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XENON AND COOLING THERAPY IN BABIES AT HIGH RISK OF BRAIN INJURY FOLLOWING POOR CONDITION AT BIRTH: RANDOMISED PILOT STUDY (COOLXENON2 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 STUDY (COOLXENON2 STUDY)**
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Study Centre	St Michael's Hospital Southwell Street Bristol BS2 8EG
Test Article	Xenon Neonatal Breathing Device
Objectives	Primary Objective: This is a randomised two group pilot study comparing; i) xenon inhalation for 18 hours in combination with whole body cooling to 33.5 °C for 3 days, to ii) the established treatment of cooling the body down 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. Our hypothesis is that during the first 2 weeks after birth using a combination of validated (in our own and others cohorts) early predictors of outcome we may observe an enhanced neuroprotective effect of the xenon-cooling combination over cooling alone and this will allow us to perform power calculations for a larger definitive neurological outcomes study of otherwise similar design. 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). Time to Normal trace and SWC are both very good (PPV>90%) outcome predictors

2) MRI before 2 weeks of age (T1W and T2W) has a PPV of 84% in cooled infants in the TOBY trial in which we were a major recruiting centre [2] Resistance Index (RI) was not a definite predictor for cooled infants [3]). We will do the examination but not use it in the prediction. Peak value of Lactate Dehydrogenase within the first 72h of life [4, and Thoresen M 2012 submitted].

<u>Secondary Objectives</u>:

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 our recently completed xenon+cooling feasibility/safety study in neonates and a few more are added .

They are:

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).

Haemodynamic: Systolic, mean and diastolic arterial pressure, heart rate.

Any problems during weaning and extubation, such as post-extubation stridor, use of steroids.

We have also looked at other variables in the infants cooled in our hospital (inborn or outborn) ca 30/year since 1/12/06. We chose the best matched controls (3:1) from this cooling database and compared them with the 14 infants in the recent Xenon + cooling human feasibility study (the precursor to this study),

We will also record:

1] Coagulopathy [INR >2, APTT> 50]

2] Inotrope requirement, cardiac Troponin T

3] Hepatic impairment, [raised Alanine aminotransferase > 40, Alkaline phosphatase > 100, peak Lactate dehydrogenase within 72h of age

4] Renal impairment, oliguria [urine output < 1 ml/kg/h after day 1 of life and raised creatinine > 100]

5] Infection [culture proven, need for antibiotics $\geq$ 5 days, raised CRP ~ within the first 24h and at 4 days of life of life]

6] Number of anticonvulsive drugs needed to treat seizures

7] Length of hospital stay

8] Time to full oral feed by bottle or breast

9] MRI assessment by T1 and T2 weighted images using the scoring system by Rutherford et al [2].

10] 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 [5]. 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 investigation for the administration of xenon by inhalation. Cooling should be started within 3h of age and xenon within 5. Use of a shorter treatment delay in these entry criteria will optimize the possibility of finding an effect if there is one.
Population Size	16 babies in each of 2 groups
Structure	Single centre prospective randomised clinical pilot study
Duration of Participation	Xenon administration period of 18 hours. Recruitment period up to 18 months from start of study. Short and long term monitoring until 18 months of age. Therefore total study duration 3 years.
Method of Assignment	Comparative study. Neonates meeting all entry criteria can be included.
Randomisation	Patients will be randomised between cooling (standard treatment) and xenon+cooling groups as this is a comparative study. Patients will be randomised on a 1:1 basis to receive either conventional cooling therapy or cooling plus xenon therapy.
Statistical Analysis	This is a randomised pilot 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 16 infants in each group. In accordance with the paper by Julious (Appendix 11), 12 is a large enough number to estimate the mean value of short term outcome variables for the group. The study does of course not have the power to test outcome. The main purpose of this randomised pilot study is to examine whether early recruitment is feasible, secondly whether the short term biological markers show the expected range of values in the cooled only group as compared to our historical controls. Our advisory statistician for this study is Professor Lars Walloe who also advised all our experimental xenon work as well as the recent clinical feasibility study. By April 2013 we have recruited 8 babies to standard cooling therapy and 7 babies to xenon plus cooling; we have failed to recruit approximately the same number due to failure to commence xenon delivery at St Michael's Hospital within 5 hours of birth. This is due to the time it takes to retrieve infants from the regional hospitals. Since April 2013, our xenon delivery system has been transportable and we have so far successfully started xenon treatment on two babies in the

	local hospital and transferred them to St Michael's Hospital while xenon was being delivered en route. This ability to commence xenon at the referring trust enabled us to recruit consecutive infants that would otherwise have been missed. We wish to assess the feasibility of recruiting now we have xenon in the ambulance by increasing the number of babies by 8.
Adverse Events	Volunteered and Elicited

Overview

The proposed study is planned to be a single centre study at St. Michael's Hospital Bristol, prospective randomised pilot clinical study. It may include Southmead Hospital Bristol at a later stage if recruitment is low. We have included a shorter delay for recruitment to optimize the effectiveness of hypothermia and potentially xenon. We will accept cooled infants that are <3h old and start xenon within 5h of age. In an experimental study we did not find any improvement of adding xenon if both were started at 5 hours after the insult [6], however 2h delayed xenon when HT was started immediately was more effective than HT alone [7].

The study examines the effect of inhaled xenon gas used in the treatment of newborn infants with hypoxic-ischemic 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. 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.

We propose to study 32 full term infants with moderate or severe HIE who, on clinical grounds, require mechanical ventilation and hypothermia for 72 hours. The hypothermia (cooling) will be routinely applied if the standard existing criteria for this are met except recruitment needs to be earlier, <3h rather than 6h as noted above. 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, the cooling then 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. 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 [8], without which this putative therapy would be completely unfeasible on cost grounds. It has recently been successfully used in 14 babies with the same entry criteria in our recent MHRA approved human feasibility/safety study [9]. Meetings of an independent Data Review Group will take place after the recruitment of every 4th baby during enrolment of infants to the xenon+cooling group.

1.0 Background and Introduction

1.1 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 hypothermia (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 [10] followed by the UK "TOBY" trial.[11] In both trials Bristol was a major recruiting centre (PI:MT). The trials have shown that HT significantly reduced disability and cooling has now become standard treatment. Because the protection from clinically delayed HT (by 4.5h) is only partial, (absolute risk reduction 15% [12], enhancing its effectiveness as well as starting earlier is paramount. More recent clinical trials have also shown effectiveness of HT [13]. 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.[14, 15] 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. We experienced no technical problems due to the breathing system or use of the xenon gas (Appendix 4) nor any adverse effects related to these.

1.2 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 hypothermia (HT) as a neuroprotective treatment through all the steps of therapeutic development starting with small (rat)[16,17] and large (pig),[18,19] animal models to a clinical feasibility study in Bristol (body or head cooling in 10 infants)[18]. 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.[1] 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.[22] 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.[11] We have received further MRC funding to carry out long term follow up of this cohort which is ongoing. Other trials in Europe [23] and Australia (ICE trial) [24] have also shown significant improvement in cooled infants.

In these 5 big trials [10,11,21-23], HT reduced death or disability from 66% to 51%. The number needed to treat (NNT) to prevent death or disability is 9. 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% [25] 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 relative 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 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 [26,27] 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 have 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 05/04/2011 (Appendix 4).

We add a recent, relevant paper [48] by Sabir et al which illustrates the safety of xenon. There was no increased apoptosis after breathing xenon for 24 hours, as compared to a 10x increase breathing isofluorane.

1.3 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.[14, 28, 29] Since 2006 we have investigated xenon-hypothermia (XeHT) in our newborn piglet model of global hypoxia-ischemia.[8, 31] With this model we have extensively examined the effect of XeHT, 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 [15]
- Better cardiovascular stability (reduced need for inotropic support) [31]

- No extubation delay [15]
- No increase in oxygen requirements [15]

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 5/4/2011. 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 and are given in more detail in Appendix 4. The design was such that the xenon duration increased from 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.

2.0 Objectives

The aim of this study is to examine using primarily short term measures and secondarily long term outcomes, the potential neuroprotective effects of the cooling+xenon combination when compared to cooling alone. We will examine the feasibility of recruiting earlier than previous trials, <3h for HT and <5h for starting xenon and assess what proportion of the eligible infants we can recruit. We will compare the short term biomarkers (aEEG recovery, MRI score, LDH and time to full feeds) in the two groups with those obtained previously in our randomised dataset (infants from CoolCap and TOBY) as well as the large group cooled since 1/12/2006 under the same protocol. In these groups of children we have both short term markers as well as Bayley outcome and have shown that our combined biomarkers has a PPV of 93%.

Primary Objective:
This is a randomised two group pilot study comparing;

i) Adding xenon inhalation for 18 hours in combination with the (existing) whole body cooling to 33.5 °C for 3 days.
ii) The established treatment of cooling the body down 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, the purpose being to reduce long term neurological impairment resulting from their condition.

Our hypothesis is that early predictors of outcome* will show a trend towards an enhanced neuroprotective effect of the xenon-cooling combination over cooling alone. The early predictors will allow us to perform power calculations for a much larger neurological outcomes study of otherwise similar design.
In addition we will examine the feasibility of earlier recruitment and start of xenon and whether we obtain the same early predictor data in the hypothermia-only group as we have in our previous hypothermia database.
(*the total clinical follow up period will be much longer than this as is already the case for cooled babies in Bristol)

2.1 Primary Endpoint

This is a randomised two group pilot study comparing;

1) xenon inhalation for 18 hours in combination with whole body cooling to 33.5 °C for 3 days, to
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.

Our hypothesis is that we may observe an enhanced neuroprotective effect of the xenon-cooling combination over cooling alone assessed initially by early outcome predictors. This will allow us to perform power calculations for a much larger neurological outcomes study of otherwise similar design. We have tested a combination of our early predictors (using our TOBY registry cooling data in 80 infants) against Bailey outcome at 18 months and found they have a positive predictive value of 94% in cooled infants

Early predictors of outcome
For the main analysis we will use
Time to recovery after birth of a normal aEEG [1]
MRI scan before 14 days of life [33, 2]
Peak LDH value within 72h of life [4, 34]
We will also collect data on:
Plasma glucose at birth (in cord blood)
Age in hours after birth when plasma lactate has fallen to 5mmol/L
Different inotropic drugs needed and the total duration in hours of any inotropic support
Number of anticonvulsants given
Amount and duration of sedation (e.g.morphine)
Age at full oral feeds (breast or bottle or tube)
Neonatal hearing screening result before 14 days of life
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 examination at 18 months of age.
Disability will be defined as any of:
Bayley II mental developmental scale (MDI) score less than 70.
Bayley II motor development scale (PDI) score less than 70. MDI and PDI will also be calculated as DQ, developmental score as this allows a larger range of score for those functioning at a low level [35]. Bilateral cortical visual impairments
Hearing loss needing amplification > 40 DB.

In addition any Adverse Events will be recorded and criteria have been devised for stopping the study early if necessary. This includes a Data Review Panel to review data when 4 xenon infants have undergone xenon treatment.

2.2 Secondary Endpoint

A series of variables that are among the most relevant for evaluation of safety (see endpoints below) were examined closely in the previous 14 baby feasibility/safety study. 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 variables 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),FiO2. Average percentage xenon in inspired gas mixture (when applicable). Tracheal tube cuff pressure.

- Endpoint 2 – defined as *Haemodynamic parameters*

- The following variables will be measured before, during and after xenon administration: Systolic, mean and diastolic arterial pressures, Heart rate.

- Endpoint 3 – defined as *Inotropic drug requirements*
  - These (drug, dose and duration) will be recorded before, during and after xenon administration until 12h after end of cooling.

- 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*
  - These 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*
  - These will be recorded before, during and after xenon administration until 12h after end of cooling: oliguria is 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*
  - These 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

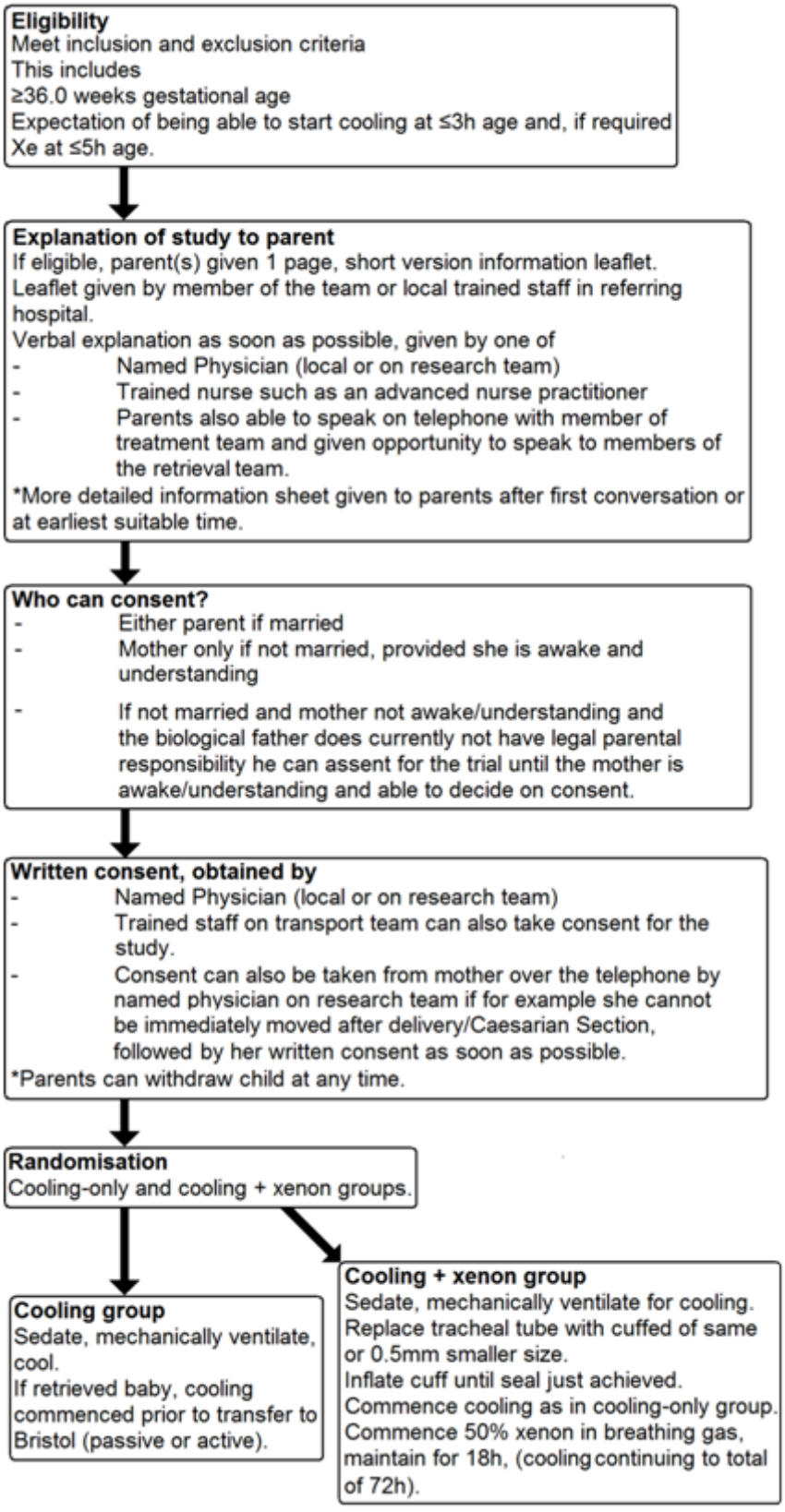
- Endpoint 10 – defined as *Haematology measurements*
  - These will be recorded before and after xenon administration (these are routinely recorded 24 hrly): Coagulopathy [INR > 2 and/or APTT> 50]. Full blood count.

- Endpoint 11 – defined as *Serum chemistry measurements*
  - These 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*
  - These will be recorded before, during and after xenon administration until 12h after end of cooling: Evidence from continuous aEEG/EEG recording and any anticonvulsant treatments given.

3.0 Study Design

This is a single centre prospective randomised clinical pilot study.


Eligibility
Meet inclusion and exclusion criteria
This includes
≥36.0 weeks gestational age
Expectation of being able to start cooling at ≤3h age and, if required Xe at ≤5h age.
Explanation of study to parent
If eligible, parent(s) given 1 page, short version information leaflet.
Leaflet given by member of the team or local trained staff in referring hospital.
Verbal explanation as soon as possible, given by one of
- Named Physician (local or on research team)
- Trained nurse such as an advanced nurse practitioner
- Parents also able to speak on telephone with member of treatment team and given opportunity to speak to members of the retrieval team.
*More detailed information sheet given to parents after first conversation or at earliest suitable time.
Who can consent?
- Either parent if married
- Mother only if not married, provided she is awake and understanding
- If not married and mother not awake/understanding and the biological father does currently not have legal parental responsibility he can assent for the trial until the mother is awake/understanding and able to decide on consent.
Written consent, obtained by
- Named Physician (local or on research team)
- Trained staff on transport team can also take consent for the study.
- Consent can also be taken from mother over the telephone by named physician on research team if for example she cannot be immediately moved after delivery/Caesarian Section, followed by her written consent as soon as possible.
*Parents can withdraw child at any time.
Randomisation
Cooling-only and cooling + xenon groups.
Cooling group
Sedate, mechanically ventilate, cool.
If retrieved baby, cooling commenced prior to transfer to Bristol (passive or active).
Cooling + xenon group
Sedate, mechanically ventilate for cooling.
Replace tracheal tube with cuffed of same or 0.5mm smaller size.
Inflate cuff until seal just achieved.
Commence cooling as in cooling-only group.
Commence 50% xenon in breathing gas, maintain for 18h, (cooling continuing to total of 72h).

3.1 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.

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:

1 Apgar score of ≤5 at ten (10) minutes after birth

2 Continued need for resuscitation, including tracheal or mask ventilation, at ten minutes after birth

3 Acidosis defined as either umbilical cord pH or any arterial, venous or capillary pH within 60 minutes of birth less < 7.00

4 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 --

1 Altered state of consciousness (reduced or absent responses or pathological irritability and hyper responsive and at least ONE or more of the following:

2 Hypotonia

3 Abnormal reflexes including oculomotor or pupillary abnormalities

4 Absent or weak suck

5 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:

1 Normal background with some (> 5 min) electrical seizure activity

2 Moderately abnormal activity (upper margin of trace >10µV and lower margin <5µV)

3 Suppressed activity (upper margin of trace <10µV and lower margin of trace <5µV)

4 Definite seizure activity

Exclusion criteria for cooling in the CoolXenon2 study

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

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):

1) Intubated, ventilated, sedated, being cooled.
2) <5 hours old
3) Any seizures under control.
4) Weight > 2nd centile for gestational age
5) Stable cardiovascular parameters; Mean arterial pressure >40mmHg.
6) Oxygen requirement via mechanical ventilator < 40%.
7) Positive End Expiratory Pressure (PEEP) requirement < 6cm H_2O
8) Arterial/capillary/venous pCO2 <7.0kPa (ideally arterial). Venous can be higher if peri-arrest and tissues have been very recently Ischaemic
9) Postnatal age <5 hours
10) 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.

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 will be born outside the study hospital (Outborn). Outborn patients are estimated to be >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 CoolXenon2 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 CoolXenon2 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 (eg 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).

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.

Clinical management

1) 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.
2) 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).

Specific clinical management of cooling group

1. This group will be cooled according to the normal protocols of the hospital as this is standard treatment.

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. The experience from our recent neonatal feasibility/safety study was that of the 14 babies, one baby developed post-extubation stridor requiring steroids.* See Appendix 4.

*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. See Appendix 11.

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 16 patients. The mortality rate in the patients referred for cooling to Bristol is ~25%.

4) Cooling will be for 72 hours using the clinically chosen cooling device in both groups, as is current standard practice for cooling.

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 equal that of air i.e. 21%. No neonate would ever receive less than 21% oxygen and the fraction delivered would be specific to each individual according to clinical need.

Explanation:

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. This is why although humans are designed to breathe air (21% oxygen), under anaesthesia in someone with normal lungs we would traditionally use 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 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 [37] 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.

i) It is worth noting that regional 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:
ii) While 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 [38].
iii) 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 21% (as in room air) 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.

Vigilance and monitoring
This would be ongoing during every case. It would include

- Blood gases 2 hourly or as clinically requested up to 1 hourly.
- (cardiac echo /doppler) where available
- Blood Pressure

- Heart rate
- Oxygen saturation by pulse oximeter SaO_2
- Xe concentration and O_2 concentration in gas mixture
- End Tidal CO_2 ($ETCO_2$) (Respironics mainstream monitor with very low deadspace in neonatal connection)

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/hr) followed by Dobutamine (up to 20mcg/kg/hr), then Hydrocortisone up to 2.5mg/kg 6 hourly or other inotrope as indicated.
If not effective then Xe delivery is 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 End-Tidal CO_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.

Risks/benefits and risk minimisation

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.[37-40] A slight slowing of pulse rate has been seen in some studies - 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 [15]
- Better cardiovascular stability [31]
- No extubation delay (delay coming off the mechanical ventilator) [15]
- No increase in oxygen requirements [15]

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. [37-40]
Because 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. 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 [9]
- 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 4

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:

iii) 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*, i.e. 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. (see section 4.0).

<u>Potential risks from breathing system:</u>
We are using a new breathing circuit to deliver this gas. 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. 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:

i) The equipment design is being evaluated for safety by the MHRA. It will be professionally constructed from all medical grade materials.

ii) 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.

iii) The gas composition, in particular oxygen, of the circuit gas will be continuously measured, as will xenon and the end-tidal carbon dioxide (which reflects the arterial value).

iv) The breathing system has an overpressure release valve in the event of any mishap.

v) 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. Finally:

vi) The xenon will be delivered by trained personnel who have also been involved with laboratory trials using xenon in animal models

vii) 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.

viii) A full risk assessment in accordance with standard medical device regulatory requirements has been undertaken and thoroughly documented

This design of breathing system has now been evaluated in this same patient population as in our recently completed 14 baby human feasibility/safety study. The findings are summarised in Appendix 4.

Use of cuffed tracheal tube:

After onset of cooling and attainment of a stable state, 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.

To minimise the gas pressure in the cuff:

i) The cuff 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."* [41] (See Appendix 11 for abstract of this paper).

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.

N.B. Any Adverse Events 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. Any such Adverse Events at or around the time of extubation will also be recorded.

Experience with this 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. See Appendix 11.

Further information can be found in Appendix 4.

Cleaning/disinfection:

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

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.

3.2 Follow Up

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
4. Hypoxic-ischaemic or other injury on MRI scan (standard sequences for this injury) preferably aged 7-12 days of age and Magnetic Resonance Spectroscopy as a part of the MRI examination if available
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

<u>Short term assessment neurological evaluation will include:</u>
1) Time to recovery after birth of a normal aEEG [1]
2) MRI scan before 14 days of life [33, 2, 44]
3) Peak LDH value within 72h of life [4, 34]

We will also collect data on:
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 (eg morphine) 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

Long term follow up (at 18 months)

Neurodevelopmental follow up is a part of the routine follow up of infants with Neonatal Encephalopathy. Bayley II 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) [10, 11] where Bayley II was also used: we were a main recruiting centre for these trials.

Disability will be defined as any of:
Bayley II mental developmental scale score (MDI) less than 70.
Bayley II motor development scale score (PDI) less than 70. MDI and PDI will also be calculated as developmental quotients (DQ), as this allows a larger range of score for those functioning at a low level [35]. Bilateral cortical visual impairments.
Hearing loss needing amplification > 40 DB.

Additionally:

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 [4] predict long-term outcome with a positive predictive value of 84%, 92% and 80% respectively. In this study we will be able to examine these predictors for cooled babies receiving xenon treatment. By defining this relationship one can calculate the sample size for a large trial using short-term outcome.

3.3 Justification of Design

A human neonatal randomised pilot study comparing cooling (the established treatment) with the cooling+50% xenon combination is now the next logical step (see "Justification of Treatment" 3.4 below). This study is necessary for 3 reasons:

1) To validate our ability to recruit a large proportion of infants needing cooling as early as 3h of age and start xenon by 5hrs.
2) To examine the positive predictive value of our early outcome biomarkers proposed from the previous 100 cooled babies and compare with these.
3) Use these data for the statistical power calculations to estimate the group sizes for a larger randomised clinical trial of cooling+xenon. This approach follows a similar pattern to the early studies of cooling in neonates; a human technical feasibility/safety study [42] was performed using different target temperatures, followed by a study using a fixed temperature protocol for 72hours [20]. This protocol was then used in CoolCap [10], modified slightly for NICHD (National Institute of Child Health and Human Development)[43] and again used in TOBY, which were the 3 first large randomised controlled cooling trials. [12]

Group size calculation

Sixteen 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.
For pilot studies of this type the recommendation is a sample size of 12 per group. The justifications for this sample size are based on

i) Feasibility – allows convenient block sizes, for example our intention for a data review committee to review data sets at block intervals of 4 neonates.
ii) Precision about the mean – little additional precision to be gained by having a larger group size than 12 [44].
iii) Precision about the variance – little additional precision to be gained by having a larger group size than 12 [44].

Reference [44] presented in full in Appendix 10.

The first 15 neonates were recruited in a period where the xenon ambulance transfer system was not ready for use. The clinical staff employed to recruit and deliver the xenon in the ambulance needed time to gain experience using the system before using it independently away from the base hospital without the supervision of Dr John Dingley. This has led to a similar number of babies which we failed to recruit (due to the time limitation) as the number recruited to

xenon. We therefore wish to recruit a further 8 babies to assess the feasibility to recruit, more reliably, consecutive babies fulfilling the entry criteria within the confines of the protocol

3.4 Justification of Treatment / Procedure

Even with the best available treatment (hypothermia) infants with HIE have a considerable risk of death or disability of 51%. 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 has very recently concluded with a very positive outcome [15] supporting the result we found in rats, ie adding xenon to HT doubles the neuroprotection [14, 17].

This equipment has recently been used in 14 human neonates during our recently completed human feasibility/safety study (Appendix 4).

4.0 Study Therapies

4.1 Test Article: Description

Overview

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.

4.2 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 Figure 1.

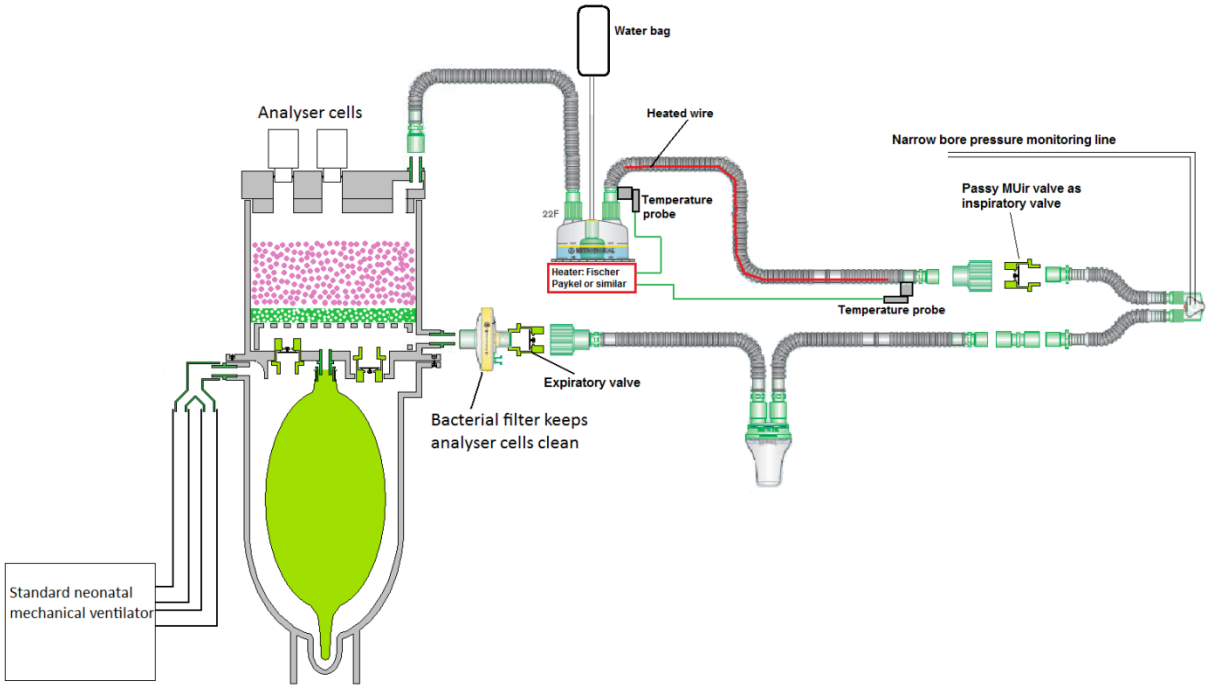


Figure 1a. Diagram of the breathing system

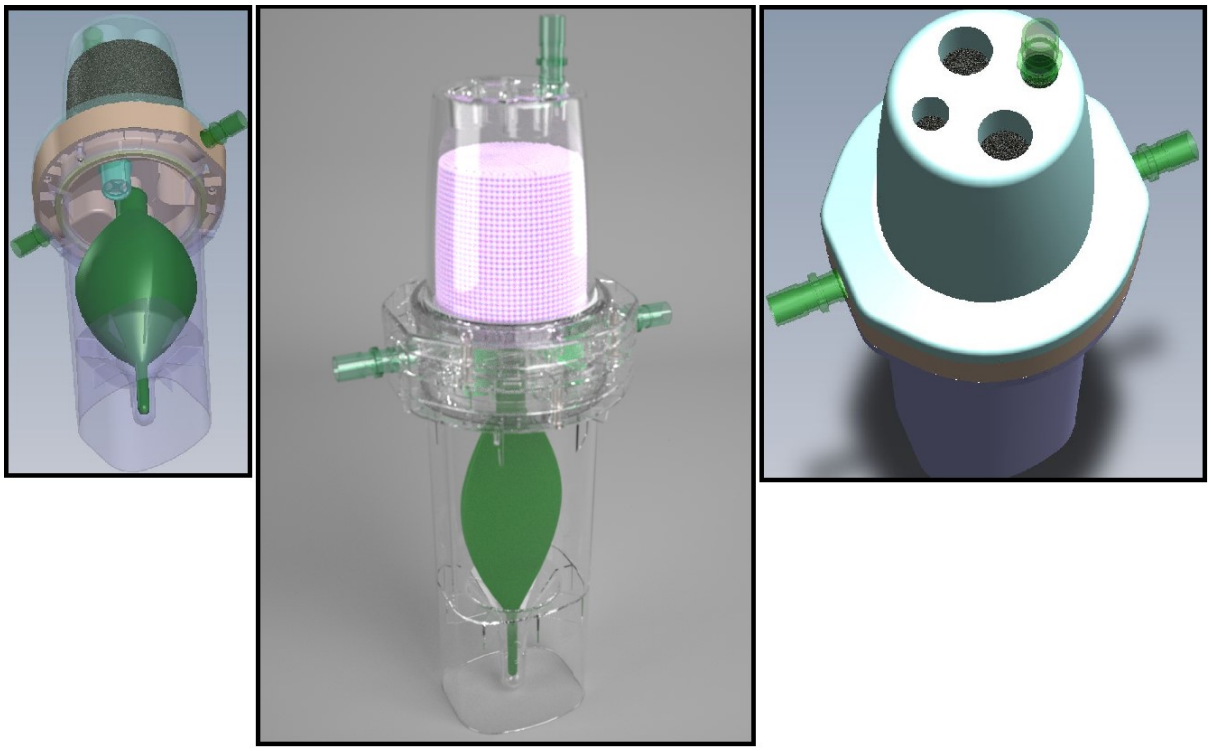


Figure 1b. Views of breathing system (without hoses)

The xenon delivery system is designed to be fitted between a conventional neonatal intensive care unit (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.

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).

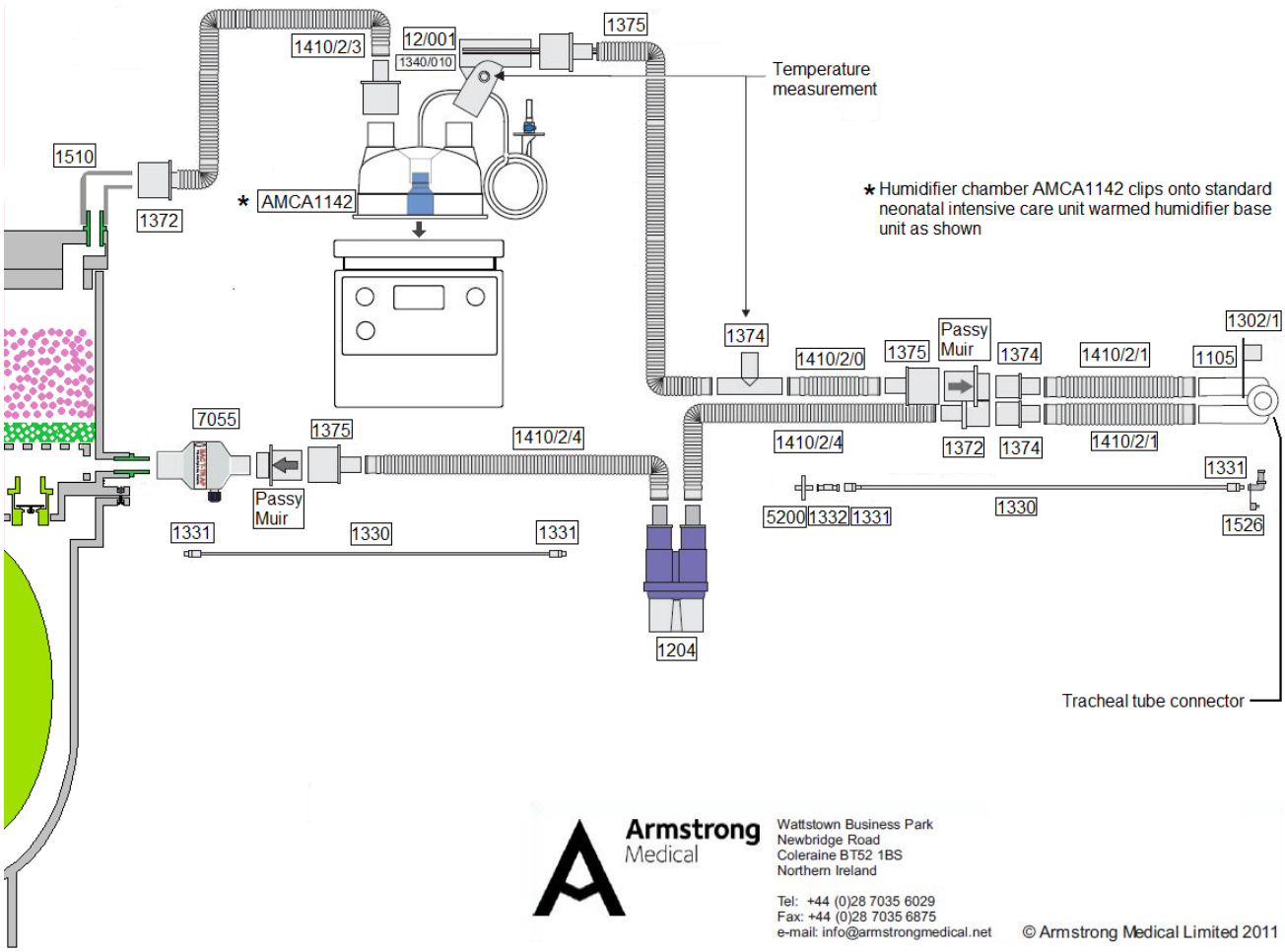


Figure 1c. Details of exact medical components used to construct the hoses of the breathing circuit, with part numbers. Hoses, humidification chamber and connectors all manufactured by Armstrong Medical Ltd, UK

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).

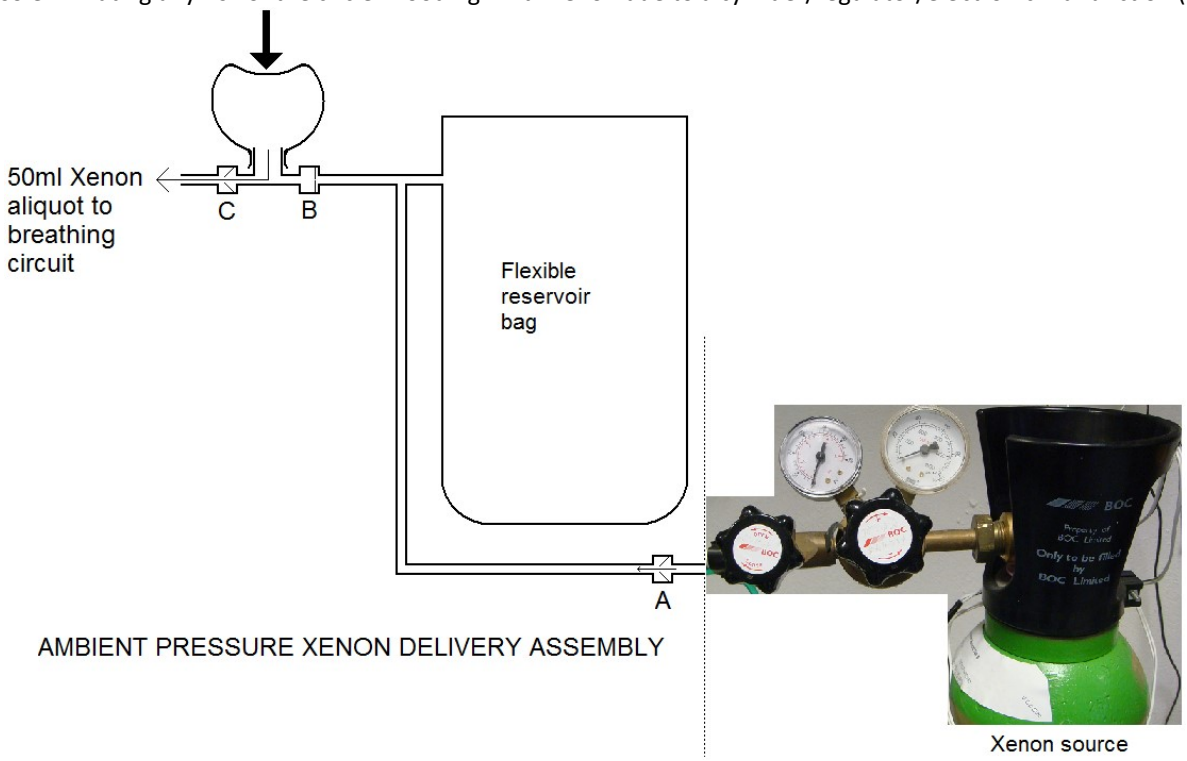


Figure 2.

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.

4.3 Test Article: Clinical Development

The research paper (Reference 15) is 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. It describes the early clinical development and performance of the delivery system and is attached in full as Appendix A2.

Appendix 5 summarises the data obtained when this breathing system design was used in our recent 14 baby human neonatal feasibility/safety study.

4.3.1 Proof-of-Concept Pre-clinical Studies

Following the technical feasibility study [15] above, physiological data has been obtained by the research group in a much larger laboratory experiment over 5 years in neonatal piglets who were subjected to a previously described global hypoxic-ischemic insult, resuscitated, and then randomised to receive varying combinations and durations of cooling and xenon. The xenon delivery system used for this large pre-clinical study was the same as the one proposed for the clinical feasibility study.

This pre-clinical study has also been is published as a paper:
Xenon enhances hypothermic neuroprotection in asphyxiated newborn pigs. Chakkarapani E, Dingley J, Liu X, Hoque N, Aquilina K, Porter H, Thoresen M. Ann Neurol. 2010 Sep;68(3):330-41.[15]

Additional details on the conduct of this laboratory pre-clinical study are given in Appendix 5 and a large quantity of physiological data is also supplied in this appendix demonstrating the physiological effects of cooling alone and then combinations of xenon + cooling in neonatal pigs after this severe global hypoxic-ischaemic insult.

4.3.2 Proof-of-Concept Clinical study

In March 2011 this research group successfully completed a clinical feasibility/safety study of inhaled xenon in combination with cooling using this equipment design to deliver xenon in 14 human neonates.
A large quantity of physiological data was acquired and this is presented in Appendix 5.

4.4 Test Article: Regulatory Status

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

4.5 Test Article: Packaging and Labelling

The Neonatal Xenon Breathing Device 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”.

4.6 Test Article: Component Tracking

Use of the Neonatal Xenon Breathing Device 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 Department Level 3, Education Centre, Bristol BS2 8AE. Tel 0117 3420233 (office hours)
- Dr John Dingley Mobile: 07735 379471 (all other times)

4.7 Control/Comparator Therapy

This is a randomised comparative pilot study. The control group will receive the standard treatment for this condition which is whole body cooling for 72 h 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.

5.0 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). Outborn patients are estimated to be >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 CoolXenon2 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 CoolXenon2 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.
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 (eg 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).

5.1 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.

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 (clinical assessment) with at least ONE of the following:
1 Apgar score of <5 at ten (10) minutes after birth

2 Continued need for resuscitation, including endotracheal or mask ventilation, at ten minutes after birth

3 Acidosis defined as either umbilical cord pH or any arterial, venous or capillary pH within 60 minutes of birth less than pH 7.00

4 Base deficit greater than or equal to 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 --

1 Altered state of consciousness (reduced or absent responses or pathological irritability and hyper responsive and at least ONE or more of the following:

2 Hypotonia

3 Abnormal reflexes including oculomotor or pupillary abnormalities

4 Absent or weak suck

5 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 section of the aEEG, not the best (al Naqeeb, et al, 1999) or seizures (clinical or electrical) thus meeting ONE of the following:

1 Normal background with some electrical seizure activity

2 Moderately abnormal activity (upper margin of trace >10µV and lower margin <5µV)

3 Suppressed activity (upper margin of trace <10µV and lower margin of trace <5µV)

4 Definite seizure activity

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):

1) Intubated, ventilated, sedated, being cooled.
2) <5 hours old
3) Any seizures under control.
4) Weight > 2nd centile for gestational age
5) Stable cardiovascular parameters; Mean arterial pressure >40mmHg.
6) Oxygen requirement via mechanical ventilator < 40%.
7) Positive End Expiratory Pressure (PEEP) requirement < 6cm H_2O
8) Arterial/capillary/venous pCO2 <7.0kPa (ideally arterial). Venous can be higher if peri-arrest and tissues have been very recently ischaemic
9) Postnatal age <5 hours
10) 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. Neonates with congenital syndromes affecting the brain should be excluded when diagnosed (see exclusion criteria below)

5.2 Exclusion Criteria

Exclusion criteria for cooling

1. Infants expected to be greater than 5 hours of age at the time of starting cooling treatment.
2. Futility. Where prognosis is considered to be hopeless e.g. no cardiac output for 20 minutes.

Additional exclusion criteria for xenon

Failure to meet any of the additional inclusion criteria for xenon listed in section 5.1.
Patients meeting the following criteria are NOT eligible for the study:
Presence of any 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. (Neonates with congenital syndromes affecting the brain should be excluded when diagnosed).

5.3 Prohibited Therapies

Not Applicable.

6.0 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.

7.0 Methods and Procedures

7.1 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 will be born outside the study hospital (Outborn). Outborn patients are estimated to be >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 CoolXenon2 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 CoolXenon2 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 (eg 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 childs 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).

7.2 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 (Prof Marianne Thoresen or Dr James Tooley) involved in the study or a trained member of the Transport team.

7.3 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 Michaels 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.

7.4 Initial Assessment

The following information will be recorded on the appropriate CRFs:

7.4.1 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

7.4.2 Patient History and Physical

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

7.4.3 Concomitant Medications

All concomitant medications will be recorded as follows:

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

7.5 Study Phases

The study consists of:

7.5.1 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).

7.5.2 Phase 2

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

7.5.3 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.

7.5.4 Patient Follow-up

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
4. Hypoxic-ischaemic or other injury on MRI scan (standard sequences for this injury) preferably aged 7-12 days of age and Magnetic Resonance Spectroscopy as a part of the MRI examination if available
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

Short term assessment neurological evaluation will include:

1) Time to recovery after birth of a normal aEEG [1]
2) MRI scan before 14 days of life [33, 2, 44]
3) Peak LDH value within 72h of life [4, 34]

We will also collect data on:

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 (eg morphine) 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

Long term follow up (at 18 months)

Neurodevelopmental follow up is a part of the routine follow up of infants with Neonatal Encephalopathy. Bayley II 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) [10, 11] where Bayley II was also used: we were a main recruiting centre for these trials.

Disability will be defined as any of:
Bayley II mental developmental scale score (MDI) less than 70.
Bayley II motor development scale score (PDI) less than 70. MDI and PDI will also be calculated as developmental quotients (DQ), as this allows a larger range of score for those functioning at a low level [35].
Bilateral cortical visual impairments.
Hearing loss needing amplification > 40 DB.

Additionally:

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 normalisation of aEEG [1] and peak LDH before 72h [4] predict long-term outcome with a positive predictive value of 84%, 92% and 80% respectively. In this study we will be able to examine these predictors for cooled babies receiving xenon treatment. By defining these relationships one can calculate the sample size for a large trial using short-term outcome.

7.6 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.

7.7 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.

8.0 Assessment of Safety

8.1 Data Review Group.

An external Data monitoring Group 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 4th xenon patient has been recruited i.e. every 4 xenon babies and in the light of this make appropriate recommendations to the study team.
The group will meet when every 4 xenon-treated babies have been enrolled. The Data Monitoring Group 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.
N.B. In our previous neonatal feasibility/safety study of xenon+cooling a partial external committee met at intervals of every other baby enrolled. One outcome of this study was that the xenon gas itself did not cause any significant physiological or biochemical changes or other problems and neither did the use of a breathing system functionally the same as the one to be used in this study. This explains our choice of a 4 enrolled xenon baby interval for the xenon+cooling group in this study.

8.2 Composition of Data Review Group

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
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Lay member:

Paula Windsor BMedSci (Hons) SR Paramedic
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Dr Steven Sale MB ChB FRCA
Paediatric Anaesthetist
University Hospitals Bristol NHS Foundation Trust
Bristol Royal Hospital for Children
Upper Maudlin Street
Bristol BS2 8BJ
steven.sale@uhbristol.nhs.uk

The Data Monitor Group can invite one member from the research team to attend their meeting:

Professor Marianne Thoresen MD PhD FRCPCH
Professor of Neonatal Neuroscience, University of Bristol
Honorary Consultant Neonatologist, University Hospitals Bristol NHS Foundation Trust
Dept of Clinical Science at South Bristol, Child Health
Level D, St Michael's Hospital
Southwell Street, Bristol BS2 8EG
marianne.thoresen@bristol.ac.uk

Dr John Dingley MD FRCA
Reader in Anaesthetics, University of Swansea
Consultant Anaesthetist
Honorary Consultant Anaesthetist University Hospitals Bristol NHS Foundation Trust
Department of Anaesthetics and Intensive Care
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JDingley98@aol.com

Dr James Tooley MBBS MRCP MRCPCH
Consultant Neonatologist, University Hospitals Bristol NHS Foundation Trust
Clinical lead, Department of Neonatal Intensive Care
Level D, St Michael's Hospital
Southwell Street, Bristol BS2 8EG
James.Tooley@UHBristol.nhs.uk

Or other named individuals in the research team

The Data Review Group will meet to assess the data from each enrolled infant at intervals of every 4th enrolled xenon+cooling infant or greater.

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

The underlying mortality is approximately 25% 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.

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 we are unable to keep 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.

Stopping rule:

Mortality since cooling became standard of care at St Michaels hospital (inborn and outborn) fell from 34%to 25%. With this (25%) underlying mortality it is not unlikely, for example, that the two first infants might die (p= 0.06). If the study is allowed to continue and also the third infant in the xenon group dies, the probability that this is a random event is very small (p=0.016) and recruitment should be stopped and the protocol reviewed.

From then on (n is the number of 'xenon' babies included in the study):

n	deaths
3	3 of 3 stop
4	3 of 4 stop
5	4 of 5 stop
6	4 of 6 stop
7	4 of 7 stop
8	5 of 8 stop
9	5 of 9 stop
10	5 of 10 stop
11	5 of 11 stop
12	6 of 12 stop

If a few more than 12 infants were to be included in the xenon arm of this study the number of deaths *used as the stopping number should be 40% of the total number.*

This stopping rule is based on two considerations:

1. Probabilities given 25 % mortality without Xe
2. Stricter rule with more babies included

(See Appendix A6)

8.4 Adverse Events (AEs)

Adverse events will be recorded in accordance with UH Bristol's Research Related Adverse Event Reporting Policy.

8.5 AE Definitions

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

Serious adverse event
"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
"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)"

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

8.6 AE Classification

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

8.7 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.

8.8 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

8.9 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 Ethics Committee 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 Ethics Committee approval to continue the trial".

9.0 Data Analysis and Statistical Plan

9.1 Statistical Analysis

This is a randomised pilot 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 16 infants in each group. In accordance with the paper by Julious (Appendix 11), 12 is a large enough number to estimate the mean value of short term outcome variables for the group. The study does of course not have the power to test outcome. The main purpose of this randomised pilot study is to examine whether early recruitment is feasible, secondly whether the short term biological markers show the expected range of values in the cooled only group as compared to our historical controls. Our advisory statistician for this study is Professor Lars Walloe who also advised all our experimental xenon work as well as the recent clinical feasibility study.

9.2 Interim Analysis and Stopping Rules

See 8.0 – 8.3 above which describes interim analysis and stopping rules in detail.

10.0 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

11.0 Modification of Protocol

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

12.0 Discontinuation of Study

See "stopping rule" in section 8 above.

13.0 Administrative Requirements, Ethical and Regulatory Aspects and Quality Assurance

13.1 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.
- International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines.
- Research Governance Framework for Health and Social Care

13.2 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.

13.3 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.

13.4 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.

13.5 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.

13.6 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.

13.7 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.

13.8 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.

13.9 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.

13.10 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.

14.0 Study Sponsor

All costs associated with the running of the study will be borne by University Hospital Bristol NHS Foundation Trust.

15.0 Publication

We intend to report / disseminate the results of the study in the following ways:
Peer reviewed scientific journals.
Internal report.
Conference presentations.
Publication on website.

16.0 References

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