother can consent if parents are not married) after a full verbal and written explanation of the study by a named physician or trained nurse such as an advanced nurse practitioner. Such staff will also meet with parents during the intervention period to ensure that they understand the study procedures and continue to consent to participate in the study. Information can be given by local trained staff and consent can also be taken over the phone with the mother if she is unable to be available for the consent process (e.g. cannot be moved shortly after Caesarean Section).

Approval for the study is being obtained from the Regional South West Research Ethics Committee covering St Michael's Hospital.

Most of the patients (>90%) will be born outside the study hospital and are brought to the hospital by a dedicated retrieval team and ambulance from Bristol. The local delivery hospitals will have medical staff (e.g. consultants, specialist registrars, advanced nurse practitioners) trained in consenting for the CoolXenon3 trial. A training log with signatures, a short CV and competency approval will be kept centrally.

Parents will receive a written information leaflet (1 page, short version, Appendix 5) when the infant is eligible for cooling treatment. Staff will discuss CoolXenon3 with parents as soon as practically possible. Parents will be able to speak on the phone to a member of the treatment team if they need further information and help with the consent process. Parents will also be offered the opportunity to speak to members of the treatment team that are picking up the baby for transport.

Trained staff on the transport team can take consent for the study.

A more detailed information sheet regarding the trial will be given to parents after the first conversation or at the earliest suitable time.

<u>Which of the parents can consent to letting the child enter the trial?</u>

- Either parent if they are married
- If the parents are not married, only the mother can consent - provided she is awake and understanding (e.g. not under the influence of anaesthetic drugs)
- If the mother is not in a state to understand the situation and the biological father does currently not have legal parental responsibility he can assent for the trial until the mother is awake/understanding the child's condition and able to decide on consent.

A parent who is eligible to consent can, at any time and without a specific reason, withdraw the child from the study. In this situation, Xenon treatment will then be stopped as soon as possible and medically safe (within minutes).

8.2 Pre-Study

Consent will be obtained as described above from parents of the neonates. Prior to this consent they will have been given an information leaflet describing the study and the discussion will have taken place with a Consultant Neonatologist involved in the study or a trained member of the Transport Team.

8.3 Screening

All infants born in St. Michael's Hospital or retrieved by ambulance from other centres to St. Michael's Hospital Neonatal ICU will have undergone a basic screen of their condition at birth as part of standard procedures, the commonest example being the APGAR scoring system, cord blood analysis and clinical examination. Any infant suspected of being in a poor condition at birth will, by locally present neonatal staff, be assessed for possible cooling as a treatment to limit brain injury using the approved entry criteria (as used in CoolCap and TOBY). Local hospitals will typically contact the St Michael's team within 1 h of age to discuss eligibility, start cooling, transport and potential inclusion in Xenon study. At this point on advice from Bristol, information on the Xenon study can be given to parents.

8.4 Initial Assessment

The following information will be recorded:

8.4.1 Demographics

PATIENT

- Date of birth
- LMP last menstrual period
- EDD expected date of delivery
- Gestational age weeks, days e.g. 40 weeks, 3 days
- Race
- Gender
- Weight

MATERNAL

- Years in education
- Labour induced on maternal indication?
- Maternal medical treatment during pregnancy

8.4.2 Patient History and Physical

- Delivery and resuscitation details , Review of Systems, Transport details if retrieved from another centre, Current diagnoses
- Clinical examination

8.4.3 Concomitant Medications

All concomitant medications will be recorded as follows:

- Medication; Start (and Stop date(s) if applicable); Indication; Dose

8.5 Study Phases

The study consists of 3 phases.

8.5.1 Phase 1

Identification of potential subjects – initially using same identification method used in St. Michael's Hospital Bristol to identify infants who would benefit from cooling.

Compliance with all inclusion/exclusion criteria for use of Xenon in addition to cooling (the standard treatment).

8.5.2 Phase 2

Formal recruitment, information supplied to / discussions with parents.
Consent.

8.5.3 Phase 3

Completion of all screening / demographics / baseline data collection immediately pre-Xenon, reintubation with cuffed tube, x-ray for tube placement and blood gas

Administration of 50% Xenon for 18 hours to infant according to randomisation protocol.

Completion of all data collection, serum chemistry and examinations immediately post-Xenon and post-cooling.

8.5.4 Patient Follow-up

8.5.4.1 Short term information and investigations (*before discharge from hospital*):

These are part of routine clinical practice.

1. Survival
2. Duration of hospitalisation
3. Time to full oral feeds, breast, bottle or tube.
4. Hypoxic-ischaemic or other injury[2] and MRI biomarkers including quantification of NAA in thalamus and fractional anisotropy in white matter using tract based spatial statistics[4] on MRI scan, which includes Magnetic resonance spectroscopy, undertaken around 48 hours of life and around day 7 of life.
5. Intracranial or intraventricular haemorrhage (on MRI, CT or ultrasound)
6. Major venous thrombosis (on MRI or ultrasound)
7. Resistance Index (Doppler examination of cerebral blood flow velocities)
8. Amplitude integrated EEG (aEEG) continuously recorded until after rewarming, or 82h in cooled-only infants. Assessment of time to normalisation of background activity, seizure occurrence and duration

9. Pulmonary hypertension needing treatment
10. Pulmonary haemorrhage
11. Pneumonia
12. Pulmonary air leak
13. Systemic hypotension needing inotropic support
14. Prolonged blood coagulation time assessed before Xenon and at ~24 hours and/or at clinically indicated timepoints
15. Cardiac arrhythmia (not temperature induced low Heart Rate)
16. Thrombocytopenia (platelets <100,000/ul)
17. Culture proven sepsis
18. Necrotising enterocolitis or intestinal perforation, medical, radiological or surgically proven
19. Plasma markers of global (increased lactate dehydrogenase (LDH)) or cardiac compromise (increased cardiac Troponin T (cTnT)) or plasma lactate
20. Renal failure treated with dialysis

8.5.4.2 Short term assessment neurological evaluation will include

1. Time to recovery after birth of a normal background aEEG[1]
2. MRI scan before 14 days of life[2,4,35]
3. Peak LDH value within 72h of life[5,36]
4. First plasma glucose after birth (usually from cord blood)
5. Age in hours after birth when plasma lactate has declined to <5mmol/l
6. Number and type of inotropic drugs needed during stay on the neonatal intensive care unit (typically the period from birth to 3-5 days of age) and the total duration in hours of any inotropic support
7. Number of anticonvulsant drugs given during stay on the neonatal intensive care unit.
8. Amount and duration of sedation (e.g. plasma morphine levels) while ventilated mechanically on the neonatal intensive care unit.
9. Stress levels (saliva, plasma and urinary cortisol and adrenocorticotropic hormone)
10. Age at full oral feeds (breast or bottle or tube)
11. Outcomes of neonatal hearing screening before 14 days of life
12. Clinical examination at birth, at 7 days of age and at discharge including weight and head circumference

8.5.4.3 Long term follow up (at 18 months)

Neurodevelopmental follow up is a part of the routine follow up of infants with Neonatal Encephalopathy. Bayley III examination at 18 months of age is used to compare the results from this study with our large local patient database and previous randomised controlled trials (CoolCap, Gluckman 2005; TOBY, Azzopardi 2009)[13,14] where Bayley II was also used: we were a main recruiting centre for these trials.

Disability will be defined as any of:

- Bayley III Cognitive and Language Composite score less than 85.
- Bayley III Motor Composite score less than 85
    - Bayley III will also be expressed as developmental quotients (DQ) calculated from Developmental Age scores as this allows a larger range of score for those functioning at a low level[37]In this study a Bayley III assessment[11] will be undertaken and data will be converted to Bayley II MDI and PDI for comparison with historical controls.
- Bilateral cortical visual impairments.
- Hearing loss needing amplification > 40 dB.

8.5.4.4 Additional follow up

We will do short-term (14 days), intermediate (6 months) and long-term (18 months survival) follow-up.

8.6 Study Data

All the patient study information will be coded by patient study number, anonymised and securely stored until the study is complete. Electronic data will be stored on a secure portion of a Hospital Trust computer. The Chief Investigator will ensure that all the patient identifying details have been removed prior to statistical analyses being undertaken.

The identification key linking study numbers to patient details will be kept by the CI (Prof Marianne Thoresen) in a locked filing cabinet in a locked office in the Neonatal Neuroscience office of St. Michael's Hospital Bristol, which also has two separate secure swipe card entry systems.

8.7 GP Notification

The General Practitioner will be informed via a letter of the participation of the infant in the study when the infant is discharged from hospital.

8.8 Definition of End of Study

The end of the trial as a whole will be when all recruited participants have completed the treatment and follow-up period ***and*** all data has been collected and analysed, with data queries answered.

9.0 Assessment of Safety

9.1 Data Review Group

An IDRG will be set up and appropriately constituted with clearly defined terms of reference. Their role will be to independently review the safety of the study after every 8th Xenon patient has been recruited and in the light of this make appropriate recommendations to the study team.

The IDRG will also meet in the event of a death, regardless of cause. This meeting will take place within ~14 days of the death and recruitment into the study will continue in the meantime. This meeting could be in person or take the form of a teleconference. In our initial study of Xenon+cooling a partial external committee met at intervals of every other baby enrolled. For the CoolXenon2 study, the IDRG met after every 4th Xenon patient had been recruited.

In the CoolXenon1 or CoolXenon2 studies there were no significant physiological or biochemical changes or other problems attributable to the Xenon gas or the use of the NXBD. An identical NXBD will be used in this study.

Given the excellent safety record of the previous two studies involving Xenon use and the NXBD, it is considered appropriate that for this study the IDMG should meet after every 8th Xenon patient is recruited.

9.2 Composition of IDRG

External Members (not involved with delivering the treatment)

Dr David Evans BM BCh (Oxon) MA (Cantab) MRCP(UK) FRCPCH Consultant Neonatologist North Bristol NHS Trust Neonatal Intensive Care Unit Southmead Hospital Bristol BS10 5NB UK	Contact details: david.evans@nbt.nhs.uk david.evans@bristol.ac.uk
Professor Colin Morley Professor of Neonatal Medicine (Retired) 23 High St Great Shelford Cambridge CB22 5EH UK	Contact details: Mobile: 07591 647097 Home: 01223 846944 colin@morleys.net
Dr Steven Jones MB FRCPCH Director Southwest Neonatal Network Consultant Neonatologist Royal United Hospitals Coombe Park Bath BA1 3NG	Contact details: Tel: 01225 825405 Steve.Jones@RUH.nhs.uk

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The IDMG should invite at least one member from the research team to attend their meeting.

9.3 Criteria for stopping the study (stopping rule) have been developed according to the following logic:

The underlying mortality is approximately 10.3% in this cohort of infants (without Xenon). Usually care is redirected based on futility, i.e. the clinical condition and state of the brain is such that no meaningful life can be expected.

9.3.1 Reasons for stopping Xe delivery before the 18h timepoint

Cardiovascular problems sometimes present as untreatable hypotension. In this situation the heart has also suffered hypoxia-ischaemia and sometimes it is impossible to maintain a perfusion pressure even with the use of maximum inotropic support.

Another problem would be if the neonate develops a very high oxygen fraction (up to 100%) in the breathing gas over time, >40% according to the study entry criteria. An example would be those who have aspirated meconium.

9.3.2 Stopping rule

Mortality since cooling became standard of care at St Michaels hospital (inborn and outborn) fell from 34%to 10.3% (17 out of 165 infants born since December 2006 and undergoing cooling died). If, with this (10.3%) underlying mortality, the two first infants receiving Xenon die, the probability that this is a random event is very small (p=0.01) and recruitment should be stopped and the protocol reviewed. The same applies if 2 out of the first three babies randomised to Xenon die (p=0.03).

Thereafter, (n is the number of 'Xenon' babies included in the study):

n	Number of Deaths	Action
2	2 of 2	Stop study and review
3	2 of 3	Stop study and review
4	3 of 4	Stop study and review
5	3 of 5	Stop study and review
6	3 of 6	Stop study and review
7	3 of 7	Stop study and review
8	3 of 8	Stop study and review
9-14	4	Stop study and review
15-22	5	Stop study and review
23-26	6	Stop study and review

This stopping rule is based on two considerations:

i. Probabilities given 10.3 % mortality without Xe
ii. Stricter rule with more babies included

9.4 Adverse Events (AEs)

Adverse events will be recorded in accordance with UH Bristol's Research Safety Reporting SOP and standard national regulatory requirements.

9.5 AE Definitions

Adverse Event (AE)

"Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment"

Adverse Reactions (ARs)

"All untoward and unintended responses to an investigational product related to any dose administered"

Serious Adverse Event (SAE)

"Any untoward medical occurrence or effect that at any dose results in death, is life – threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity or is a congenital anomaly or birth defect."

Unexpected Adverse Reaction (UAR)

"An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unauthorised medicinal product or summary of product characteristics for an authorised product)"

9.5.1 Assessment of causality

1. **Not related** – temporal relationship of the onset of the event, relative to the administration of the product, is not reasonable or another cause can by itself explain the occurrence of the event.
2. **Unlikely**
3. **Possibly related** – temporal relationship of the onset of the event, relative to administration of the product, is reasonable but the event could have been due to another, equally likely cause.
4. **Probably related** – temporal relationship of the onset of the event, relative to the administration of the product, is reasonable and the event is more likely explained by the product than by another cause.
5. **Definitely related** – temporal relationship of the onset of the event, relative to the administration of the product, is reasonable and there is no other cause to explain the event or a re-challenge (if feasible) is positive.

Of the above definitions,
"possibly", "probably" and "definitely" related to an investigational medical product are considered adverse reactions
"unlikely" and "not related" do not qualify as a causal relationship.

The severity and causality of a serious adverse event, adverse event or SUSAR (Suspected Unexpected Serious Adverse Reaction) should be assessed by a qualified medical practitioner, usually (but not always) the Chief Investigator (CI).

Adverse events or reactions that are not life threatening and do not result in death or hospitalisation may also be considered serious if they jeopardise the subject or require intervention.

All adverse events must be reported to the sponsor immediately, unless they are identified as not requiring this in the trial protocol. It is then the responsibility of the sponsor to report the incident to the MHRA.

9.6 AE Classification

The Investigator(s) will establish the causality of AEs.

9.7 AE Recording

All Adverse Events volunteered by study Patients or elicited by the Investigator must be recorded on the AE forms provided. All AEs must be recorded whether or not considered drug / treatment related.

9.8 AE Reporting

The Investigator must report by telephone all serious AEs to the Sponsor immediately upon discovery, and forward the completed AE form as soon as it is available. If the event has been classified serious / severe, or there is any suspicion

that the AE was caused by a test article, details of medical history, concomitant medication and an assessment of compliance with therapy should accompany the AE form. The Investigator must also report the resolution reported AEs promptly.

Non-serious AEs should be reported at the next routine contact.

The time period allowed for reporting such events to the MHRA are as follows:
Reaction which is fatal or life threatening, as soon as possible but no later than 7 days after first occurrence
Reaction which results in hospitalisation /disability/congenital anomaly, as soon as possible, but no later than 15 days after first occurrence

9.9 Reporting Responsibilities to Regulatory Authorities, Investigators and Ethics Committees

The study will be performed subject to Research Ethics Committee (REC) approval, including any provisions of Site Specific Assessment (SSA), and local Research and Development (R&D) approval. The Sponsor will send appropriate safety notifications to regulatory authorities in accordance with applicable laws and regulations.

The Investigator must comply with any applicable site-specific requirements related to the reporting of SAEs involving his/her subjects to the REC that approved the trial.

In accordance with ICH GCP guidelines, the Sponsor will inform the Investigator of "findings that could adversely affect the safety of subjects, impact the conduct of the trial or alter the REC approval to continue the trial".

10.0 Data Analysis and Statistical Plan

10.1 Statistical Analysis

This is a prospective randomised comparative outcome study. Data presentation will include descriptive statistics only. These will be compared in the 2 treatment groups but also to recordings of the same variables in a similar group of 45 infants (recruited to cooling only from the same entry criteria) recently treated in our department (historical control data). Differences between the groups will be estimated

Sample size is 26 infants in each group. The advisory statistician for this study is Professor Lars Walløe who has also advised on all aspects of the Xenon work (pre-clinical and clinical) undertaken to date.

10.2 Interim Analysis and Stopping Rules

No interim analyses are planned. Stopping rules are detailed in Section 9.3.1.

11.0 Withdrawal of Subjects from Study

Patients may be withdrawn from the study for any of the following reasons:

- Experiences clinically significant Adverse Event as determined by the Chief Investigator
- Requests to be withdrawn from the study
- Is unable to complete the study because of unforeseen circumstances
- Develops other conditions for which, in the Investigator's opinion, it is in the Patient's best interest to be withdrawn from the study

12.0 Modification of Protocol

Any amendments to this protocol must be approved by MHRA, the appropriate REC, the Sponsor and Investigators.

13.0 Discontinuation of Study

See Section 9.3.1.

14.0 Administrative Requirements, Ethical and Regulatory Aspects and Quality Assurance

14.1 Study Approvals: MHRA, Research Ethics Committee (REC) and NHS Trust R&D Approval

Before the study can begin the Chief Investigator must provide the Sponsor with:

i. a signed copy of a letter of "No Objection" from the MHRA
ii. a copy of the approval notice for the protocol from the appropriate Research Ethics Committee, signed by the Chairperson;
iii. a copy of the research governance approval from the University Hospitals Bristol NHS Foundation Trust.

This study will be conducted in accordance with:

- The Medicine for Human Use (Clinical Trial) Regulations 2004.
- The EU Medical Device Directive, 93/42/EEC of 14 June 1993
- BS EN ISO 14155 (2011) Clinical investigation of medical devices for human subjects. Good clinical practice
- International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines.
- Research Governance Framework for Health and Social Care

14.2 Responsibilities of the Investigator

The Investigator shall be responsible for ensuring that the clinical study is conducted in accordance with the protocol, the ethical principles enshrined in the Declaration of Helsinki (**Appendix 9**), ICH guidance on Good Clinical Practice and all applicable regulatory requirements.
In all cases the informed consent of subjects or their legal representatives is an essential precondition for participation in any clinical study.

14.3 Subject Information

An unconditional prerequisite for a subject participating in the clinical study is his/her written informed consent. Therefore, adequate information must be given to the subject by the Investigator (in this case parent) before informed consent is obtained. A subject information sheet will be provided by the Sponsor for the purpose of obtaining informed consent. In addition to this written information, the Investigator will verbally inform the subject. The wording used in the information sheet will be such that it can be fully and readily understood by laypersons. The subject information sheet will be revised whenever new information becomes available that is relevant to the consent of the subjects.

14.4 Subject Consent

The written informed consent of the subject (in this case subject's representative – a parent) to participate in the clinical study has to be given before any study-related activities are carried out. It must be signed and dated by the subject and by the Investigator or person designated by the Investigator to conduct the informed consent discussion.

14.5 Source Data and Subject Files

The Investigator must keep a written subject file for every subject participating in the clinical study. In this subject file, the available demographic and medical information of a subject has to be documented, in particular the following: name, DOB, sex, height, weight, medical history, concomitant diseases and medications (including changes during the study), statement of entry into the study, study identification, randomisation number (if applicable), date of informed consent, all study visit dates, predefined performed examinations and clinical findings, observed AEs and reason(s) for withdrawal from the study, if applicable.

It should be possible to verify the inclusion and exclusion criteria for the study from the available data in the file. It must be possible to identify each subject by using this subject file.

Additionally, any other documents with source data have to be filed. This includes ECG tracings, X-ray films, CT and MRI scans, laboratory value listings and QoL questionnaires. All these documents have to contain a subject identifier and details of the study procedure to which the document belongs.

14.6 Reporting and Recording of Data

The data recorded during the course of this study must be documented on the Case report Forms (CRFs) and forwarded to the Sponsor. The Investigator must ensure the CRFs do not contain the names of any study subjects. All fields on the CRFs must be completed.

All study data will be recorded on the CRFs. All CRF's must be made available to the Study Monitor as soon as they have been completed so that the validity and completeness of the forms can be determined. Where possible all CRF data

should originate from a verifiable medical record. All data should be recorded in black ink on the CRF for ease of duplication, interpretation and analysis.

Any corrections should be made by scoring through the original value with a single line and writing the new value next to the original entry with the Investigator initialling and dating the new entry. Only the Investigator or designated staff may amend or otherwise alter any data entered onto the CRF. In addition any changes must be made on all copies of the document so that there is no difference between copies. If the reason for the correction is not obvious then, when appropriate, a brief explanation of the reason for the correction should be made. **Correction fluids should never be used on any document**. Before providing copies of completed CRF's to the Study Monitor, the Investigator should review their completeness, accuracy and legibility. The Chief Investigator must always retain a copy of all completed CRF's in the site Study Master File.

14.7 Monitoring

The study will be monitored and audited in accordance with UH Bristol's policy. All trial related documents will be made available on request for monitoring and audit by UH Bristol, the relevant Research Ethics Committee and for inspection by the Medicines and Healthcare products Regulatory Authority or other licensing bodies.

The Chief Investigator will permit the Study Monitor to visit the Investigational Site at regular intervals to review all the CRFs, study management and GCP compliance.

During monitoring visits, the Study Monitor will:

- Help resolve any problems
- Examine CRFs for omission of data, compliance and possible AEs
- Discuss inconsistencies in the study data
- Ensure that all study materials are correctly stored and dispensed
- Check adherence to the obligations of the Investigator
- Review consent forms, in particular the date of consent and signature
- Perform source data verification as described below

In line with ICH guidelines, monitoring will include verification of data entered in the CRF against original subject records. This verification will be performed by direct access to the original subject records and the Sponsor guarantees that subject confidentiality will be respected at all times. Participation in this study will be taken as agreement to permit direct source data verification. The Study Monitor must be kept informed of all issues pertinent to the study.

At the final monitoring visit the Study Monitor must resolve any outstanding data deficiencies and retrieve all used and unused test articles.

14.8 On-site Audits

The various National Regulatory Authorities, and the Sponsor, in the person of a scientifically trained and properly authorised employee, have the right to inspect all study records, including source documents. In the event of a notification of audit being received, the Chief Investigator should immediately notify the Sponsor.

14.9 Record Storage and Retention

Data will be collected and retained in accordance with the Data Protection Act 1998. Study documents (paper and electronic) will be retained in a secure location during and after the trial has finished. All source documents will be retained for a period of 15 years following the end of the study. Where trial related information is documented in the medical records - those records will be identified by a 'Do not destroy before dd/mm/yyyy' label where date is 15 years after the last patient last visit. Good clinical practice requires that a copy of all study data and documentation must be retained in the files of the responsible Investigator for a minimum of 15 years following notification by Sponsor that all Investigations (not merely the Investigators' portion) are completed, terminated, or discontinued. If the Chief Investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept responsibility.

14.10 Indemnity

This is an NHS-sponsored research study. For NHS sponsored research HSG(96)48 reference no. 2 refers. If there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS Indemnity

covers NHS staff, medical academic staff with honorary contracts, and those conducting the trial.

NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. *Ex-gratia* payments may be considered in the case of a claim.

15.0 Study Funding

All costs related to the standard care of treating a newborn infant suffering from hypoxic-ischaemic brain damage with therapeutic hypothermia will be borne by the University Hospitals Bristol NHS Foundation Trust. Any additional costs related to this study will be funded by the Moulton charitable foundation (breathing circuits, xenon, research staff).

16.0 Publication

The results of the study will be reported / disseminated in the following ways:

- Peer reviewed scientific journals
- Internal report
- Conference presentations
- Publication on website

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Liu X, Dingley J, Scull-Brown E,Thoresen M Delayed Hypothermia-Xenon Treatment Improves Function but not Brain Pathology in Neonatal Rats Surviving to Adulthood.
Submitted for publication: Ped Res

18.0 Appendices

Appendix A1

Summary of Study Procedures

1) *Initial screening* process using St. Michael's Hospital existing screening protocol for administration of therapeutic cooling (now standard treatment in this group of infants).

2) *Additional screening*: inclusion/exclusion criteria for administration of xenon.

3) *Discussion* with parent(s) of infant. *Patient information sheet*

4) *Informed consent*.

5) *Pre-xenon data collection*:
i) Collection of demographic information.
ii) Documentation of concomitant medications.
iii) Collection of baseline data pre-xenon, i.e. vital signs, mechanical ventilation requirements, serum chemistry, full blood count, arterial blood gases.

6) *Delivery of xenon* according to protocol for 18 hours.

7) *Post-xenon data collection*:
i) Full blood count.
ii) Serum chemistry.
iii) Assessment of level of consciousness, tone, reactions.

8) *Follow up. Bayley clinical neurodevelopmental score at 18m.*

Appendix A2 Pre-clinical technical feasibility study in pigs.

Technical Communication

A Closed-Circuit Neonatal Xenon Delivery System: A Technical and Practical Neuroprotection Feasibility Study in Newborn Pigs

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BACKGROUND: Asphyxia accounts for 23% of the 4 million annual global neonatal deaths. In developed countries, the incidence of death or severe disability after hypoxic-ischemic (HI) encephalopathy is 1–2/1000 infants born at term. Hypothermia (HT) benefits newborns post-HI and is rapidly entering clinical use. Xenon (Xe), a scarce and expensive anesthetic, combined with HT markedly increases neuroprotection in small animal HI models. The low-Xe uptake of the patient favors the use of closed-circuit breathing system for efficiency and economy. We developed a system for delivering Xe to mechanically ventilated neonates, then investigated its technical and practical feasibility in a previously described neonatal pig model approximating the clinical scenario of global HI injury, prolonged Xe delivery with and without HT as a potential therapy, subsequent neonatal intensive care unit management, and tracheal extubation.

METHODS: Sixteen newborn pigs underwent a global 45 min HI insult (4%–6% inspired oxygen reducing the electroencephalogram amplitude to <7 μV), then received 16 h 50% inspired Xe during normothermia (39.0°C) or HT (33.5°C). A conventional neonatal ventilator provided breaths of oxygen to a lower chamber compressing a hanging bag within. This bag communicated with the upper closed part of the breathing system containing soda lime, unidirectional valves, Xe/oxygen analyzers, and a tracheal tube connection. At each end-inspiration, this bag emptied fully and a bolus of oxygen, the driving gas, crossed from the lower to upper chamber via an additional valve. This mechanically substituted the gas uptake from the circle during the previous breath cycle (oxygen + small volume of Xe) with an equivalent volume of oxygen creating a slow-rising inspired oxygen concentration. This was offset by manual injection of Xe boluses, infrequently at steady state, due to the low-Xe uptake of the patient.

RESULTS: Total mean Xe usage was 0.18 (0.16–0.21) L/h with no differences between Xe-HT and Xe-NT groups, which had weights of 1767 (1657–1877) g and 1818 (1662–1974) g, respectively (95% CI). HT reduced heart rate in the cooled animals; 180 (165–195) vs 148 (142–155) bpm ($P < 0.0001$) with no differences in arterial blood pressure, oxygen saturation, arterial carbon dioxide tension, or weaning times between these groups.

CONCLUSION: We describe a closed-circuit Xe delivery system with automatic mechanical oxygen replenishment, which could be developed as a single use device. Gas exchange was maintained while Xe consumption was minimal (<$2/h at $10/L*). We have shown it is both feasible and cost-efficient to use this Xe delivery method in newborn pigs for up to 16 h with or without concurrent cooling after a severe HI insult.

(Anesth Analg 2009;109:451–60)

Xenon (Xe) is a rare noble gas with anesthetic properties, also showing great promise as a neuroprotectant in both *in vitro* and *in vivo* experimental studies.[1–8] Its mechanisms include preconditioning,[8] antagonism of the *N*-methyl-D-aspartate subtype of glutamate receptor,[9,10] and reduction of neurotransmitter release,[1,11] among others.[12] Severe hypoxic-

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Dr. John Dingley is a Director of a University spin-out company, AOX Ltd., set up to develop delivery systems for gases, including xenon. The research group used technology from a patent held by AOX on part of the device described in this paper with the permission of AOX. AOX did not fund this study in any way.

*The price of xenon in nearly all previous papers on this subject has been quoted as $10/L. However, the authors would like to note that the price has now risen to approximately $30/L since this paper was written.

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ischemic (HI) brain injury occurs in 1–2/1000 live human births at term with permanent disability or death rates of 60%–70%.[13] Brain injury develops after a destructive cascade lasting hours or days that includes "excitotoxic" apoptosis by prolonged activation of *N*-methyl-D-aspartate receptors,[14] and this raises the possibility that an after insult therapy for newborn infants might be developed to limit the eventual damage. Currently, hypothermia (HT) is the only intervention that improves neurological outcome after HI injury both experimentally[15–18] and clinically.[19–21] Six infants must be treated with HT for one to derive a clear benefit (i.e., the "number needed to treat" is 6),[19] so potential adjunctive therapies are of great interest.

Xe is an attractive adjunct to cooling due to its lack of chemical reactivity, minimal side effects,[22,23] and ease of reversibility. Xe is not fetotoxic[24,25] and has been used safely, although briefly, in newborns.[26] The expense and scarcity are currently obstacles to widespread anesthetic use and need to be resolved if this gas is to enter clinical practice.

Both Xe and HT are antiexcitotoxic[4,27,28] and antiapoptotic.[4,29] The optimum Xe administration period required to maximize neuroprotection in the newborn is unclear, although HT is currently used clinically for up to 72 h. In our previous rodent studies, we found that 3 h of Xe inhalation had an additive neuroprotective effect with HT, increasing neuroprotection from 37% (HT only) to 76% when HT was combined with 50% inhaled Xe.[7] Other rodent studies have suggested synergism between HT and Xe.[4] The next step is to perform *in vivo* experiments more representative of the human neonate, and for this we need to develop and evaluate a suitably economical Xe delivery system.

Xe is extracted during industrial oxygen production from liquefaction of air at a relatively fixed rate of approximately 9–12 million L/yr. For Xe procurement alone, air liquefaction would be prohibitively expensive. The need to use Xe efficiently is therefore due not only to its expense but also to its scarcity and effectively fixed annual production. This cost, often quoted as $10/L,[30] has led to the suggestion that despite "ideal anesthetic" characteristics, Xe could never enter clinical use.[31] However, the paucity of effective neuroprotective interventions in the newborn and the promise of Xe in this regard caused us to consider how this potential drug might be used efficiently.

Xe is a relatively insoluble gas[32] so, after initial loading or "wash-in," a state of near-equilibrium is rapidly attained. Subsequent patient uptake is thereafter very low (typically 2.5–4 L/h) in the human adult,[33,34] suggesting that a cost-efficient clinical delivery system should be technically possible.[35]

A low-flow circle might appear a reasonable solution; however, if a 70% Xe anesthetic is administered for 2 h in an adult with a total fresh gas flow as low as 0.5 L/min, <20% of this Xe will be taken up, with more than 80% still being spilled as waste.[30] Russian researchers have been obliged to use Xe recovery methods to mitigate losses with breathing systems of this type.[36] The low-Xe uptake of the patient favors the use of closed-circuit (minimal flow) breathing systems for greatest efficiency, economy, and responsible use of this limited resource.

The primary objectives of this study were to (a) design an optimally gas-efficient closed-circuit Xe/O_2 delivery system suitable, in principle, for use in ventilated neonates, (b) provide inherent safety against hypoxic gas mixtures without use of complex control systems, (c) evaluate its technical and practical feasibility with and without simultaneous HT in a previously described model approximating the real human clinical scenario of global neonatal HI injury, prolonged Xe delivery, subsequent neonatal intensive care unit (NICU) management, and tracheal extubation. Secondarily, we explored the effects of HT on Xe consumption.

METHODS

The protocol was conducted under Home Office license in accordance with United Kingdom guidelines in Large White Landrace pigs <24 h old. To determine whether it was possible to deliver Xe alone and with cooling by this method in neonates after a global HI insult, two groups of eight animals were maintained and monitored before (baseline period), during (HI insult), and after (16 h Xe delivery) a severe, transient global HI insult produced using a previously described technique.[17] During the after insult Xe delivery period, one group was maintained at normothermia, whereas the other was additionally cooled to a target temperature of 33.5°C. From pilot data, the selection of $n = 16$ animals led to a power of 0.8 for detecting a 20% difference between groups in Xe consumption, using an α of 0.05.

Equipment Design, Selection, and Technical Considerations

Three machines and monitoring systems were constructed for this study.

The Breathing System

If no fresh O_2 is supplied to a conventional anesthesia circle system, the patient will consume all O_2 present until a hypoxic mixture develops. Therefore, in a closed-circle system, fresh gases must be added at a rate exactly matching patient uptake of each gas. There are various ways to achieve this. In the simplest of closed underwater diving systems, the user will breathe pure O_2 around a circuit similar to an anesthesia circle. In time, by metabolic O_2 consumption, the reservoir bag will completely collapse part-way through an inspired breath. At this point, by continued inspiratory effort, a demand valve on an attached O_2 cylinder will open, providing the missing volume of O_2 to complete the inspiration phase. If we now imagine this circle to contain a mixture of Xe (slow

uptake) and O_2 (metabolic uptake), a different situation develops. It can be envisioned that substitution of these two uptake volumes with pure O_2 at each end-inspiration would produce a slowly increasing inspired O_2 fraction (F_{IO_2}). It was our intention to incorporate these concepts into a ventilator driven closed circuit in which a standard ventilator, set to deliver 100% O_2 in each breath to the circuit, provided not only the motive power but also, via a special volume substitution valve, the required O_2 replenishment in place of the demand valve in the diving example. When delivering a Xe/O_2 mixture, there would then be a background tendency for the F_{IO_2} to slowly increase, whereas the inspired Xe concentration would decrease at a similar rate. This would provide a degree of hypoxic mixture protection without use of O_2 sensors, servo loops, or electronic valves, any of which could fail and produce a life-threatening situation. Xe could then be delivered to the circle by computer or manual control to offset this trend, replacing the minimal patient Xe uptake. The design of a circuit to achieve these design aims is described in Figures 1 and 2. All components of this closed-circuit breathing system are original designs with the exception of the flexible bag and patient connector (Intersurgical, Wokingham UK).

The Xe delivery system is designed to be fitted between a conventional NICU mechanical ventilator (SLE 2000, SLE, South Croydon, UK) and the tracheal tube. The latex-free bag in the lower chamber has a volume of 500 mL, larger than a neonatal tidal volume, providing free space to accommodate added Xe bolus volumes without overspill. The removable soda lime canister has a volume of 800 mL permitting long periods of ventilation without replacement. The inspiratory and expiratory hoses are made of smooth bore medical polyurethane. Relative to conventional corrugated hoses these had minimal compliance and formed reliable leak-free connections.

In this design, a small aliquot of O_2 enters the closed circle upper part of the circuit at each end-inspiration from a lower bag-in-bottle chamber (where it is present as the driving gas from the ventilator) via a dedicated O_2 substitution valve.

Modern NICU ventilators maintain a bias gas flow throughout the breathing cycle. At end-expiration, this displaces any residual CO_2 from the "Y" connector, reducing CO_2 rebreathing. However, with a closed circuit, the absence of a bias flow combined with small neonatal tidal volumes means that an efficient self-closing, leak-free, low-opening pressure inspiratory valve was required to prevent rebreathing. Our inspiratory valve was derived from a military design (IDA71 diving rebreather, Russian Navy)*. A very thin valve disc was, by virtue of its negligible mass, adequately retained by a very fine coil-spring to provide the desired low opening pressure while retaining the ability to function in any orientation—important in this application as we sited it in the inspiratory hose close to the tracheal tube. Although reusable in this study (Fig. 1), the breathing system was designed to allow possible redevelopment as a molded single-use device, explaining, for example, the selection of a low-cost hanging bag rather than a vertical bellows. The compressible volumes of the complete upper closed circuit with soda lime and the lower bag-in-bottle chamber were 1100 and 2100 mL, respectively. These volumes were mainly dictated by the diameters of polycarbonate tubing available for construction, giving an overall system compliance of 3 mL/cm H_2O. The design aims were that it should (a) be driven by a standard NICU ventilator, (b) avoid the

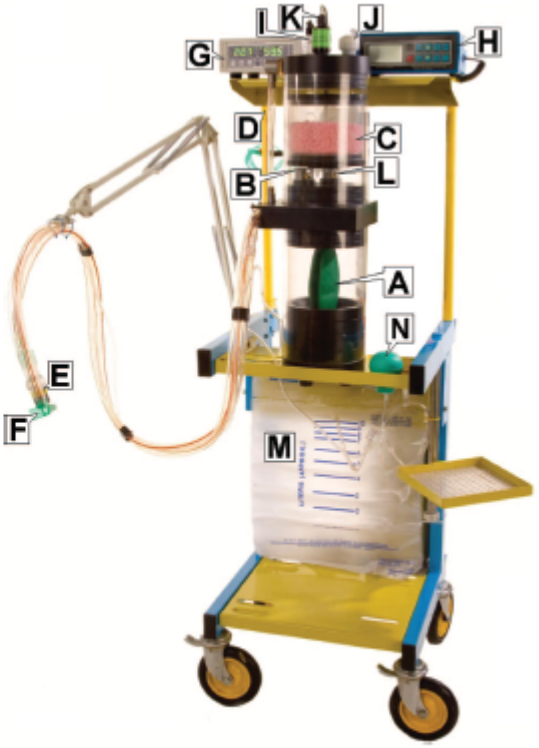

Figure 1. Full neonatal closed-circuit assembly (overall height 122 cm). A neonatal ventilator set to deliver 100% O_2 is attached to lower chamber of the circuit. During inspiration, bag (A) is compressed. Gas passes to upper part of machine, cannot pass through expiratory valve (B) so passes through soda lime (C), via inspiratory hose (D) and inspiratory valve (E) to patient connection (F) inflating the lungs. Gas monitoring includes xenon/O_2 analyzer (G), auxiliary O_2 analyzer (H), O_2 cells for these analyzers (I,J), and a xenon sensor (K). Bag (A) fully empties before end-inspiration due to patient uptake of gas from circle during the previous breath cycle, at this moment O_2 substitution valve (L) opens to replenish this volume deficit with O_2. During expiratory cycle gas passes through expiratory valve (B) into bag (A). Aliquots of xenon from flexible bag (M) are manually added via a valve arrangement by pressing flexible bulb (N).

*Available at: www.therebreathersite.nl/Zuurstofrebreathers/Russian/ida-71.htm.

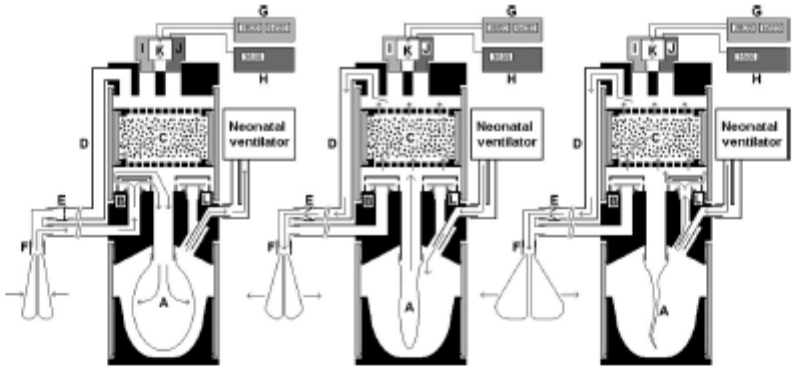

Figure 2. Mechanism of operation. Labeling matches Figure 1. Although pressure-controlled ventilation was used in the study, it is easier to describe mechanism in terms of volume-controlled ventilation. End-expiration (left): Lungs deflated, exhaled gas into bag (A) via expiratory valve (B). Bag only partially full (exaggerated in diagram). Gas volume in (A) slightly less than tidal volume set on ventilator due to gas uptake by patient during previous breathing cycle. Mid-inspiration (center): Bag A deflating, gas passing through soda lime, hose, inspiratory valve to lungs. Oxygen from ventilator entering lower chamber through an elongated port. This, by an entrainment effect on surrounding gas, creates a mild pressure reduction beneath the O_2 substitution valve (L) ensuring it remains closed until end-inspiration without spring-loading. End-expiration (right): Ventilator delivers remainder of tidal volume to lower chamber. Bag (A) is already empty so small aliquot of O_2 driven through O_2 substitution valve (L) into closed circuit so replacing gas volume consumed by patient. A safety valve spills gas from upper chamber to the lower if circle is overfilled for any reason (not shown).

need to develop a specialized Xe-specific ventilator, (c) have inherent hypoxic mixture prevention properties not dependent on electronics, (d) reduce misassembly risk, and (e) provide the user with the most intuitive treatment reversal method: reconnection of the neonate directly to the NICU ventilator.

Gas Delivery to Breathing System

This also followed the single-use concept. The slow-rising F_{IO_2} tendency was deliberately offset by occasional manual delivery of Xe boluses to maintain the target Xe concentration; the combination of closed circuit, low patient weight, and low-Xe uptake suggest that these boluses should be infrequent. We have previously delivered Xe using high-pressure Xe cylinders with electronic valves actuated by both computer[37] and manual control.[23] After using servo control in the past, we realized that, because of the slow uptake of Xe, manual addition of Xe boluses should not be particularly onerous,[23] facilitating a much simpler design devoid of any computer control systems. In this study with neonatal subjects, we anticipated a very low-Xe uptake and therefore adopted a manual method. On this occasion, it was designed to function at ambient pressure for additional simplicity and safety, eliminating any risk of the circle "flooding" with Xe due to a cylinder/regulator/electronic malfunction (Fig. 3).

This design, in turn, facilitated the use of small low-pressure cylinders of 5-L Xe to fill an ambient pressure Xe reservoir bag, thus eliminating the need for a large high-pressure Xe cylinder with its attendant risk of costly accidental gas loss. A flexible bulb and valve arrangement allowed manual addition of approximately 50 mL Xe boluses from reservoir bag to breathing system. These were added to the expiratory limb of the circuit to allow maximum mixing with

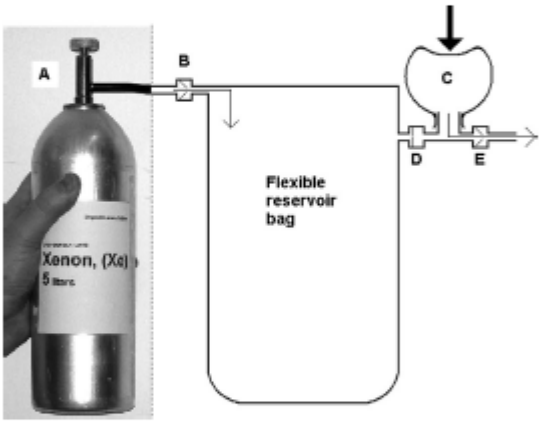

Figure 3. Xenon delivery to closed circuit. Xenon cylinder is completely discharged (5 L Xe) into the flexible ambient pressure reservoir bag via stopcock (A) and self-sealing unidirectional valve (B). Flexible bulb (C) refills itself from the reservoir bag via unidirectional valve (D) and then by manual pressure on bulb (C) 50 mL xenon boluses can be delivered via unidirectional valve (E) into the breathing system as required.

existing gases in the circle before entering the lungs. The flexible bulb volume of 50 mL was selected not only because of the paucity of suitable designs available but also because its slow refill properties and small volume ensured more even gas mixing within the circle, as it was impossible to rapidly add larger xenon boluses. Erring on the side of caution, our circuit was primed with O_2. During the first few minutes of Xe delivery, the F_{IO_2} was increased by tolerating some overspill of circle gas as it was added. Once the target Xe concentration had been reached, the system was allowed to run in a steady closed state by the O_2 substitution valve mechanism and then only occasional Xe boluses

were required. The Xe/O_2 monitors were constantly observed as would be the case during the conduct of a conventional anesthetic. Deviations in Xe concentration of more than 2%–4% from target were corrected by the operator adding Xe to the circuit, each bolus typically producing a 2% increase in Xe concentration. These corrections offset the inherent tendency of this circuit design to generate a very slowly increasing O_2 concentration (O_2 + minimal Xe consumptions being replaced with equal volume of O_2 via substitution valve). The total (cumulative) Xe consumption pattern in each experiment was derived from a record of the number and timing of each bolus.

Leak Test Procedure

Before use, an infant test lung (Imtmedical, Switzerland) was fitted to the tracheal tube connector and the ventilator started. A small volume of gas (approximately 100 mL) was added to the breathing system; at end-expiration, the hanging bag would then contain this exact volume of gas and, in the absence of leaks, this volume would not decrease with time.

Circuit Priming Before Use

One liter per minute of O_2 was delivered to the ventilator connector port on the lower chamber. The O_2 entered the lower chamber, collapsed the hanging bag, flowed upward via the O_2 substitution valve through the soda lime, and out via the inspiratory hose, substantially replacing all contained gases with O_2, which we confirmed by examination of the O_2 analyzer displays.

Gas Monitoring

Concentrations of Xe/O_2 in the breathing gas and $ETco_2$ concentration needed to be measured for safety and to guide manual Xe administration. Our previous experience with closed circuits and side-stream monitors highlighted problems with sample line occlusion, humidity-induced unreliability, gas leakage, and nitrogen contamination of gas returning to the circuit. We therefore used mainstream monitoring wherever possible. A mainstream Xe/O_2 anesthesia analyzer (GKM-03-INSOVT, Moscow, Russia obtained via Alfa-Impex Oy, Finland) was used and 22-mm female taper fittings in the upper chamber of the breathing system were created to accept the two sensors of this monitor (Fig. 4). This analyzer was designed for anesthesia in Russia, where Xe has been licensed since 2002 and used in approximately 3000 anesthetics (personal communication with supplier). It has a conventional electrochemical O_2 cell and, possibly uniquely, a similarly shaped Xe sensor operating on the principle of thermal conductivity. Xe concentration is difficult to measure at acceptable cost as it is almost completely unreactive, does not absorb light, and is not paramagnetic. This sensor works on the principle that, in a mixture of gases in which one has a very different thermal conductivity to the others, its relative concentration can be derived. The thermal conductivity of Xe

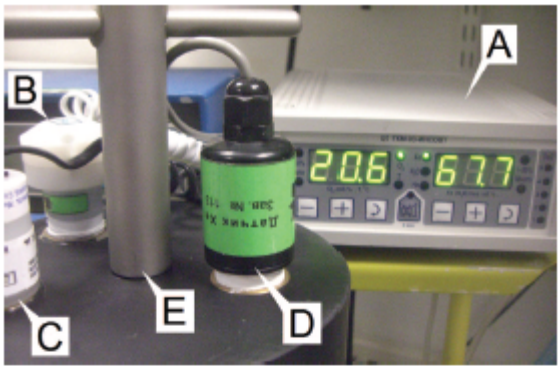

Figure 4. Gas analyzer and sensor connections. The xenon/oxygen analyzer is shown (A). Upper surface of breathing system contains main (B), auxiliary (C) electrochemical oxygen cells, and the thermal conductivity xenon sensor (D). Handle (E) is used to disassemble the main components of the upper structure.

is almost five times lower than that of O_2 or nitrogen, which is similar, and this anomaly favors Xe measurement by this method.

For safety, a secondary battery-powered anesthesia O_2 analyzer (Teledyne, Viamed, West Yorkshire, UK) was used with an electrochemical sensor, which was also mounted on the top of the breathing system. $ETco_2$ concentration was measured with a mainstream infrared analyzer (Tidal Wave™, Respironics UK, Chichester, UK), specifically chosen for its low dead space neonatal tracheal tube connector to minimize CO_2 rebreathing.

Xe Uptake Modeling

To investigate the proportion of our experimental Xe consumption rates attributable to uptake by the piglets (i.e., uptake by tissues) versus that due to losses by leaks and diffusion, we used the computer simulation program "Narkup 2000" (D C White, Northwick Park Hospital, Middlesex, UK and G Lockwood, Hammersmith Hospital, London, UK) to predict the Xe uptake by the piglets.[38] This was first programmed with neonatal cardiorespiratory values (weight 1.8 kg, cardiac output 250 mL/min, minute ventilation 310 mL/min, 35% dead space, 7% shunt), and typical adult tissue compartment parameters (the relative content of each tissue type within the body). The simulation was then repeated using neonatal tissue compartment parameters derived from anatomical tables[39] in case the lower fat content of neonates had any major effect on the predicted Xe uptake.

Conduct of Experiment

Anesthesia/Ventilation

Unrestrained animals were anesthetized with halothane/O_2/nitrous oxide followed by orotracheal intubation with cuffed 3-mm internal diameter tubes (Mallinckrodt Medical, Athlone, Ireland) and mechanical ventilation (SLE 2000, SLE) commenced. The

cuff was inflated with air during ventilation until an audible leak just ceased. The animals were not paralyzed. Vascular access was obtained and monitoring of core temperature, electrocardiogram, and amplitude integrated electroencephalogram (aEEG) was established. aEEG (CFM 6000, Olympic Medical, Seattle, WA and BrainZ™, BrainZ instruments Limited, Auckland, New Zealand) was required as a monitoring tool during the HI insult.[40] Ventilation settings were adjusted to maintain an $ETco_2$ between 35 and 45 mm Hg, confirmed by intermittent blood gas analyses during the study, except during the insult. Before and after the HI insult, the Fro_2 was adjusted to maintain a saturation on pulse oximetry of >95%.

Fluid Management

A 3.75% dextrose/0.45% saline maintenance infusion was commenced at 15 $mL \cdot kg^{-1} \cdot h^{-1}$ before the insult, after which 5% dextrose/0.45% saline was run at 10 $mL \cdot kg^{-1} \cdot h^{-1}$.

Temperature Control

The piglets were kept in purpose-built neonatal incubators. During the initial baseline period and HI insult periods, the normal piglet core temperature of 39°C ± 0.2°C was maintained with a radiant warmer. HT was induced to a rectal temperature of 33.5°C ± 0.2°C as required, using a clinical cooling mattress (Tecotherm, TecCom, GmbH, Munich, Germany).

Cardiac Monitoring

Continuous mean arterial blood pressure (MAP) was measured from an umbilical artery catheter and three-lead electrocardiogram monitoring (Passport 2, Datascope, Montvale, NJ) was undertaken. During the insult, no lower limit for MAP was set, unless followed by bradycardia (<80 bpm); otherwise, a MAP less than 40 mm Hg during the study period was treated with a 10 mL/kg bolus of 0.9% saline followed by a second identical bolus if still unsatisfactory. If hypotension persisted or bradycardia developed, inotropic support was commenced with dopamine (range, 5–20 $\mu g \cdot kg^{-1} \cdot min^{-1}$) plus, if necessary, noradrenaline (range, 0.1–1 $\mu g \cdot kg^{-1} \cdot min^{-1}$).

After placement of all monitoring equipment, the piglet was laid prone and rested for 60 min under anesthesia after which a 45 min global HI insult was applied.[41]

HI Insult and Resuscitation

Briefly, the Fro_2 was reduced abruptly to 4%–6% for 45 min to reduce the aEEG background amplitude to <7 μV with an inspired halothane concentration of 0.7%–1.0%.[41] The piglet was then resuscitated for 30 min in air or the lowest inspired O_2 concentration required to achieve an O_2 saturation of more than 95%, and anesthesia was being maintained using a total IV technique comprising a 4 mg/kg propofol bolus, propofol (4–12 $mg \cdot kg^{-1} \cdot h^{-1}$), and remifentanil (3–20 $\mu g \cdot kg^{-1} \cdot h^{-1}$). Although an air/$O_2$ mix helped remove any residual volatile anesthetics, 100% O_2 would have been theoretically better, allowing formal denitrogenation before closed circuit use. This was, however, undesirable in terms of reperfusion injury immediately after such a severe insult. We had also discovered from pilot work that the small piglet nitrogen content versus the volume of the closed circuit meant that the lack of a denitrogenation maneuver did not, in practice, present a nitrogen accumulation problem.

After insult, the pigs were randomized to receive Xe while normo or hypothermic: 16 h 50% Xe at T_{rectal} 39.0°C for 16 h, or 16 h 50% Xe at T_{rectal}33.5°C for 12 h, followed by rewarming, respectively.

Xe Delivery After Insult

The closed-circuit Xe/O_2 mixture was delivered for 16 h after insult. Once the 50% target Xe concentration had been reached, the propofol infusion was stopped and background remifentanil infusion continued. Pressure-controlled ventilation was used with inspiratory pressures of 18–20 cm H_2O and positive end-expiratory pressure of 4 cm H_2O, measured at the tracheal tube connector. After this, all anesthetics were stopped, analgesia was provided by IM buprenorphine (10–20 μg/kg every 12 h), the tracheal tube cuff deflated, the piglets were reconnected directly to the ventilator, gradually weaned, and tracheally extubated.

Analysis of Data

The data are mainly descriptive. Mean (95% CI) was used to describe normally distributed data, and median (nonparametric 95% CI) was used to describe skewed data. For statistical analysis, linear regression, and the Mann–Whitney test were used to explore differences between groups (SPSS 15.0 for windows, SPSS, Chicago, IL, and Minitab 15, Coventry, UK). With respect to Xe consumption data, linear regression was performed for each animal with cumulative Xe requirement as the dependent variable and time as the independent variable. A two-tailed P value <0.05 was considered significant.

RESULTS

The mean weight of the pigs was 1767 (1657–1877) g and 1818 (1662–1974) g for the Xe-NT and Xe-HT groups, respectively. The median age of the pigs in the Xe-NT group was 12.25 (2–23.39) h and 17.25 (11.9–23.84) h in the Xe-HT group.

During the Xe administration period, physiological variables were stable. Mean (95% CI) heart rates were 180 (165–195) and 148 (142–155) bpm ($P < 0.0001$) with MAPs of 54 (51–57) and 54 (49–59) mm Hg for the Xe-NT and Xe-HT groups, respectively. The O_2 saturations on pulse oximetry for Xe-NT and Xe-HT were 99.3% (99–99.6) and 99.2% (98.8–99.5), respectively. CO_2 removal was adequate with blood gas values of 41.3 (38.6–43.9) and 43.9 (36.1–51.8) mm Hg in the

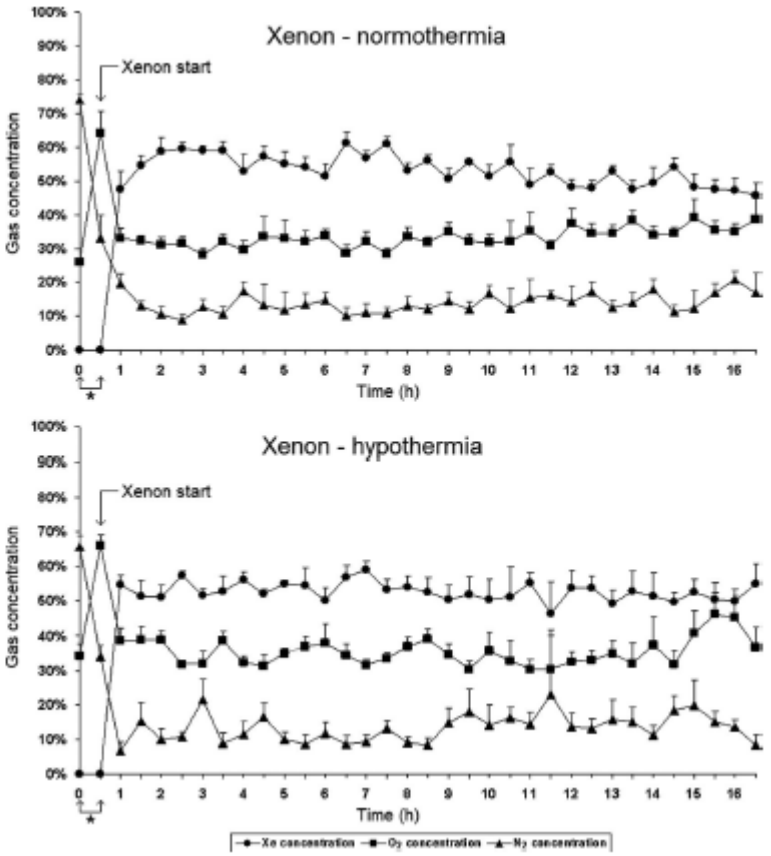

Figure 5. Concentrations of oxygen, xenon, and nitrogen (calculated as 100 − O_2% − Xe%) versus time. * = 30 min recovery period after insult.

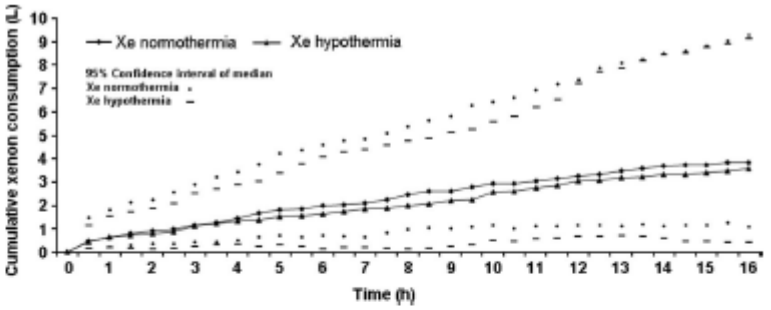

Figure 6. Cumulative xenon (XE) dose in both the Xe-normothermia and Xe-hypothermia groups during the 16 h of XE administration. Median (95% CI).

Xe-NT and Xe-HT groups. No animals died during the conduct of the experiment, with median (95% CI) times to successful weaning and tracheal extubation after cessation of the 16-h period of Xe administration of 3 (0.5–12.1) and 8.5 (2.5–40) h for each group, respectively. Except heart rate, none of these differences were significant.

The median (95% CI) Xe concentration in the circle was 52.3% (50.9–53.9) and 52.17% (50.9–53.5) in the Xe-NT and Xe-HT groups, respectively. O_2 and nitrogen concentrations in the circle were 33.1% (32.1–34.5) and 13.4% (12.2–15.6), respectively, in the Xe-NT group. In Xe-HT group, O_2 and calculated nitrogen concentrations were 34.7% (32.6%–36.9%) and 13.5% (11.2–15.1), respectively (Fig. 5).

The predicted tissue Xe uptake using NARKUP software programmed for a 1.8-kg patient with an adult tissue compartment distribution was 25.5 mL/h over the 16-h period. This decreased further to 16.1 mL/h when neonatal tissue compartment parameters were used.

The cumulative fresh Xe expenditure is shown in Figure 6. The overall median (95% CI) hourly Xe consumption was 0.19 (0.15–0.24) and 0.18 (0.16–0.21) L/h in the Xe-NT and Xe-HT groups, respectively, with an overall value for both groups of 0.18 (0.16–0.21) L/h. There was no significant difference between the slopes of the Xe consumption curves of the Xe-NT 0.0028 (0.0022–0.0053) and Xe-HT 0.0035 (0.0025–0.0056) groups (P = 0.5). Multiple linear regression showed that the temperature during Xe

delivery, weight, age, or sex of an animal had no influence on the Xe requirement over time.

DISCUSSION

Closed breathing systems may appear as an exotic method of anesthesia delivery; however, circle breathing systems with CO_2 absorption for acetylene anesthesia were described by Carl Gauss as long ago as 1924/1925, and use of these systems "closed" with basal O_2 flows is usually attributed to the anesthesiologist Brian Sword (CT) 4 yr later. Closed systems become particularly attractive whenever an expensive anesthetic is to be used, which has a low-patient uptake. This situation existed in the past with respect to cyclopropane, which was originally very expensive and the same principles apply now with respect to Xe. The advent of computer control systems has allowed production of closed circuit machines, which are easier to use, a current example being the Drager Zeus. A predecessor of this machine, the PhysioFlex (Physio, The Netherlands then Dräger, Lübeck, Germany), had a variant, the PhysioFlex-Xe, which was capable of closed-circuit Xe delivery.[42] At least one computerized closed circuit machine for Xe anesthesia will be launched again soon in Europe. Closed systems have also been used successfully for many years in mine rescue, firefighting, military diving, and aerospace.

We sought to develop a relatively straightforward closed circuit delivery system suitable for cost-effective delivery of Xe to neonates as a potential neuroprotectant after perinatal asphyxia and to investigate its technical and practical feasibility in a realistic model of this clinical situation, which included the induced HT currently in clinical use.

The overall Xe utilization rate was 0.18 L/h which, at approximately $10/L, equates to a running cost of under $2/h. This included some Xe losses incurred during the initial wash-in procedure to increase the inspired Xe fraction as rapidly as possible in which some gas overspill was tolerated. This initial loss could be avoided in the future by priming the circuit with a Xe/O_2 mixture rather than with O_2 alone. If, hypothetically, 1 million L/yr of the global Xe production could be set aside solely for medical use, then this would allow approximately 231,000 neonatal 24 h treatments to be provided for less than $45 Xe per treatment. In contrast, as an example, a very low-flow regime of 200 mL/min Xe would consume this entire supply within 3472 neonatal 24 h treatments at a Xe cost of $2,880 per treatment. We therefore believe our aim of effective cost containment for clinical practicality and responsible use of this scarce resource was achieved.

Satisfactory gas exchange was maintained in both groups during the 16 h after insult Xe delivery period. Our target Xe concentration was 50%, raising the possibility that the remaining gas in the circle could be 50% O_2. In neonates with minimal lung damage, this might produce hyperoxia which, in addition to the well-known retinopathy, could be harmful after an HI insult via reperfusion injury and other mechanisms. In practice, babies with perinatal HI can have abnormal gas exchange from meconium aspiration, for example, and often do require an elevated F_{IO_2}. The residual nitrogen present in our breathing gas mixture advantageously helped reduce the F_{IO_2} to approximately 34%. Maneuvers, if required, to attenuate an elevated F_{IO_2} include increasing the Xe fraction or deliberately injecting of small volumes of air. In a human neonatal trial, there might be a precautionary requirement to flush the circuit at intervals of approximately 2 h to prevent accumulation of foreign gases emerging from the lungs, such as methane from gut fermentation and acetone from liver metabolism as the clinical effects of these gases, if any, are currently unclear. The clinical implications of this action are unclear. As the optimum therapeutic duration of Xe delivery is not currently known, our soda lime container was intended to last up to 72 h without replenishment as HT is currently being induced clinically for similar periods. The maximum volume of 800 mL that it can carry is probably excessive for such small subjects, especially if cooled. This volume will be reduced in future versions. If periodic circle flushes are considered necessary, any reductions in circuit volume would also be advantageous to minimize the Xe requirement of this maneuver.

We account for the reduced heart rate in the Xe-HT group relative to the Xe-normothermia group by the clinical observation that therapeutic HT in human neonates consistently decreases heart rate.[43] Despite using cuffed tracheal tubes, weaning and tracheal extubation were possible without postextubation stridor. We used cuffed tracheal tubes (commercially available for human neonatal clinical use) to prevent excessive Xe loss via leakage at this point. We acknowledge past concerns over cuffed tubes in infants and potential airway edema. However, such tubes are now available in sizes as small as 2.5 mm internal diameter and finding a role in some centers with few reported problems.[44] The effect of Xe diffusion into tracheal tube cuffs has been investigated by others and, although it does occur, the effect is much less pronounced than that seen with nitrous oxide.[45]

The predicted patient tissue Xe uptake using NARKUP software programmed for a 1.8-kg patient with an adult tissue compartment distribution was 0.026 L/h over the 16 h period, i.e., less than one seventh of our experimental measured Xe requirement. The uptake decreased even further to 0.016 L/h, i.e., 16 mL/h if neonatal tissue compartment parameters were used in the simulation, reflecting the lower proportion of body fat in neonates. In addition, our measured *in vivo* Xe requirement was linear with time rather than reaching an expected plateau as tissues saturated and was found to be unrelated to the weight of the animal. These observations collectively suggest that a steady, slight Xe loss from the breathing system

was occurring in addition to this almost negligible predicted patient uptake. Despite being very low, we, therefore, suspect that a majority of our ongoing Xe requirements were still being expended replacing minor Xe losses. Because each machine was leak tested before use, we suggest that potential mechanisms of Xe loss might include diffusion through the silicone rubber hanging bag or minor leaks past the tracheal tube cuff. This is encouraging despite an overall Xe requirement of only 0.18 L/h (<$2/h), as it appears that with further development there may be the potential to reduce this even further.

The Xe consumption was sufficiently modest to render the concept of using gas cartridges a technically feasible method of refilling the Xe reservoir. Although we used small single-use gas cylinders in this study, we envision small pressurized gas cartridges containing 1–5 L Xe would be an ideal method of supply. Such cartridges already exist for carbonated drink (CO_2) and food industry (N_2O) use. One cartridge could provide sufficient Xe for an entire treatment of many hours duration.

The PhysioFlex-Xe was a closed system of 3.5 L volume, with small increments of O_2 and other gases given by computer-controlled injection and excess gases evacuated as necessary. Inspired gases were measured as follows: O_2, paramagnetic analyzer; carbon dioxide and volatile anesthetics, infrared spectrometer; Xe, thermal conductivity. The system incorporated a blower circulating the contents at 70 L/min, dual soda lime containers, a charcoal canister to produce rapid reductions in volatile and/or Xe concentrations as required, and four membrane chambers allowing controlled ventilation/system volume sensing. The system we describe is also a closed circuit. However, by design, it has no computerized gas-injection system (although it easily could), relying instead upon mechanical substitution of patient gas uptake with O_2 to ensure a constant or slow-rising O_2 concentration, offset by manual injections of Xe, which are infrequent at steady state due to the very low-Xe uptake of the neonate. It currently has a volume approximately 1/3 that of the PhysioFlex, which we intend to greatly reduce. Gas monitoring is by mainstream methods for reliability. As with the PhysioFlex, we measure Xe concentration by thermal conductivity electing to use a robust Russian device for this purpose having built and used Xe monitors of various designs in the past. It has no gas blower, membrane chambers, or computer, and the concept is that our unit could be redeveloped as a molded single-use circuit. Any compliant regions of compressible gas would be greatly reduced by molding the housings closely around the internal structures. A preassembled device has far fewer connections and seals, especially around the soda lime refill mechanism, all of which can leak. Leakage rates, which would normally be irrelevant in anesthesia practice could materially affect running costs in a circle system intended for Xe, explaining our great attention to this issue. As examples from current medical practice, cardiopulmonary bypass circuits are preassembled for safety and at least two single-use conventional anesthesia circles are also commercially available.

It is possible that an O_2 substitution valve, if incorporated into a circle-based anesthesia machine might, by replenishing all the metabolic O_2 uptake, improve upon the performance of the current antihypoxic mixture devices where at present even if the O_2 rotameter is off, a residual flow of 50–100 mL/min O_2 remains, which may not, however, meet a patient's metabolic needs.

In summary, we have described and evaluated a closed-circuit Xe delivery system specifically intended for neonatal use, which automatically replenishes patient O_2 consumption without complex control systems, has hypoxic mixture protection, and could be designed as a molded single-use device. The overall Xe requirement, and therefore running cost, was minimal at 0.18 L/h (<$2/h), permitting responsible use of a restricted global Xe supply in the maximum number of clinical cases per year. It is both technically and physiologically feasible to use this method of Xe delivery as a potential therapeutic intervention in neonates for prolonged periods, even after a severe HI injury, either with or without the cooling that might also be used. We have shown that economic Xe delivery is not only theoretically but also practically possible, even in this challenging clinical situation.

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Appendix A3 Additional details on the conduct of the previous human clinical feasibility/safety study are given below, a summary of physiological effects is provided, a large quantity of physiological data from the cooled and xenon+cooling groups is also supplied.

Single centre prospective non-randomised clinical feasibility study "CoolXenon"

http://www.controlled-trials.com/ISRCTN75602528

UK MHRA Medicines and Healthcare products Regulatory Agency

MHRA approval for clinical use of the xenon delivery system
MHRA approval to give 50% xenon for up to 18h as a potential neuroprotective drug to babies

Entry criteria

- Undergo cooling therapy for perinatal asphyxia
- Needing <35% oxygen (Xenon is a heavy gas)
- Parental written consent for added Xenon & re-intubation with cuffed tube

Patient 1	20% Xe for 3h
Patient 2	50% Xe for 3h
Patient 3-4	50% Xe for 6h
Patient 5-11	50% Xe for 12h
Patient 12-14	50% Xe for 18h

For every 2 patients recruitment was paused
External review panel to review all patient data before commence recruitment

Objective:
Add up to 18 hours of 50% **xenon** inhalation to the established treatment of 72h of WBC cooling to 33.5°C
Hypothesis:
Observe no changes in those clinical and biochemical markers that we routinely monitor in infants who receive cooling therapy

It is technically feasible and cost effective to use our closed loop xenon delivery system

Results

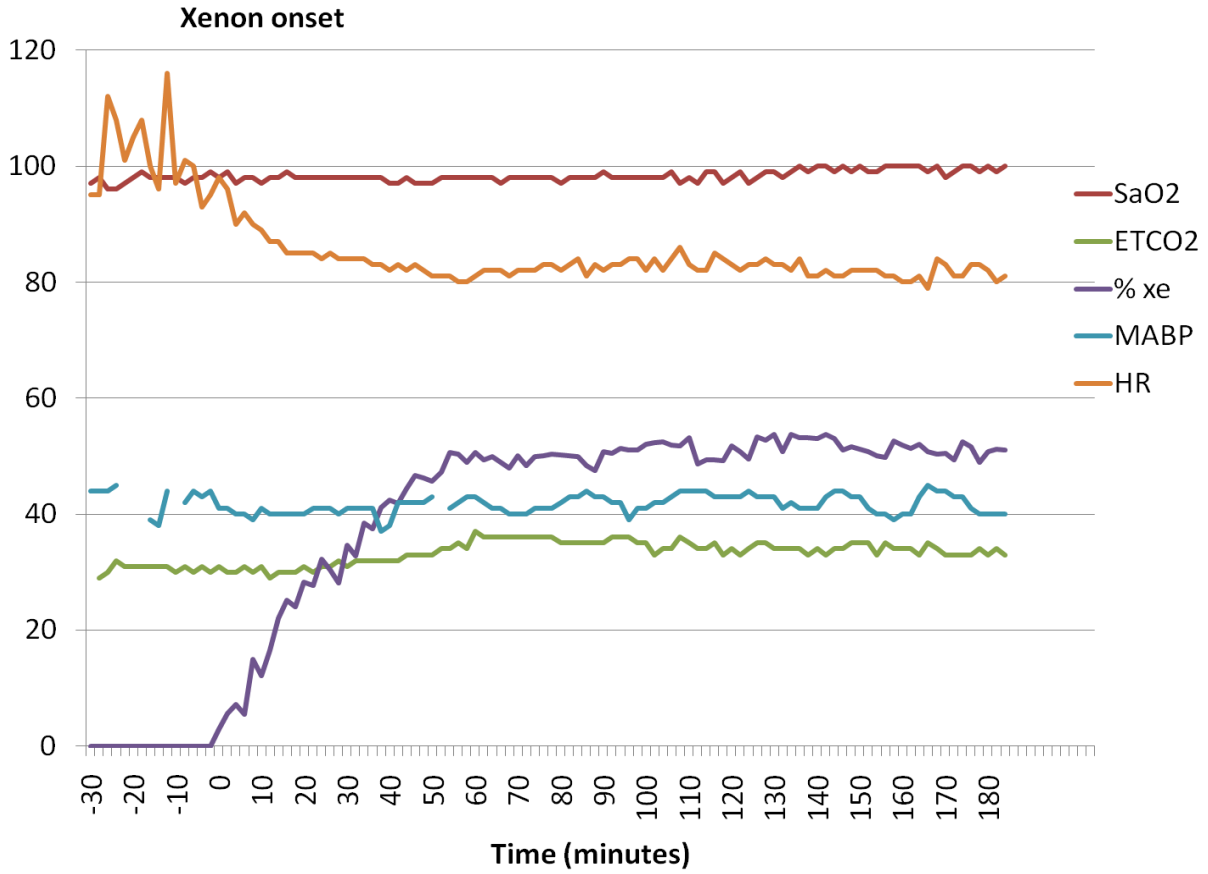
n=14

13 of the 14 babies were outborn (retrieved from other centres).

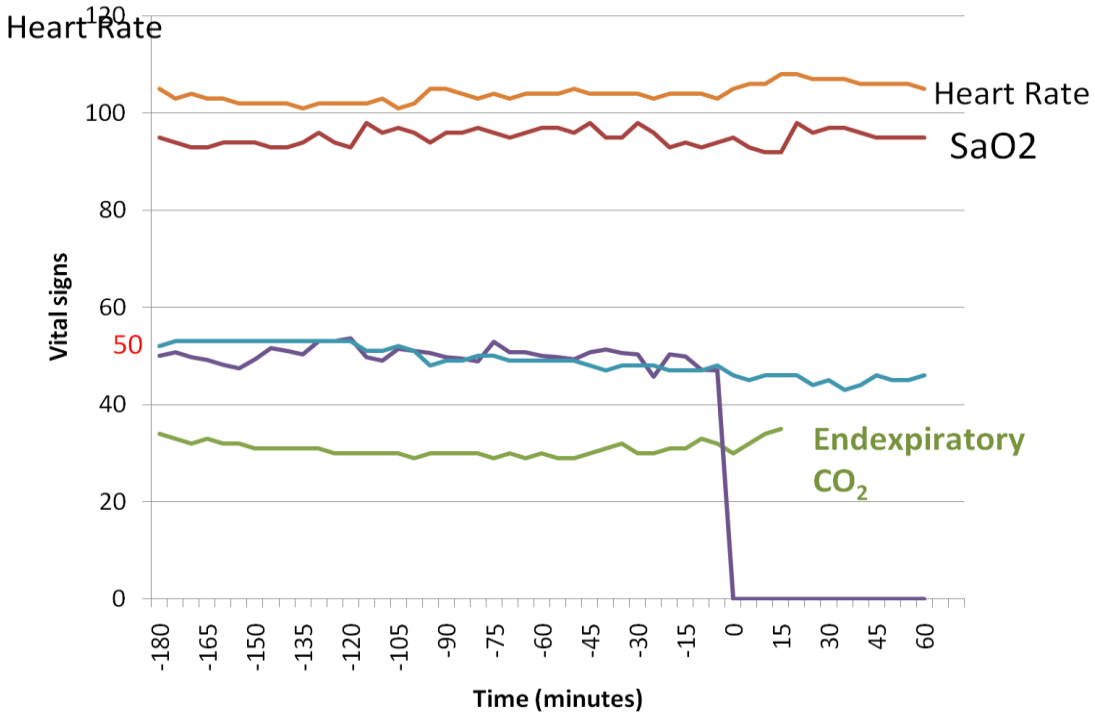
Demographics

Patient criteria n=14 female=7	**Median (range)**
Weight kg	3.438
Gestation weeks	40.2 (36-42)
Apgar 1 min & 10min	1.0 & 5.5
Cord Ph	**6.86 (6.72-7.03)**
aEEG before start cooling	90% Burst Suppression
Seizure before cooling	60%
Pheno before cool Number of AED doses	50% 2.5 (2.0)

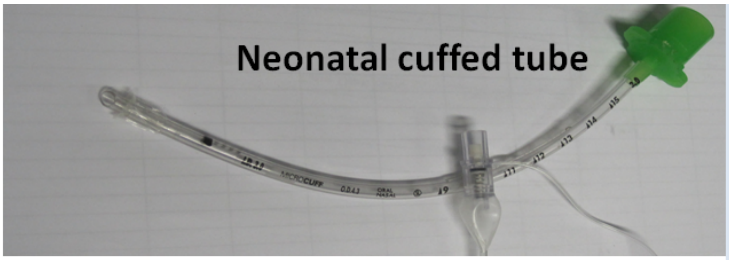
Physiological changes during and after Xenon



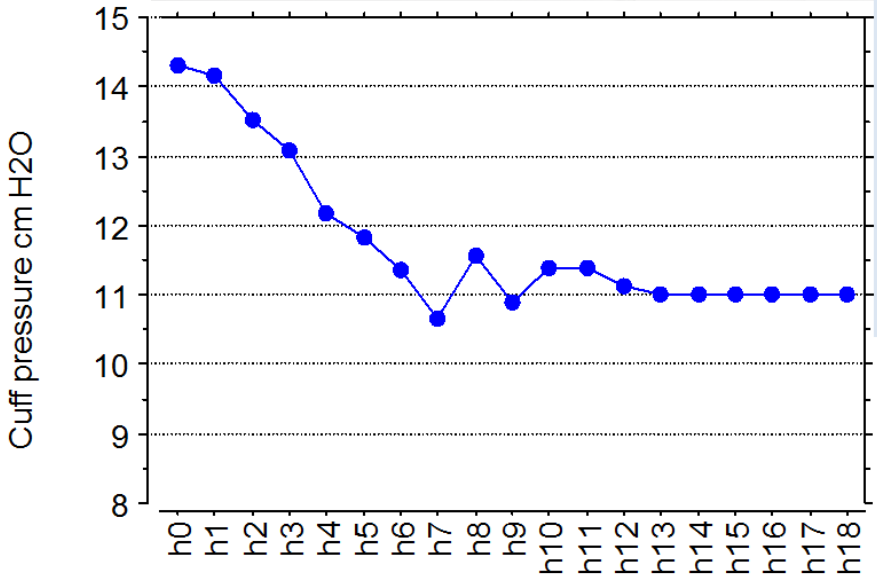
Haemodynamic changes at onset of xenon.



Haemodynamic changes at cessation of xenon.

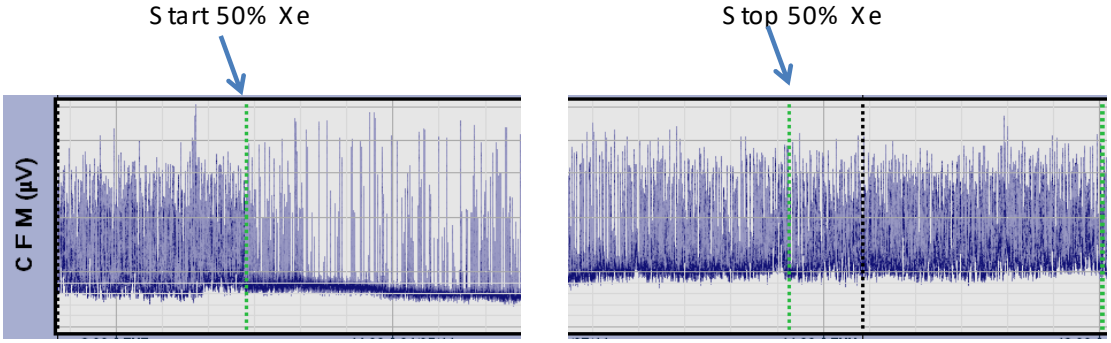


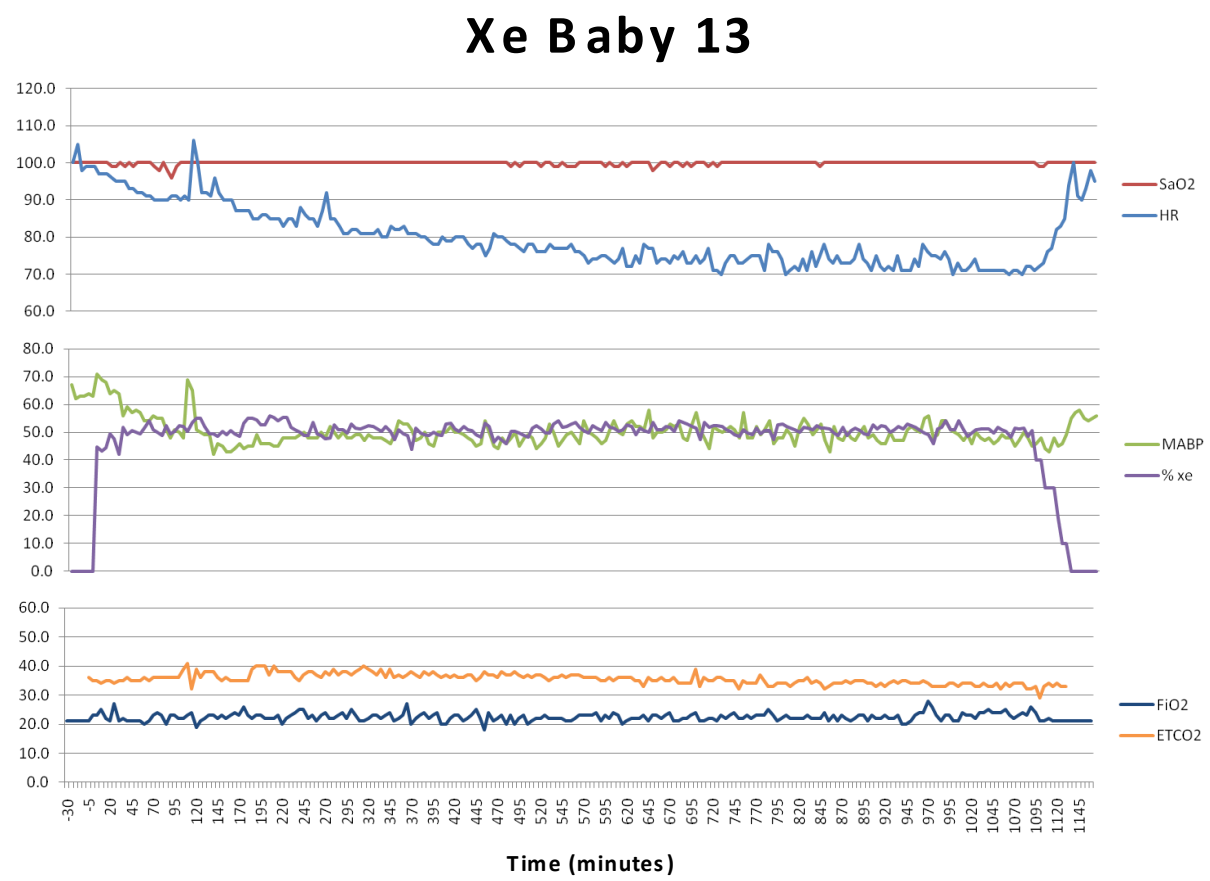
- Reintubated with tube size 0.5 smaller
- Aimed at lowest cuff pressure with no leak
  ~ 11 cm H20
- End xenon –cuff deflated, kept tube in situ



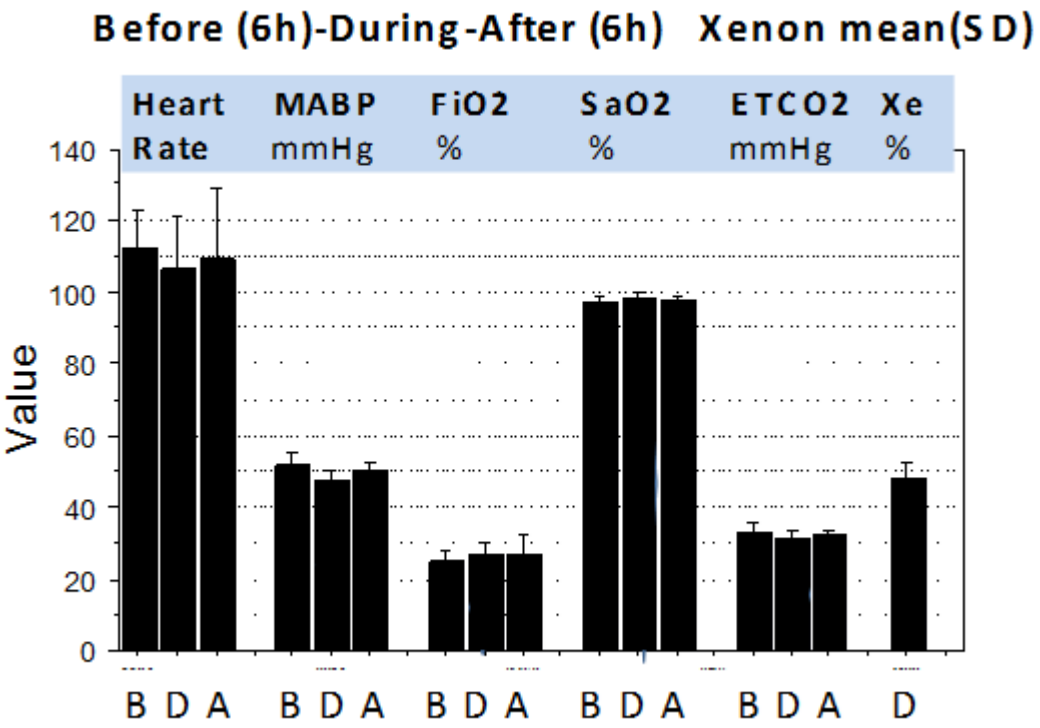
Duration of xenon inhalation with cuffed tube

Xenon is sedative in 50% concentration
Depresses the aEEG which recovers when xe is discontinued





Example of cardio-respiratory physiology throughout an entire 18hr study peri



Summary of cardio-respiratory variables before (B) during (D) and after (A) xenon.

SUMMARY

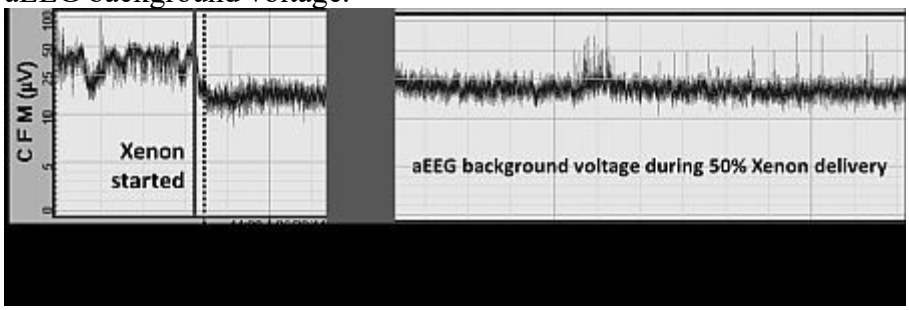
- It is feasible to give 50% xenon for up to 18h in infants undergoing therapeutic hypothermia
- Only 0.2-0.3L/hour xenon was used with a closed loop xenon recycling system
- Xenon is sedative and other sedation is needed when xenon is discontinued
- No significant cardiovascular or respiratory changes before, during and after xenon
- In one child with abnormal lungs, xenon did not increase FiO2 requirement.

Appendix A4

[1400.6] 50% Xenon Significantly Reduces aEEG Background Activity in Healthy Newborn Pigs

Hannah Gill, John Dingley, Elisa Smit, Xun Liu, Damjan Osredkar, Hemmen Sabir, Marianne Thoresen. School of Clinical Sciences, University of Bristol; St Michael's Hospital, Bristol, United Kingdom; Department of Physiology, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway; Medical School, Swansea University, Swansea, United Kingdom.

BACKGROUND: Some inhalation anesthetics reduce EEG background activity and EEG voltage-range has been used to monitor the depth of anesthesia in children[1]. The noble gas xenon is an inhalation anesthetic, which augments neuroprotection when combined with therapeutic hypothermia (HT) after hypoxic-ischemic brain injury. However, when used in its proven neuroprotective dosage (50%) xenon does not have full anesthetic potency.
OBJECTIVE: This current study aimed to examine the effect of 50% inhaled xenon on amplitude integrated EEG (aEEG) background activity in healthy newborn pigs.
DESIGN/METHODS: Six healthy newborn pigs aged <24h, receiving i.v. fentanyl sedation, were ventilated for 24h with 50% inhaled xenon at normothermia (rectal temperature 38.5°C). The 50% xenon concentration was achieved within 10min. aEEG (CFM, Natus, Seattle, USA) was recorded for 30min before the onset of xenon delivery and throughout the 24h period. Background voltage changes were analyzed.
RESULTS: Shortly after the onset of 50% xenon delivery, the aEEG background voltage range was significantly reduced in 5 pigs (Figure) and unchanged in one. Mean arterial blood pressure, heart rate and oxygen saturation were not influenced by the delivery of xenon. Throughout ventilation with 50% xenon, aEEG background voltage was constant (Figure). Fentanyl did not appear to change aEEG background voltage.



CONCLUSIONS: 50% xenon significantly reduces aEEG background voltage in healthy newborn pigs throughout prolonged delivery. As aEEG background voltage recovery predicts outcome in cooled asphyxiated newborns[2], care must be exercised when interpreting the background voltage in neonates also receiving xenon.
First Author is a Ph.D. Student

E-PAS2013:1400.6
Session: Platform Session: Neonatal Hypoxic Ischemic Encephalopathy & Neuroprotection (10:30 AM - 12:30 PM)
Date/Time: Saturday, May 4, 2013 - 11:45 AM
Room: Ballroom B - Walter E. Washington Convention Center
Course Code: 1400

Appendix A5. Patient Information sheets (abbreviated and full).

University Hospitals Bristol Foundation Trust NHS

Imperial College Healthcare NHS Trust

Xenon and cooling therapy in babies at high risk of brain injury following poor condition at birth: A randomised outcome study (CoolXenon3 study)

Version 1.5 22 July 2015

Information for parents (Abbreviated version)

Xenon and cooling therapy in babies at risk of brain injury following poor condition at birth: A randomised outcome study (CoolXenon3). Version 1.5

We know that your baby is very unwell and that this is a very difficult time for you. Your doctor will have explained what has happened and that your baby will benefit from being looked after on the Neonatal Intensive Care Unit.

Being poorly immediately after birth often occurs when a baby's supply of oxygen is reduced during pregnancy, labour and/or delivery. This can lead to injury to the brain and a risk of later disability.

Your baby is receiving full support of all bodily functions in the intensive care unit. We also give specific treatment to protect the brain by reducing the temperature of the body (and brain) from 37 to 33.5°C. Cooling has been proven to reduce the risk of later disability and has now been widely introduced as standard treatment for these babies. Your baby will receive intensive care and be cooled for 3 days (72 hours), then slowly returned to normal temperature However, this treatment does not help all infants.

As part of your baby's intensive care their breathing is currently being helped by a breathing machine (ventilator). The ventilator delivers the amount of air and oxygen your baby needs.

What is the aim of our study ? The aim of our study is to find out if adding Xenon gas to the air and oxygen mixture a baby breathes for the first 18 hours of the cooling treatment will improve the brain protection already produced by the cooling treatment and improve a baby's chances of making a full recovery.

What is Xenon? Xenon is a gas found in tiny amounts in the air that we breathe. In higher concentrations (above 50%) it makes us sleepy. Xenon has been used in adult anaesthesia and has not been found to have any side effects. There has been very limited use of Xenon in newborns so far. We have previously given Xenon to 30 babies and no negative side effects were seen.

Why am I being asked to take part? To find out if breathing xenon is helpful we will give it to a small group of babies like yours in combination with their normal cooling treatment as part of a randomised research study (all babies in the study will be cooled; half the babies will also receive xenon). Your baby may be suitable to be included in this study.

What would being in the study mean? Your baby will be looked after in the Neonatal Intensive Care Unit as with any other baby that is cooled for 72 hours. If you do decide to take part in the CoolXenon3 study, your baby will be allocated to receive either 'cooling alone' or 'cooling and xenon'. This treatment allocation is decided randomly (by chance).All babies in the trial, whether treated with cooling or cooling and Xenon will receive the same careful monitoring, examinations and follow up. Xenon will be added to the breathing gas from a specially designed breathing circuit, in a concentration of 50% for 18 hours. Trained staff will be present throughout the cooling-xenon treatment and they will stop the treatment immediately if they experience any problems. As part of their normal care, all cooled babies have a brain scan (known as an 'MRI scan') when they are one week old. Babies in our research study will also receive an MRI scan at around two days old: this scan is sometimes, but not always, something that a cooled baby would have as part of their standard care.

Your participation in this study is voluntary and you may withdraw your baby from the study at any time without giving us a reason. This will not affect the care or treatment of your baby in any way.

All the information we gather about you and your baby will be stored securely. Neither you nor your baby will be identified in any of the study reports or scientific and medical papers that we will write following completion of the study.

Who is running this study? The Chief Investigator is Professor Marianne Thoresen, a Consultant Neonatologist. Principal investigator at St Michael's Hospital, Bristol site is Dr Ela Chakkarapani, a Consultant Senior Lecturer in Neonatology who had undertaken the laboratory study with xenon and cooling. Principal Investigator at the Hammersmith, London site is Dr Sudhin Thayyil, Consultant and Reader in Neonatology. Co-Investigator is Dr John Dingley, a Consultant Anaesthetist who developed the Xenon delivery system. Both Professor Thoresen and Dr Dingley have conducted previous studies on babies using a xenon delivery system at St Michael's Hospital

What happens now? If you decide to take part in this study you will be asked to sign a consent form. We will describe the study and go through a fuller version of this information sheet with you. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care your baby will receive.

University Hospitals Bristol Foundation Trust NHS

Imperial College Healthcare NHS Trust

Xenon and cooling therapy in babies at high risk of brain injury following poor condition at birth: A randomised outcome study (CoolXenon3 study)

Version 1.4 22 July 2014 2015

Information for parents (Full version)

Xenon and cooling therapy in babies at risk of brain injury following poor condition at birth: A randomised outcome study (CoolXenon3). Version 1.4

Background Information

We know that your baby is very unwell and that this is a very difficult time for you. Your doctor will have explained what has happened and that your baby will benefit from being looked after on the Neonatal Intensive Care Unit.

Being poorly immediately after birth often occurs when a baby's supply of oxygen is reduced during pregnancy, labour and/or delivery. We do not always know what causes this decrease in oxygen supply but we do know that it can affect the ability of the whole body, particularly the brain, to function properly. This can lead to injury to the brain and a risk of later disability.

The only treatment available at present that is known to benefit these babies is to reduce your baby's body temperature from the normal level (37°C) to lower than normal body temperature (33.5°C) for 3 days (72 hours) and then slowly bring the body back to normal temperature. Trials of this cooling treatment have been shown to give some protection to the brain and to reduce the risk of later disability. This cooling treatment has now been widely introduced as a standard treatment for these babies.

Your baby is already receiving intensive care treatment. The specialist staff caring for your baby know from the tests they have performed that your baby has suffered from a lack of oxygen around the time of birth and that cooling treatment is suitable for your baby.

Cooling does not help all children and it is therefore important to investigate whether another treatment combined with cooling can give more protection to the brain.

What is the aim of the study?

The aim of our study is to find out if breathing Xenon gas for the first 18 hours of the cooling treatment will improve the brain protection already produced by the cooling treatment and improve a baby's chances of making a full recovery.

What is Xenon?

Xenon is a gas found in tiny amounts in the air that we breathe. In higher concentrations (above 50%) it makes us sleepy and it can be used to provide anaesthesia. In our laboratory studies we have shown that xenon may improve the brain protection produced by the cooling treatment following a lack of oxygen.

How is the Xenon given?

Your baby's breathing is currently being helped by a breathing machine (ventilator) which provides air and extra oxygen if required. If your baby is allocated to the **'Cooling and Xenon'** group**, 50%** Xenon will be added to the air and/or additional oxygen your baby is breathing using a safe and efficient delivery and monitoring system we have developed.

Does my baby have to take part in the study?

Your baby does not have to take part in this study. If you decide not to take part, your baby will receive the same intensive care treatment they are already receiving, which includes the cooling treatment from the specialist staff in the Neonatal Intensive Care Unit.

If you do choose to take part in the study you may withdraw your baby from the study at any time without giving a reason. **Your decision to take part or not will not affect the care of your baby in any way**.

What will happen to my baby if I agree to take part?

Your baby is already receiving intensive care treatment.

If you do decide to take part in the study your baby will be treated either with 'cooling' or 'cooling and Xenon'. **It is important to realise that taking part in the study does not mean that your baby will definitely receive Xenon; half the babies will receive cooling treatment and half will receive cooling treatment and Xenon**.

How is the treatment chosen?

This study is a 'randomised study'. This type of study is the best way to compare two different treatments. Patients are split into two different groups; one group is treated in the standard way and the other has an added treatment, in this case, the Xenon. To make sure the two groups of babies are the same at the beginning the allocation to the groups is made randomly or 'by chance'. There is an equal chance of your baby being allocated to the 'Cooling' group or the 'Cooling and Xenon' group. This is essential so that a fair comparison can be made between the groups.

Neither you nor the doctors or nurses are able to choose which treatment your baby receives.

If your baby is allocated to the **'Cooling' group** they will be looked after by the staff in the Neonatal Intensive Care Unit. They will be given **standard cooling treatment** as already described and any problems associated with your baby's condition will be monitored and treated accordingly.

If your baby is allocated to the **'Cooling and Xenon' group**, your baby will receive the same intensive care treatment as above and in addition, the ventilator that is currently helping with your baby's breathing will deliver air (and oxygen as required) plus Xenon gas at a concentration of 50% for a period of 18 hours.

In order to give the Xenon to your baby we will replace the existing breathing tube that has been placed into your baby's windpipe and attached to the ventilator with a different type of tube. The new one has a small balloon which can be gently inflated to fit the windpipe and prevent Xenon leaking from around the tube. A special breathing circuit will be connected to the ventilator to deliver Xenon to your baby. Trained staff from the clinical study will be present throughout the Xenon treatment to monitor its delivery.

When the 18 hours of Xenon treatment is over the balloon of the breathing tube is deflated and the Xenon breathing circuit disconnected. The ventilator will continue to help your baby with its breathing by delivering air and oxygen for as long as your baby needs it.

What are the possible side effects of xenon?

Xenon has been used as an anaesthetic gas for adults and has not been found to have any side effects. There has been very limited use of xenon in newborn infants so far. The risks of unwanted side effects are expected to be low due to the lack of side effects in adult anaesthesia experience. Knowledge of side effects in infants is limited to the 30 babies we have previously given xenon to, where no negative side-effects were seen.

Ongoing medical care of your baby

All babies taking part in this study will be monitored very carefully by the highly skilled staff in the Neonatal Intensive Care Unit. The unit has much experience in caring for babies who are undergoing cooling treatment. All necessary care will be given to ensure your baby is kept in a stable condition that will promote recovery. If the doctors caring for your baby feel that the xenon (if your baby is in the xenon group) or indeed the cooling needs to be stopped, they will do this immediately.

As part of their normal care, all cooled babies have a brain scan (known as an 'MRI scan') when they are one week old. Babies in our research study will also receive an MRI scan at around two days old, while they are still being cooled. This scan does no harm and may, in fact, be something that a cooled baby would have as part of their standard care. It is very important for parents and doctors to have as much information as possible at an early stage and the additional scan may help to show whether there has been any brain injury and if so, the severity of this.

Will my taking part in the study be kept confidential?

All the information we gather about you and your baby will be stored securely and only members of the study team will be able to see it. The information we need for the study includes details about your pregnancy and about your baby's stay in the neonatal unit, as well as details of your family doctor. We routinely follow up all babies who are unwell at birth and we need your name, your address and other contact details in order to do this. Your GP will also be informed of your baby's participation in this study. Data will be shared between the collaborators at the Imperial College London and the University Hospitals Bristol Foundation NHS Trust, Bristol to aid the analysis of MRI and other outcome measures.

What will happen to the results of the study?

The study will run for approximately 18 months with another 18 months for follow-up of the babies in outpatient clinic. We would also like possibly to contact you at a future time when your child is in the early school years to see how they are doing; this would be entirely voluntary on your part. At the end of the study the results will be made available to doctors and nurses caring for babies like yours throughout the world. Neither you nor your baby will identified in any of

the study reports or scientific and medical papers that will be written. If you wish, we will also send you a copy of the final results of the study. This is unlikely to be before 2016. At any time you can contact the Chief investigator of the study, Professor Marianne Thoresen.

What happens if something goes wrong?

If anything goes wrong as a consequence of taking part in the trial because negligence has occurred, University Hospitals Bristol NHS Foundation Trust will compensate you. Negligence would include, for example, a situation in which injury is caused by us not following the written study protocol properly. This means that if your child is harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against the United Bristol Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will always be available to you (if appropriate).

In the event that your child is harmed during the research study and this is not due to someone's negligence there are no special compensation arrangements. University Hospitals Bristol NHS Foundation Trust, which is sponsoring the study, cannot offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. *Ex-gratia* payments may be considered in the case of a claim.

Who is organising, leading and funding the research?

The Chief Investigator is Professor Marianne Thoresen a Researcher and Consultant Neonatologist at St Michael's Hospital Neonatal Intensive Care Unit. Professor Thoresen's research has made a large contribution to cooling treatment over the past two decades, leading to it being introduced in the UK, and many other countries, as a standard treatment for babies who have suffered from a lack of oxygen at birth. Co-Investigator Dr John Dingley is a Consultant Anaesthetist and Researcher responsible for the development and design of the Xenon breathing circuit. The study is sponsored by the University Hospitals Bristol NHS Foundation Trust. The study is part-funded by MOULTON, a UK charity supporting new treatments for medical problems in children and by the children's charity SPARKS.

Who has reviewed/approved the study?

The study has been Reviewed and approved by the NRES Committee South West - Central Bristol Research Ethics Committee and the UH Bristol Research and Innovation Department. The use of xenon and the equipment developed to deliver it have been reviewed in depth and approved by the UK regulatory authority, the Medicines and Healthcare products Regulatory Agency (MHRA). The study will be continuously monitored by an independent group of experts.

What happens now?

Thank you for reading this information leaflet. If you wish to discuss anything about the study please speak to the doctors or nurses looking after your baby. If you decide to take part in this study you will be asked to sign a consent form. We will describe the study and go through this information sheet with you. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care your baby would receive.

Contact details:

Professor and Neonatal Consultant, Marianne Thoresen (Chief Investigator) Tel: 0117 34 25607/25226/25733

St Michael's Hospital, Bristol

Dr Ela Chakkarapani, (Principal Investigator),
Consultant Neonatologist and Senior Lecturer
Tel: 01173425711

Dr John Dingley, (Co-Investigator)
Consultant Anaesthetist and Reader in Anaesthesia
Tel: 01792 703491

Mrs Emma Scull-Brown (Trial administrator, Bristol)
Tel: 0117 342 5733

Hammersmith Hospital, London

Dr Sudhin Thayyil (Principal investigator – second site)
Consultant Neonatologist and Weston Reader
Tel: 02033138515.

Ms Vânia Oliveira (Trial administrator, London)
Tel: 02033132473

Appendix A6 Informed consent document

STUDY ID nr [ ]

CONSENT FORM

Title of Project:

XENON AND COOLING THERAPY IN BABIES AT HIGH RISK OF BRAIN INJURY FOLLOWING POOR CONDITION AT BIRTH: A RANDOMISED OUTCOME STUDY (COOLXENON3 STUDY)

Version 1.4 22/7/2015

Name of Patient _____

Parent, please initial box ↓

1. I confirm that I have read and understand the information sheet dated 22 July 2015 version 1.4 (Full version) for the above study and I have been given a copy [ ]
2. I understand that my participation is voluntary and that I am free to withdraw my child at any time without my medical or legal rights being affected [ ]
3. For the purpose of this trial I am willing to allow access to my child's medical records by members of the research team and data to be shared between the collaborators but understand that strict confidentiality will be maintained. The purpose of this is to check that the study is being carried out correctly and aid analysis of investigations. [ ]
4. For the purposes of this trial, I am willing to be contacted and asked about participation in further follow up when my child is in the early school years. [ ]
5. I give permission for my GP Dr……...to receive details of my child's participation in this study [ ]
6. I agree to my child taking part in the above study. [ ]
7. I understand that data collected from this study may be transferred to, and viewed by, appropriate authorities for regulatory purposes. Any data that are transferred to such authorities will not identify me or my child nor will it contain any means of enabling me or my child to be identified. I give permission for such authorities and their designated representatives to have access to my data from this study. [ ]

Name of parent	Date	Signature (parent)
Name & designation of person taking consent	Date	Signature
Name of Researcher	Date	Signature

One copy to be retained by parent, one to be retained by investigator, one to be filed in patient's medical records

Appendix A7 Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects
Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION
1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects**,** including research on identifiable human material and data.
The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.
9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH
11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research
subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.
17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special
attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing,
the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.
26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary

characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if
the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
• The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
• Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.
35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.