PROTOCOL ALBINO

Effect of ALlopurinol in addition to hypothermia for hypoxic-ischemic Brain Injury on Neurocognitive Outcome

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Protocol

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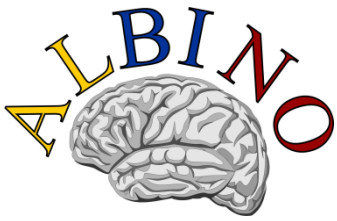

– a blinded randomized placebo-controlled parallel group multicenter trial for superiority (Phase III)

Short Title of Clinical Study	**ALBINO**
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Investigational drug	Allopurinol PFI

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An independent DMC will be established as described in section 8.2 and will be informed regularly as detailed in section 9.4 of this protocol. Further details on the composition of the DMC and processes of ongoing safety analyses will be described in the DMC Charter (a separate document).

VII Funding Agency

European Union:

This study is funded by the European Union under the Horizon 2020 framework program, call H2020-PHC-2015-two-stage, topic: PHC-18-2015: “Establishing Effectiveness of Therapeutic Interventions in the Paediatric Population”, proposal number: 667224.

Abbreviations

AMG	Arzneimittelgesetz (German Pharmaceutical Act)
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte (German Federal Institutes for Drugs and Medical Devices)
CPCS	Center for Pediatric Clinical Studies
CRF	Case Report Form
DMC	Data Monitoring Commitee
FPI	First patient in
GCP	Good Clinical Practice (Guideline ICH E6)
HIE	Hypoxic-ischemic encephalopathy
ICH	International Conference on Harmonisation
ISO	International Organization for Standardization
LPO	Last patient out
PFI	Powder for injection
PI	Principal Investigator
SCPE	Surveillance for Cerebral Palsy in Europe

1 Synopsis

Study Acronym	ALBINO
EudraCT-number	2016-000222-19
Protocol title	Effect of **AL**lopurinol in addition to hypothermia for hypoxic-ischemic **B**rain **I**njury on **N**eurocognitive **O**utcome (ALBINO)
Study design	Blinded randomized placebo-controlled parallel group multicenter trial for superiority (Phase III)
Planned sample size	846
Total study duration	60 months (5 years)
Scheduled starting date	April 1st 2016
Study duration/patient	24 months
Primary Objective	To evaluate whether in newborns with severe perinatal metabolic acidosis or ongoing cardiopulmonary resuscitation at 5 min after birth and early clinical signs of potentially evolving hypoxic-ischemic encephalopathy, early postnatal allopurinol compared to placebo (mannitol) administered in addition to standard of care (including therapeutic hypothermia if indicated) reduces the incidence of death or severe neurodevelopmental impairment (as defined herein) at 24 months of age.
Secondary Objectives	To evaluate the effect of allopurinol in addition to hypothermia (if indicated) on: - brain injury assessed by magnetic resonance imaging, - brain injury assessed by cerebral ultrasound, - amplitude integrated electroencephalogram, - full scale multichannel electroencephalogram, - laboratory biomarkers and markers of peroxidation To evaluate the safety of allopurinol in neonates treated with hypothermia. To study pharmacokinetics of allopurinol (verum) and mannitol (placebo) in neonates treated with hypothermia and not treated with hypothermia
Inclusion Criteria	Term and near-term infants with perinatal asphyxia and encephalopathy as defined herein.
Key Exclusion Criteria	- Gestational age below 36 weeks - Birth weight below 2500 g - Postnatal age >30min at the end of the screening phase, - Severe congenital malformation or syndrome requiring neonatal surgery or affecting long-term outcome - Patient considered “moribund” - Decision for “comfort care only” before study drug administration - Parents declined study participation as response to activities of community engagement
Individual termination of treatment	The study will be completed for each patient after follow-up at 2 years of age
Investigational drug	Allopurinol powder for injection (PFI) or Mannitol PFI-Placebo

2 Introduction

2.1 Summary

Neonatal hypoxic-ischemic encephalopathy (HIE) is a major cause of death or long-term disability in infants born at term in the western world, affecting about 1-2 per 1.000 life births and consequently about 5-10.000 infants per year in Europe. Hypothermic treatment became the only established therapy to improve outcome after perinatal hypoxic-ischemic insults. Despite hypothermia and neonatal intensive care, 45-50% of affected children die or suffer from long-term neurodevelopmental impairment. Additional effect is expected with adjuvant earlier neuroprotective interventions, beside hypothermia, which are warranted to further improve the outcome of affected infants.
Allopurinol is a xanthine oxidase inhibitor and reduces the production of oxygen radicals and brain damage in experimental, animal, and preliminary human studies of ischemia and reperfusion, if administered early after the insult.
This study aims to evaluate the efficacy and safety of allopurinol administered immediately after birth to near-term infants with early potential signs of HIE.

2.2 Background

2.2.1 Introduction

Neonatal **hypoxic-ischemic encephalopathy (HIE)** following birth asphyxia is a major cause of death or long-term disability in term neonates in the western world, affecting about 1-4 per 1.000 live births and consequently about **5-20.000 infants per year in Europe**. Because of less established perinatal care, HIE is even more common in less privileged settings, affecting about **1 million infants every year worldwide**.
In recent years, therapeutic hypothermia became the only established therapy to improve outcome after perinatal hypoxic-ischemic insults. Despite hypothermia and modern supportive neonatal intensive care, **45-50% of children with moderate or severe HIE** (i.e., 2.500-10.000 infants per year in Europe) **still die or suffer from long-term neurodevelopmental impairment** [Edwards BMJ 2010]. Therefore, additional early neuroprotective interventions, beside hypothermia, are warranted to further improve their outcome.
Allopurinol is a xanthine oxidase inhibitor and reduces the production of oxygen radicals and brain damage in experimental and early human studies of ischemia and reperfusion.

2.2.2 The medical problem: Hypoxic-ischemic encephalopathy (HIE)

During labour and child birth various events (such as placental abruption, uterine rupture, umbilical cord complications, etc.) may result in impaired oxygenation and/or perfusion of the newborn brain which may result in brain injury termed “hypoxic-ischemic encephalopathy” (HIE) [recently reviewed in Volpe, Ann Neurol 2012]. HIE is associated with development of long-term motor, cognitive, and neurosensory and memory disability and is one of the fundamental problems in perinatal medicine affecting about **5.000-20.000 infants/year in Europe** (or 1-4/1000 live births in western societies) and approximately **1 million infants/year worldwide**.
For infants without congenital malformations who are born at term, a recent systematic review estimated that the incidence of an umbilical arterial pH < 7.0 at birth (as one of several potential indicators of perinatal hypoxia) is 3.7 of 1000 live births, of which 17% survive with neonatal neurologic morbidity, 16% develop seizures, and 6% die during the neonatal period [Graham Am J Ob Gyn 2008]. The incidence of neonatal neurologic morbidity and/or mortality in this population was 23%. The overall incidence of HIE was estimated to be 2.5 of 1000 live births.

In term infants with perinatal asphyxia and postnatal HIE, brain injury predominantly originates in the immediate perinatal period (in contrast to a more distant prenatally acquired brain injury) as indicated by the lack of already established brain injury on early postnatal MRI [Cowan, Lancet 2003]. Consequently, brain injury in this population may potentially be ameliorated by postnatal pharmacological interventions.

2.2.3 The burden for patients and society from cerebral palsy and cognitive disability

The most common motor disability resulting from HIE is “cerebral palsy” (CP). All-cause CP is the most common motor deficiency in children, affecting 1-4 per 1000 live births [Cans, Dev Med Child Neurol 2007]. CP has been defined as a group of disorders, permanent but not unchanging, involving movement, posture and motor function and due to a non-progressive lesion or abnormality of the developing brain [*Surveillance of cerebral palsy in Europe* (SCPE), Dev Med Child Neurol 2000]. Cerebral palsy may only become apparent at 2 years of age, and its incidence is therefore not adequately reflected in the data on neonatal neurologic morbidity described above [Graham, Am J Ob Gyn 2008]. Based on their data, HIE in term infants accounts for 15% of cases of cerebral palsy [Graham, Am J Ob Gyn 2008] and it can be assumed that in Europe, 2.000 new cases of CP are caused every year by perinatal HIE.

Cognitive disability, the other major adverse outcome after HIE, prevents affected patients to lead their lives independently (without assistance and/or financial support). Survivors of HIE are at risk of developing cognitive deficits, even in the absence of CP [Gonzales, Arch Dis Child 2006; Pappas Pediatrics 2015]. It can be estimated that 2.000(-4.000) newborn infants may be newly affected in Europe each year because of perinatal HIE.

Whereas the suffering of affected children and adults and their families cannot be adequately quantified, additional health care costs attributable to cerebral palsy and/or intellectual impairment were estimated to amount to 12.000 – 30.000 € annually per affected patient [Kancherla, Res Dev Disabil 2012]. Carefully assuming a life expectancy of the disabled children of 20-40 years, these medical costs amount to about 400.000 – 1.200.000 € per child with CP and/or intellectual impairment, not yet taking into account costs for special education and life-long social support and assisted living.

For the purpose of this proposal, CP and cognitive disability are summarized and referred to as severe neurodevelopmental impairment (NDI). Because CP and cognitive disability frequently occur in combination after HIE, it is carefully assumed that about 2.000-4.000 infants in total are newly affected by severe NDI in Europe every year, adding up to additional costs of **0.8 to 4.8 billion € / year for medical care of HIE survivors in Europe**.

Beyond these most severe impairments, it is important to note that even following mild HIE up to 50% of affected infants may experience long-term memory impairments [van Handel Dev Neuropsychol 2012] or behavioral problems [van Handel J Pediatr Psychol 2010].

2.2.4 The pathophysiology of HIE and the potential role of allopurinol

The single major cause of HIE is a perinatal hypoxic/ischemic event (perinatal asphyxia). This hypoxic insult can cause immediate (necrosis) and delayed death (apoptosis) of (especially neuronal) cells, the latter responsible for a substantial amount of HIE-associated permanent brain damage. Whereas no intervention is known to prevent necrosis, the delayed cell death by apoptosis can be reduced by therapeutic interventions:

Apoptosis is in part caused by secondary energy failure which can be reduced by hypothermic treatment [recently reviewed in Jacobs, Cochrane Database Syst Rev 2007 and Edwards BMJ 2010, Tagin Arch Pediatr Adolesc Med 2012].

Apoptosis is also caused by xanthine oxidase-mediated production of cytotoxic oxygen radicals during reperfusion, and there is evidence that allopurinol, a xanthine-oxidase inhibitor, reduces delayed cell death in animal models of perinatal asphyxia and ischemia/reperfusion [reviews by Palmer Pediatr Res 1990, Warner, J Experiment Biol 2004, and Braunersreuther Curr Pharmaceut Biotechnol 2012].

Other factors such as exitotoxicity, additional hypoglycemia or hyperthermia, etc. are also important determinants of brain injury [reviewed in Volpe, Ann Neurol 2012] and will (in part) be taken into account in this proposal.

2.2.5 *Experimental evidence* for a reduction of brain injury in HIE by allopurinol

Allopurinol, a xanthine-oxidase inhibitor, blocks purine degradation. It also seems to result in the accumulation of adenosine during hypoxia, since allopurinol treatment increases brain tissue levels of adenosine after hypoxic-ischemic injury [Marro, Brain Research 2006]. Adenosine is a potent inhibitory neuromodulator providing additional neuroprotection in HIE. In higher concentrations, allopurinol acts as an iron-chelator and direct scavenger of free radicals [Shadid, Neurosci Lett 1998].
Allopurinol **pretreatment** preserves cerebral energy metabolism as shown by 31P NMR during perinatal hypoxia-ischemia in immature rats [Williams, Neurosci Lett. 1992] and thus, prevents cerebral damage [Palmer, Pediatr Res. 1990].

The timing of xanthine oxidase-mediated production of cytotoxic superoxide free radicals was most clearly determined by Ono et al. [Brain Research 2009] in a rat model of forebrain ischemia and reperfusion using a chemo-electric sensor placed in the jugular bulb:

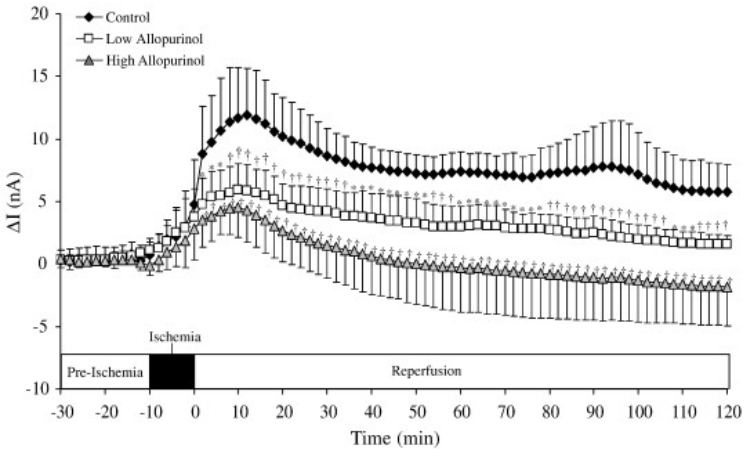


Figure 1: Change from baseline of superoxide-anion current [Ono, Brain Research 2009]

Figure 1 illustrates that the superoxide free radical production starts at low level during ischemia and increases dramatically during or immediately after reperfusion. Most of this superoxide anion production can be prevented by high-dose allopurinol pre-treatment – but a very early allopurinol treatment (within 10-30min after birth) could be almost equally effective as a pre-treatment.
These results confirm previous reports of early oxygen radical production after total brain ischemia which followed the cerebral blood flow post-ischemic hyperperfusion peak after about 10min (see Figure 2 from [Dirnagel, J Cereb Blood Flow Metabol 1995]) and continued oxygen radical production for at least 2 hours after onset of reperfusion after more severe ischemia.

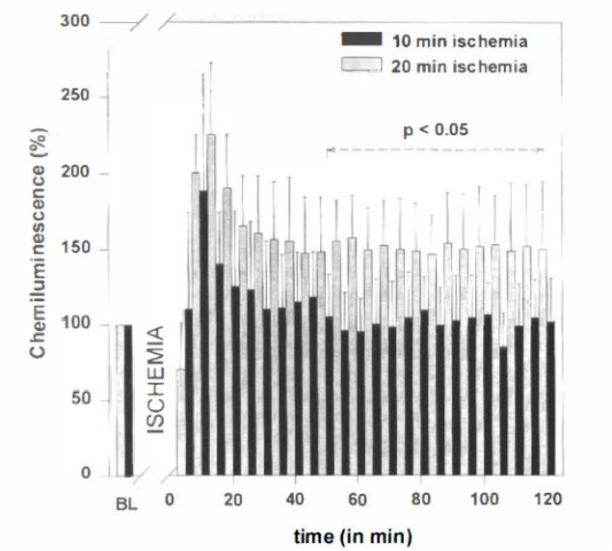


Fig. 2: Oxygen Radical production measured online and in vivo by chemiluminescence in rat whole brain ischemia as increase over baseline [Dirnagel, J Cereb Blood Flow Metabol 1995].

In agreement with the finding that xanthine-oxidase mediated oxygen radical injury largely occurs *early after* ischemia, allopurinol administered **after** inducing hypoxia-ischemia reduces brain injury in 7-day-old rats [Palmer, Pediatr Res 1993]. Vasogenic edema as assessed by T2-weighted magnetic resonance imaging was reduced [Peeters-Scholte, Pediatr Res 2003] and cerebral energy state was preserved in allopurinol-treated piglets [Peeters-Scholte, Exp Brain Res 2004]. Although these data are promising, allopurinol should certainly be administered as early as possible to achieve optimal efficacy.

More recently, it has been shown that maternal treatment with allopurinol during the ischemia/reperfusion challenge of acute birth asphyxia in fetal sheep restored the fetal neuronal damage toward control scores; indicating that maternal treatment with allopurinol offers potential neuroprotection to the fetal brain in the clinical management of perinatal asphyxia [Kaandorp Reprod Sci 2014]. Furthermore, allopurinol reduces oxidative stress in the ovine fetal cardiovascular system after repeated episodes of ischemia-reperfusion [Derks Ped Res 2010].

Finally, there is experimental evidence that the integrity of the NMDA receptor, which is involved in excitotoxic brain injury, may be preserved by allopurinol in models of hypoxic-ischemic brain injury [recently reviewed in Boda, J Perinatol 2009].

In summary, allopurinol prevents adenosine degradation, oxygen radical formation, preserves NMDA receptor integrity, and consequently may reduce brain injury in HIE by several mechanisms of action which are independent from the proven beneficial effect of hypothermic treatment on cellular energy metabolism. An additional beneficial (or even synergistic?) effect of allopurinol in addition to hypothermia can therefore be expected.

2.2.6 *Clinical evidence* and *evidence from systematic reviews* for a reduction of brain injury in HIE by allopurinol

Because of the strong experimental evidence and the (scarce) available clinical data which suggest a beneficial effect, a larger trial is mandatory to confirm (or disprove) that allopurinol treatment is of significant benefit for newborn infants with HIE.

Following a favorable pilot study [Torrance, Pediatrics 2009], a randomised, blinded, placebo controlled ALLO-2 Trial was conducted to investigate the effect of **antenatal allopurinol** for reduction of postasphyctic HIE in 222 women with suspected fetal hypoxia indicating immediate delivery [Kaandorp, BMC Pregnancy and Childbirth 2010]. This recently completed ALLO-2-Trial suggested a trend towards lower cord serum concentrations of the calcium binding protein S100ß, which was the primary outcome of this study and is an established biochemical surrogate marker of brain injury and subsequent neuro-developmental disabilities: 44.5 pg/mL (IQR 20.2-71.4) in the ALLO group versus 54.9 pg/mL (IQR 26.8-94.7) in the CONT group (difference in median -7.69 (95% CI -24.9 to +9.52)), which was not statistically significant. Post hoc subgroup analyses showed a potential treatment effect of allopurinol on the proportion of infants with a cord S100ß value above the 75th percentile in girls (ALLO n=5 (12%) vs CONT n=10 (31%); risk ratio (RR) 0.37 (95% CI 0.14 to 0.99)) but not in boys (ALLO n=18 (32%) vs CONT n=15 (25%); RR 1.4 (95% CI 0.84 to 2.3)). Also, cord neuroketal levels were lower in girls treated with allopurinol as compared with placebo treated girls: 18.0 pg/mL (95% CI 12.1 to 26.9) in the ALLO-2 group versus 32.2 pg/mL (95% CI 22.7 to 45.7) in the CONT group (geometric mean difference -16.4 (95% CI -24.6 to -1.6)). [Kaandorp, Arch Dis Child F&N 2015]. Although already encouraging, it is unlikely that umbilical cord blood biomarker concentrations reflect the complete effect of allopurinol on brain injury in asphyxiated infants, because the surge in oxygen radicals occurs during reperfusion, i.e. during and after resuscitation. Therefore, neither the contribution of oxygen radicals to brain injury nor the effect of allopurinol by preventing oxygen radical formation will be apparent fully in cord blood biomarker concentrations.

Most importantly, the **prenatal administration of allopurinol** at a dose of 500mg to mothers **was safe** and no adverse effects were observed in their offsprings [Kaandorp, Arch Dis Child F&N 2014].

Because the infant's condition in utero cannot be evaluated with high precision and HIE can only be diagnosed after birth and may even occur unexpectedly, prenatal administration is difficult in clinical routine. Prenatal administration of allopurinol to every mother at risk of perinatal asphyxia will likely expose numerous mothers and children unnecessarily – as demonstrated in the ALLO-2 trial [Kaandorp, Arch Dis Child F&N 2015]. Therefore, a randomised trial of **early postnatal allopurinol** treatment in newborns with HIE is required.

Up-to-date, three small trials (including 114 infants altogether) [van Bel, Pediatrics 1998, Benders, Arch Dis Child 2006, Gunes, Pediatr Neurol 2007] examined postnatal allopurinol for HIE [for systematic review see: Chaudhari, Cochrane Database Syst Rev 2008]. Long-term outcome data of two of these preliminary studies suggest a reduction in the combined outcome of death or severe neurodevelopmental impairment from 65% in the control group to 25% in the allopurinol group in moderately asphyxiated infants, whereas severely asphyxiated infants do not seem to benefit [Kaandorp, Arch Dis Child 2012].
These studies examined allopurinol administration up to 4 hours postnatally and were performed without concomitant hypothermia treatment which meanwhile has become state-of-the-art for HIE treatment. This proposal therefore aims to study the **very early postnatal administration of allopurinol (within 30min after birth) in addition to hypothermia** and the impact of hypothermia on allopurinol metabolism and pharmacokinetics.
In line with this proposal, an international panel of experts very recently ranked allopurinol among the top 5 candidates for pharmacological prevention of brain injury in newborn infants with HIE "ready for bench to bedside translation" [Robertson, J Pediatr 2012]. Furthermore, a Cochrane systematic review on postnatal allopurinol to reduce brain damage comes to the conclusion: "*The available data are not sufficient to determine whether allopurinol has clinically important benefits for newborn infants with hypoxic-ischemic encephalopathy and, therefore, larger trials are needed. Such trials could assess allopurinol as an adjunct to therapeutic hypothermia in infants with moderate and severe*

encephalopathy and should be designed to exclude clinically important effects on mortality and adverse long-term neurodevelopmental outcomes." [Chaudhari Cochrane Database Syst Rev 2008]

Because infants with moderate HIE (i.e. those who will likely benefit the most) may not be clearly identifiable very early after birth, the proposed trial needs to study allopurinol in all infants with severe birth asphyxia and early clinical signs of hypoxic ischemic encephalopathy, similar to those criteria used for screening in former large hypothermia trials [Shankaran NEJM 2005, Azzopardi NEJM 2009].

2.2.7 *Clinical evidence* for a reduction of brain or tissue injury in human patients with other forms of organ ischemia/reperfusion injury

Randomised controlled trials have found evidence of benefit from high dose allopurinol (>10mg/kg) in limiting tissue reperfusion injuries in adult patients undergoing coronary bypass surgery [Johnson, Am Heart J 1991; Sisto, Ann Thoracic Surg 1995, for review Braunersreuther, Curr Pharmaceut Biotechnol 2012].
High-dose allopurinol reduced oxygen radical production and peroxidation product formation in newborn infants undergoing extracorporal membrane oxygenation (ECMO) for severe respiratory failure [Marro, Pediatr Res 1997].
Moreover, allopurinol pre-treatment reduced a composite outcome of death or adverse neurological or cardiac outcomes in newborn infants undergoing surgical correction of hypoplastic left heart syndrome [Clancy, Pediatrics 2001].

2.2.8 No evidence of significant harm from allopurinol in newborn populations

As far as reported in the previous trials of antenatal [Torrance, Paediatrics 2009: n=27 exposed to allopurinol, Kaandorp, Arch Dis. Childhood F&N 2015: n=111 exposed to allopurinol] and postnatal [van Bel, Pediatrics 1998: n=11 exposed to allopurinol, Gunes, Pediatr Neurol 2007: n=30 exposed, Benders, Arch Dis Child 2006: n=17 exposed] allopurinol in HIE and high dose allopurinol in other clinical settings in neonates and infants [McGaurn, Pediatrics 1994: n=12 infants exposed to allopurinol; Marro, Pediatric Research 1997: n=11 exposed to allopurinol; Clancy, Pediatrics 2001: n=155 exposed to allopurinol], there is no evidence for significant adverse effects of allopurinol in newborn infants even at high doses. The Cochrane review concludes: „The available data have not raised major safety concerns related to use in newborn infants." [Chaudhari, Cochrane Database Syst Rev 2008]

2.2.9 Need for an adequately powered clinical trial to resolve uncertainties about safety and efficacy of allopurinol to reduce NDI in infants with HIE

The above described experimental and preliminary clinical evidence indicates that **early postnatal allopurinol in infants at high risk of HIE has a potential for improved outcome and a very low risk of adverse effects** (based on 114 infants treated postnatally and additional 276 infants treated by administration to the mother). The remaining clinical uncertainty about safety and missing proof of efficacy can only be resolved by a large, adequately powered, pragmatic clinical trial. A small trial will add very little in terms of additional safety data and will not be able to resolve uncertainty about efficacy.
Given the enormous costs for long-term medical care and social and special educational support in infants with disability following HIE, public funding of such a trial with a low cost medication appears justified and the costs of the proposed trial for society would be settled, if its results would help to save just 10 infants from neurodevelopmental impairment. Therefore, **there seems to be a high potential for return of investment from a societal point of view**.

The ALBINO consortium therefore proposes such a large clinical trial for safety and efficacy:

Justification for one large conclusive clinical trial instead of a small pilot study:
ALBINO will be the largest trial for neonatal hypoxic ischemic encephalopathy to date and will allow appropriate assessment of potential risks associated with treatment. Care was also taken to complement the stringent primary outcome measure of paramount clinical importance with potentially more sensitive biomarkers of brain injury, particularly with beyond-the-state-of-the-art advanced magnetic resonance imaging, as is currently also done by other investigators (e.g., TOBYXe-trial (NCT00934700)) again enabling comparison of treatment results between studies. Advanced MRI techniques show good (yet not perfect) correlation with long-term outcome (e.g., [Rutherford Paediatrics 1998, Barkovich, AJNR 1998, Shah Pediatrics 2006, Martinez-Biarge, Neurology 2011, Tusor, Pediatr Res 2012; Shankaran Pediatrics 2015]) – but 1) a clinical outcome appears more meaningful to patients and caregivers and 2) available data in populations with HIE are insufficient for appropriate sample size analyses. Furthermore, smaller studies measuring surrogate outcomes will not be able to add substantially to current knowledge on the safety profile of the study medication.

This proposal deviates from the EMA's suggestion of proof of principle by proofing benefit from allopurinol administration in patients with HIE on surrogate markers of brain injury before performing a study on safety and efficacy [EMA: Revised priority list for studies into off-patent pediatric medicinal products 2012] for the following reasons:
a) There are already clinical data from post- and prenatal trials suggesting a probable reduction in brain injury by allopurinol. (see above: *Clinical evidence* and *evidence from systematic reviews* for a reduction of brain injury in HIE by allopurinol)
b) The most (and only?) meaningful proof of benefit / efficacy is a reduction in the composite outcome of death or neurodevelopmental impairment which can only be ascertained at 2 years after birth/treatment, making an adaptive design (such as proposed by [Bauer, Biometrics 1994]) unfeasible.
c) Although biomarkers, particularly advanced imaging techniques (referenced above), correlate with long-term outcome and are essential to quantify the degree of brain injury early on, they are not developed to the point that an improvement in brain injury score / white matter microstructures can be translated into a clinically meaningful benefit in long-term outcome. Hence it is unclear how a pilot study applying such biomarkers as primary outcome measures could be adequately designed and powered.
Instead of a pilot study for proof of principle or an adaptive design, we therefore suggest close follow-up of MR-imaging along with all safety relevant data by a Data Monitoring Committee (which will include experts for brain imaging not otherwise involved in the study). This DMC would then be able to suggest discontinuation of the study in case of evidence of harm or lack of benefit at pre-defined milestones.

2.2.10 Orphan Drug Designation for allopurinol for treatment of perinatal asphyxia

In January 2015, our group (lead: ACE pharmaceuticals) has submitted an application for **orphan drug designation (ODD) for allopurinol sodium for treatment of perinatal asphyxia** to the European Medicines Agency (EMA). In the COMP meeting of April 16, 2015 a positive opinion has been issued for the orphan drug designation for allopurinol sodium for treatment of perinatal asphyxia (**EMA/OD/004/15**) to the EC. The "COMP opinion letter" is attached to this application as supporting document.
Meanwhile (June 2015) ODD has been granted by the EMA. The public summary of the opinion of the Committee for Orphan Medicinal Products at the EMA (COMP) on ODD is available at:

http://www.ema.europa.eu/docs/en_GB/document_library/Orphan_designation/2015/06/WC500188864.pdf.

2.2.11 Scientific Advice from the European Medicines Agency (EMA)

In November 2015, ACE Pharmaceuticals has requested Scientific Advice and Protocol Assistance from the EMA for the study with allopurinol sodium for treatment of perinatal asphyxia, including questions specifically related to this study protocol and the intended procedure of deferred consent.
Scientific advice was received confidentially by ACE Pharmaceuticals in May 2016 and issues relevant to this study protocol have been communicated to the Steering Committee of the ALBINO-trial and subsequently incorporated after careful consideration.

3 Aims of the study

3.1 Primary Objective

To evaluate whether in newborns with asphyxia and early clinical signs of hypoxic ischemic encephalopathy, early postnatal allopurinol compared to placebo administered in addition to standard of care (including therapeutic hypothermia if indicated) reduces the incidence of death or severe neurodevelopmental impairment (defined as cerebral palsy, or cognitive or language impairment, the latter defined as cognitive- and the language-composite scores of the Bayley Scales of Infant and Toddler Development (3rd edition) <85) at 24 months of age.

Justification of the chosen primary objective:

Why long-term neurodevelopmental outcome?
The long-term neurocognitive outcome of newborn infants determines their ability to lead their lives independently without assistance. In a review on outcome measures in randomised controlled trials in infants, long-term neuro-developmental outcome measures were clearly identified as most meaningful [Zhang, J. Pediatr 2001].

Why cerebral palsy?
As outlined in 1.1.1.2, cerebral palsy is the major outcome after HIE in the term born child. Especially, when associated with basal ganglia and thalamus lesions, which are the main lesion types after HIE, CP can be expected in all cases [Kraegeloh-Mann, Dev Med Child Neurol 2002].
Severe visual and hearing impairment are not to be expected without motor and cognitive impairment, e.g. CP, and hence are not included in the primary outcome measure.

Why cognitive / language impairment defined as cognitive- and the language-composite scores **<85 (mean – 1 SD)?**
Cognitive impairments can be observed without cerebral palsy following HIE, particularly if the lesion type on MRI shows a watershed predominant pattern involving the cerebral white matter, particularly in the frontal and parieto-occipital vascular watershed areas [Gonzales & Miller Arch Dis Child F&N 2006; Pappas Pediatrics 2015]. Furthermore, cognitive impairments may even follow mild HIE, where memory impairments or behavioral problems are observed in up to 50% of affected infants [van Handel Dev Neuropsychol 2012; van Handel J Pediatr Psychol 2010].
The cut-off for the cognitive- and the language-composite scores of <85 for the definition of a cognitive and/or language delay is meaningful, as infants who remain at a level of more

than one standard deviation below the mean are likely to fail or will at least have major difficulties with regular school education. If an intervention increases the proportion of infants above this cut-off, it will likely reduce the need for special education and life-long assistance for these individuals and thereby substantially reduce special education and social care costs for society.

Why performing neurocognitive outcome assessment at 24 months of age?
At the age of 24 months, the standardized cognitive tests will be reasonably predictive of longer-term outcome and overall academic achievements [Peralta-Carcelen, Pediatrics 2009].
Most infants will walk and all cases with HIE-associated CP will be apparent by 24 months. Whereas very mild or atypical forms of CP may not always be recognized before 2 years of age, the brain lesions following HIE in the term born child are expected to lead to clear spastic or dyskinetic CP of higher severity, which can reliably be diagnosed at 2 years of age.

Nevertheless, longer-term neurocognitive outcome at 6 years is considered important and consequently will be pursued, provided adequate funding can be obtained (see secondary outcome measures).

Why choosing a composite outcome?
Because the cognitive-composite-score (as well as the language-composite-score) may fail to capture important competing outcome events such as severe physical impairments (here CP) or cognitive impairments that preclude psychomotor testing, a composite outcome measure (composed by language-composite-score < 85 or cognitive-composite-score <85 or cerebral palsy present) will be evaluated as primary endpoint.

Death will be included as a separate, mutually exclusive endpoint [Engel and Franz IJSMR 2016, accepted].

The proposed outcome is in line with the suggestion brought forward in the Cochrane Review on postnatal allopurinol: "*Such trials […] should be designed to exclude clinically important effects on mortality and adverse long-term neurodevelopmental outcomes.*" [Chaudhari, Cochrane Database Syst Rev. 2008]

Why not (yet) relying on MR-Imaging brain injury scores (or biochemical biomarkers) as primary outcome?
Brain injury scores on MRI have been shown to correlate with long-term outcome [Shankaran J Pediatr 2015]. As continuous outcome measures they may result in a higher power to prove efficacy and a lower sample size would be required to prove efficacy. However, the agreement between biomarkers / MRI-scores and clinical outcome need further investigation. Available data show that the severity of basal ganglia/thalamic lesions is associated with the severity of motor impairment (Spearman rank correlation 0.77; $p<$ 0.001 [Martinez-Biarge, Neurology 2011]) – but this means that only 60% of the variability in motor outcome can be explained by the variability in MRI-Score. Abnormal signal intensity in the posterior limb of the internal capsule predicted the inability to walk independently by 2 years, but specificity was only 77% (indicating incorrect prediction in 23% of cases with abnormal signal) [Martinez-Biarge, Neurology 2011]. However, using quantitative approach of MRI images, such ADC values, as a measure of restricted diffusion, as a sign of ischemic tissue, show that ROC analysis of ADC values of the corpus callosum of infants treated with hypothermia, data obtained at 1.5 and 3.0T were combined, the area under the curve was 0.87 with a cut-off value of 0.969 x 10^{-3} mm^2/s.
Nevertheless, in the end, to-date clinical benefit is most relevant for patients and their families and should also be most relevant for prescribing physicians. Furthermore, available

data on MRI scores in populations with HIE are currently still insufficient for appropriate sample size analyses required for a sound clinical trial.
Finally, reliable assessment of safety (i.e. to rule out potential, yet unknown adverse effects) of allopurinol also requires a sample size of several hundred infants, consequently it clearly is preferable to study the more meaningful clinical outcome.
Despite this clear commitment of the ALBINO investigators to seek neurodevelopmental outcome as primary outcome measure, advanced quantitative analyses of MRI of the brain and advanced quantitative analyses of multichannel EEG have the potential to show treatment effects and will become more and more relevant for outcome prediction and will hence be determined during the ALBINO study as secondary endpoints (below).

3.2 Secondary Objectives

To evaluate the effect of allopurinol in addition to hypothermia (if indicated) on:

- brain injury assessed by magnetic resonance imaging,
- brain injury assessed by cerebral ultrasound
- amplitude integrated electroencephalogram,
- full scale electroencephalogram,
- laboratory biomarkers and markers of peroxidation.

To evaluate the safety of allopurinol in neonates treated with hypothermia.

To study pharmacokinetics of allopurinol and mannitol in neonates treated with hypothermia and not treated with hypothermia

3.3 Future Objectives

Additionally, provided that additional funding can be ascertained, to evaluate the effect of early postnatal allopurinol on neurological, developmental and anthropometric outcome variables (including the Kaufmann ABC) at 6 years of age. (This will require another source of funding and is beyond the scope of this proposal/application):

4 Study design

4.1 Design

This is a placebo-controlled, (double-)blinded, randomised, parallel-group comparison for superiority (Phase III study).

4.2 Study duration

The study is scheduled to begin in April 2016, recruitment should start in autumn/winter 2016.

The individual participation in the study will be 2 years (24 hours of treatment with an additional follow up for 2 years).

Start of the study:	April 1st 2016
FPI:	December 1st 2016
LPO:	November 30th 2020
End of study.	December 31st 2020

5 Study population

5.1 Screening and Recruitment

Patients will be recruited in more than 60 centres in 13 European countries. These centres currently treat on average 500-600 newborn infants with hypothermia for moderate or severe HIE per year.
It is therefore anticipated that 1200 infants with umbilical blood pH<7.0 or base deficit >15 mmol/l will be screened and 846 infants meeting all inclusion criteria and no exclusion criterion will be recruited for the ALBINO trial.
We estimate a recruitment of about 35 patients per month in 13 European countries, therefore recruitment will last for 24 months (for details about number of cases refer to chapter 9.1 Sample Size).

All patients who meet at least one inclusion criterion (listed below under section 5.2 Inclusion Criteria) have to be screened for the study.
Each screened patient is given a **patient identification number (PIN)** according to the screening log in the ISF. This number is the overall identifier of the pseudonymised patient throughout the study.
For every screened patient, a screening form has to be filled in the eCRF. This enables the documentation of non-biased recruitment according to CONSORT-statement later on.

Deferred Consent:
Because of the need to administer allopurinol as early as possible (described in detail in section 2.2.5 "*Experimental evidence"*) it is anticipated to be impossible (or at least extremely difficult) to obtain meaningful written informed consent before administration of study medication (although a "declaration of intent" will be sought). The ALBINO consortium believes that this study falls under §30 of the Declaration of Helsinki (2014) which supports research of emergency interventions even without prior written consent and has already received supporting statements of national as well as European parent organisations.

> Art 30 of the Declaration of Helsinki (2014):
> „*Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.*".

This is discussed in detail in the section 10 "*Ethical Issues*".

Deferred written informed consent will be obtained during the first day of life – and before a second dose of allopurinol is administered if the child meets the national/local criteria for therapeutic hypothermia.

To enable very early pharmacokinetic analysis (0.75ml in n=40 patients) and very early determination of oxidative damage (0.5ml in n=100 patients) – blood samples will be collected together with clinically indicated blood samples (i.e., without study-driven needle stick) before written informed consent was obtained and will be destroyed if written informed consent is not given.

Community Engagement / Opt-out:
Local Investigators will issue press releases (translated into the national language based on English templates provided by the CPCS) to inform the community about the conduct of the trial and the deferred consent procedure. The first press release will be issued after approval of the deferred consent procedure by the relevant ethics committee and regulatory authority and before the first patient is enrolled at the respective site.
Furthermore, local Investigators distribute flyers and posters (translated into the national language based on English templates provided by the CPCS) in prenatal clinics, delivery rooms, obstetric private practices relevant to the study sites. Furthermore every women admitted to the delivery room should be given a short study information (flyer) to enable them to make a 'declaration of intent' – and particularly to take the opportunity to opt-out from the study.

5.2 Inclusion criteria

Term and near-term infants with a history of disturbed labour who meet at least one criterion of **perinatal acidosis (or ongoing resuscitation) and** at least two early clinical signs of potentially evolving **encephalopathy** as defined herein:

Severe perinatal metabolic acidosis or ongoing cardiopulmonary resuscitation at 5 min after birth:
At least 1 out of the following 5 criteria must be met
- Umbilical (or arterial or reliable venous) blood gas within 30 min after birth with pH<7.0
- Umbilical (or arterial or reliable venous) blood gas within 30 min after birth with base deficit ≥16 mmol/l
- Need for ongoing cardiac massage at/beyond 5 min postnatally
- Need for adrenalin administration during resuscitation
- APGAR score ≤5 at 10min

AND

Early clinical signs of potentially evolving encephalopathy:
At least 2 out of the following 4 criteria must be met:
- Altered state of consciousness (reduced or absent response to stimulation or hyperexcitability)
- Severe muscular hypotonia or hypertonia,
- Absent or insufficient spontaneous respiration (e.g., gasping only) with need for respiratory support at 10 min postnatally,
- Abnormal primitive reflexes (absent suck or gag or corneal or Moro reflex) or abnormal movements (e.g., potential clinical correlates of seizure activity)

5.3 Exclusion criteria

- gestational age below 36 weeks
- birth weight below 2500 g
- postnatal age >30min at the end of screening phase

- severe congenital malformation or syndrome requiring neonatal surgery or affecting long-term outcome
- patient considered “moribund”
- decision for “comfort care only” before study drug administration
- parents declined study participation as response to measures of community engagement
- parents haven’t had the chance to appraise the conduct of the ALBINO study at the study site and to refute that their child may receive study drug in the event of asphyxia/HIE
- both parents are insufficiently fluent in the study site’s national language(s) or English or do not have the intellectual capacity to understand the study procedures and to give consent as judged by the personel who had been in contact with the mother/father before delivery.
- both parents/guardians less than 18 years of age, in case of single parent/guardian this one less than 18 years of age

Justification for in- and exclusion criteria:
Because preclinical data strongly suggests benefit and early clinical data suggests safety and potential efficacy, it is now essential to study safety and efficacy of early postnatal allopurinol to prevent brain injury in infants at high risk for HIE, in exactly the population concerned: i.e. in infants at high risk of HIE.
The definition of the study population is similar to previous studies for evaluation of hypothermic treatment for HIE (in part summarized by [Volpe, Ann Neurol 2012]) and to ongoing studies on additional therapy for HIE (e.g., TOBYXe-trial (NCT00934700)) to enable comparability of results.
The inclusion criteria are not identical to the above referenced trials concerning aEEG-criteria and the assessment of clinical scores of HIE because of the need for immediate administration of allopurinol to enable maximum effect of allopurinol (as described under section 1.1.1.4). Immediate administration, before the reperfusion associated surge in oxygen radicals has occurred, will prevent complete assessment of the clinical recovery after resuscitation and before initial administration of study medication: in particular: 1) an aEEG-trace, an important diagnostic tool to assess the severity of HIE used in above referenced trials, will not be available in most infants within 30min. and 2) clinical scores to assess the severity of HIE used in above referenced trials (Thompson Score, Sarnat Score) may overestimate severity of HIE at that early age.
Hence, it has to be anticipated that a recovery of the neurological status may occur in about 20% of infants recruited into the ALBINO trial after meeting all in- and no exclusion criteria, who will thereafter not develop moderate to severe HIE and will not qualify for hypothermia. In fact, this early recovery may already be part of a neuroprotective effect of early allopurinol in the active treatment group.
Since also mild HIE has been recognized to negatively influence long-term outcome [van Handel et al, J Pediatr Psychol. 2010], brain imaging (MRI) and assessment of neurocognitive outcome at 24 months of age are justified in infants who do not develop moderate to severe HIE and will not qualify for hypothermia to enable assessment of a potential benefit from the intervention.

5.4 Enrollment into the study

All patients who meet the inclusion criteria but none of the exclusion criteria will be enrolled into the study.
A check-list (separate document – not part of the protocol), which lists all in- and exclusion criteria, must be ticked / filled-in and signed before the next package of the study medication (of consecutively numbered packages) is opened.

5.5 Gender Aspects

It is well known that female preterm infants at any given gestational age at birth develop more favourable than male preterm infants, although the mechanism is not yet established. Furthermore, gender-dependent pathways of hypoxia/ischemia induced cell death and neuroprotection have been identified in immature P3 rat model [Nijboer Dev Neurosci 2007, Nijboer J Cereb Blood Flow Metab. 2007].
In that line, female fetuses seemed to benefit somewhat more from antenatal allopurinol than male fetuses as suggested by data from the Allo-2-trial [Kaandorp Arch Dis Child F&N 2014]. The mechanism of such a potential gender difference in the efficacy (and safety?) of allopurinol is not yet known and will be addressed by appropriate subgroup analyses verifying an interaction between gender and treatment group assignment on all outcome and safety variables.
Because of the preliminary nature of the data referred to above (which may still be a chance finding), the ALBINO study was designed anticipating an even distribution of male and female patients and an equivalent effect of allopurinol for both boys and girls.

5.6 Randomisation

Randomisation lists will be prepared by the CPCS IV and sent to ACE Pharmaceuticals for blinded labelling and packaging of the study medication.

Randomisation will be done in blocks of four.

Each shipment of study medication to study centres will comprise complete blocks of 4, thereby achieving stratification by centre.

Justification:
Although a variable block size would have been desirable for best allocation concealment, a fixed block size of 4 was selected for prevention of an uneven distribution of verum/placebo in this study with a low anticipated recruitment rate per centre (on average < 10-15) – as well as for practical reasons of study medication distribution to numerous study sites.

Stratification for therapeutic hypothermia – although desirable – is impossible, because the clinical indications for therapeutic hypothermia will evolve with time and may not be apparent at the 1st dose of study medication.

6 Study medication

6.1 Treatment

Intervention: Allopurinol, powder for injection (PFI), administered in two doses. First dose (20 mg/kg in 2ml/kg sterile water for injection) given as soon as intravenous access is established. Start of infusion of study medication should be within 30min (no later than 45min) after birth and second dose (10mg/kg in 1ml/kg sterile water for injection) 12 hours after the first dose. The second dose will only be administered to infants treated with therapeutic hypothermia. Infants who recover quickly and do *not* qualify for and hence do *not* undergo hypothermia will *not* receive a second dose. Administration will be by infusion over 10min using a syringe pump through secure venous access.

Control: Placebo (Mannitol, PFI, 20mg/kg in the same volume and at the same time intervals as the intervention group – (2^{nd} dose 10mg/kg only if infant undergoes therapeutic hypothermia)).

Dosing according to body weight: Dosing of study medication should be done according to actually ***measured*** body weight – if the infant's health permits weighing. Otherwise the clinical estimate of the team will be sufficient (estimated weight must be documented).

If the infant's birth weight is / is estimated to be >5000g – the 1^{st} dose of allopurinol / placebo is 100mg in 10ml sterile water for injection and the 2^{nd} dose is 50mg in 5ml.

Justification of allopurinol dosage:
The proposed dose will result in drug levels higher than those aimed for in the treatment of gout [van Bel Pediatrics 1998, van Kesteren, Ther Drug Monit 2006]. Given the favorable safety profile even at that dose (see section 2.2.8 for details) and the observation that allopurinol at high concentrations scavenges free oxygen radicals and chelates free iron in addition to xanthine oxidase inhibition [Pacher, Pharmacol Rev 2006], this seems to be justified.

Because the optimal plasma concentration for maximum therapeutic effect and minimum adverse effect are unknown to date, dose finding studies based on plasma concentrations do not seem reasonable. A comparison of several doses of allopurinol for clinical effects is not feasible because of the high sample size required for such a study.

Pharmacokinetics during hypothermia treatment need yet to be determined. Because preliminary data suggest that metabolism of allopurinol will be slowed-down during therapeutic hypothermia, the 2^{nd} dose is reduced to only 10mg/kg (in comparison to another 20mg/kg administered in previous trials).

The second dose of study medication is ***not*** administered to infants who recover quickly after perinatal asphyxia, i.e., who do ***not*** develop moderate or severe hypoxic-ischemic encephalopathy and hence do ***not*** qualify for and do ***not*** undergo hypothermia treatment, because (so far) an effect of allopurinol on longer-term neurocognitive outcome has only been suggested for infants with moderate HIE (i.e., infants qualifying for hypothermia treatment). Nevertheless, it appears very likely that infants with mild HIE (i.e., those who recover quickly after asphyxia) also benefit from allopurinol, too.

Adaptation of Dosage:
Finally, pharmacokinetic analyses will be done early (within the first year of recruitment) in this study to ensure adaptation of dosing if plasma concentrations turn out lower than those in the preliminary clinical studies [vanBel Pediatrics 1998; van Kesteren Therapeutic Drug Monitoring 2006].

Justification of mannitol placebo:
Mannitol powder for injection (pfi) has the same appearance as Allopurinol pfi, but the dose is so low that a clinically relevant effect is not expected.
Mannitol at 10 times the dose anticipated for ALBINO (or even higher doses) has been used in the past for treatment of cerebral edema and as an osmotic diuretic. Both actions would not be of harm for the study patients, and, if at all, would be expected to be of benefit. More importantly, at the much lower dose administered during the ALBINO study, the before mentioned effects (diuretic / anti cerebral-edema) are not expected at all.

Mannitol is also used as an excipient, e.g. in paracetamol iv-preparations. The 1000mg/100ml vial Perfalgan (Bristol-Myers Squibb) contains (according to information received from the manufacturer, Axel Franz personal communication with Mrs. Geslinde Walter at +49 89 12142314 on 21.07.2016) 3850mg Mannitol. The recommended single dose of 7.5 mg/kg paracetamol i.v. consequently results in the administration of 28.9mg/kg of Mannitol i.v.. A typical cumulative daily dose of 4*7.5mg/kg paracetamol i.v. hence results in a dose of 115.5mg/kg mannitol (slightly higher than the single dose of mannitol used as placebo in this trial). During routine care, the prescribing physician does not even considering that mannitol is administered simultaneously to paracetamol.

In parallel of the determination of pharmacokinetics of allopurinol, determination of the pharmacokinetics of mannitol will be considered in the placebo group to inform its use as excipient in the future.

6.2 Blinding, packaging, storage instructions, labelling, shelf life of the study medication

6.2.1 Blinding of study medication

Each box of study medication (containing 2 vials of powder for injection (pfi) and 2 ampules of water for injection will be dedicated to a single patient and will be labelled with a **unique Medication Identification Number (MIN)** (4 digits) which enables re-identification of the medication / treatment allocation (together with randomisation list or the security envelops for emergency unblinding (detailed in section *8.4 Emergency Code Breaking*)).

Example: **MIN: 1001**

The study medication will be referred to as: Allopurinol or Placebo

6.2.2 Packaging of study medication

The study medication, both allopurinol (verum) as well as mannitol (placebo), will be provided as powder for injection (pfi) in 20ml vials, each containing 100mg pfi.
2 vials with pfi will be provided in a package (for a first and, in case the patient undergoes therapeutic hypothermia for HIE, a second dose of study medication).
Every package of study medication containing two vials of pfi is dedicated to a single patient. Unused 2nd doses of study medication have two be retained in a safe place (to avoid accidental use in other patients) and destroyed after appropriate documentation according to the ALBINO-SOP on Drug Accountability and Destruction of unused Study Medication.
The package of study medication also contains 2 ampulles of water for injection, with 10ml each, to reconstitute the pfi before application.

Four (n=4) individual, patient-dedicated boxes of study medication (each patient-dedicated box containing 2 vials with pfi and 2 ampulles of sterile water for injection and carrying a

unique MIN) will be distributed together in a *transport box* (also referred to as "'set-of-four' study medication boxes".

6.2.3 Transport of study medication

All arrangements for the shipment of study medication to the participating centers will be done by ACE Pharmaceuticals, Zeewolde, the Netherlands. Study medication will be shipped at ambient temperature; temperature will be logged during transport. Upon arrival of study medication a receipt along with the printout of the temperature logger should be returned to ACE Pharmaceuticals:

Fax: +31 36 5229096
Email: albino@ace-pharm.nl

6.2.4 Request for additional study medication

As soon as the 3rd individual box of study medication has been reached (= one left) of last received *transport box*, additional study medication has to be ordered immediately at:

Email: ctm-albino@ace-pharm.nl

6.2.5 Storage Instructions for study medication

Store study medication at dry, secure place, out of reach of children, at 8-25°C. Study medication can be kept for a maximum of 6 months at 30°C in the delivery room.
The temperature logger provided with the study medication should be kept at the study site and used for continuous logging of storage temperature (for that individual shipment).

6.2.6 Labelling of study medication

Labeling will be done according to GMP/GCP and national regulations, and labels will be documented in a separate document not part of this protocol**.**
Keep **"Study Medication List"** (separate document not part of this protocol), which will be provided along with the study medication, next to study medication boxes to ensure consectutive administration of consecutively numbered study medication.
After completion, the **Study Medication List** must be filed in the **Investigator Site File.**

6.2.7 Shelf Life of study medication

Study Medication has a shelf life at room temperature of 5 years. "the "Use By" date will be depicted on the label of study medication.

6.2.8 Instructions for reconstitution of study medication

Instructions for reconstitution of study medication will be provided in that study medication package.
In short: 10ml of sterile water for injection (wfi) are drawn up from the vial provided in the study medication package and injected into the vial with the study medication powder for injection (pfi). The pfi should dissolve instantaneously resulting in a clear solution. The solution is then transferred into a syringe suitable for the local syringe pumps and the dose is administered as slow intravenous infusion over 10min.

6.2.9 Instructions for destruction of unused study medication after completion of the study following appropriate documentation

Destruction of unused study medication as well as documentation of such destruction will be done according to the ALBINO-SOP on Drug Accountability and Destruction of unused Study Medication, which is not part of this protocol.

6.3 Concomitant medication

Any concomitant medication that is medically necessary for the patients will be allowed within the study, except open-label allopurinol in any dosage and any application mode.

All concomitant medication administered to study participants during the first week of life (until 7d*24h/d=168h after birth) will be logged in the eCRF with generic name, route of administration, start date/time and stop date/time. Except for vitamin K, vitamin D, Fluoride, which are administered to all newborn infants.
This procedure allows and facilitates any later check and control as well as assessing any possible related effect.

6.4 Recommendations for concomitant supportive therapy

To ensure best supportive care of all ALBINO participants, investigators have to consider the following recommendations:

Prevent Hyperoxia
Hyperoxia will increase oxidative stress (which the ALBINO intervention specifically wants to reduce). Resuscitation with room air improves outcome [Saugstad Neonatol 2008]. Furthermore, hyperoxia on NICU admission is strongly associated with adverse outcome [Kapadia J Pediatr 2013].
→ consider to start resusciation with room air
→ consider to monitor SpO_2 and titrate oxygen according to SpO_2-target [Dawson et al. Pediatrics 2010])

Prevent Hypocapnia
Hypocapnia results in cerebral artery vasoconstriction and may aggravate secondary energy failure. Hypocapnia, specifically minimum pCO2 and cumulative duration with pCO2<35mmHg were associated with adverse outcome [Pappas et al J Pediatr 2011].
→ consider to monitor tcCO2 or blood gases

Prevent Hypoglycemia:
Hypoglycemia is common following asphyxia because glycogen stores were exhausted during anaerobic metabolism. Hypoglycemia may aggravate secondary energy failure [Vanucci &Vanucci Sem Neonatol 2001].
→ consider to monitor blood glucose levels regularly

Prevent Hyperthermia
In controls of the NICHD hypothermia trial, hyperthermia was associated with adverse outcome [Laptook Pediatrics 2008].
→ consider to monitor body temperature and prevent body temperature above 37.5°C

Monitor for Seizures
The duration of seizure activity is associated with MRI brain injury score [Rooij Pediatrics 2011].
→ consider to monitor for seizures and to treat (at least) clinically apparent seizures

Prevent Undernutrition during in and out patient care

In a small RCT hyperalimentation of infants following neonatal brain injury improved head circumference growth and axonal diameter of corticospinal tracts [Dabydeen Pediatrics 2008].
→ consider to monitor nutritional intakes and to advice parents for time post-discharge

7 Study procedures and examination methods

7.1 Description of study visits and treatment

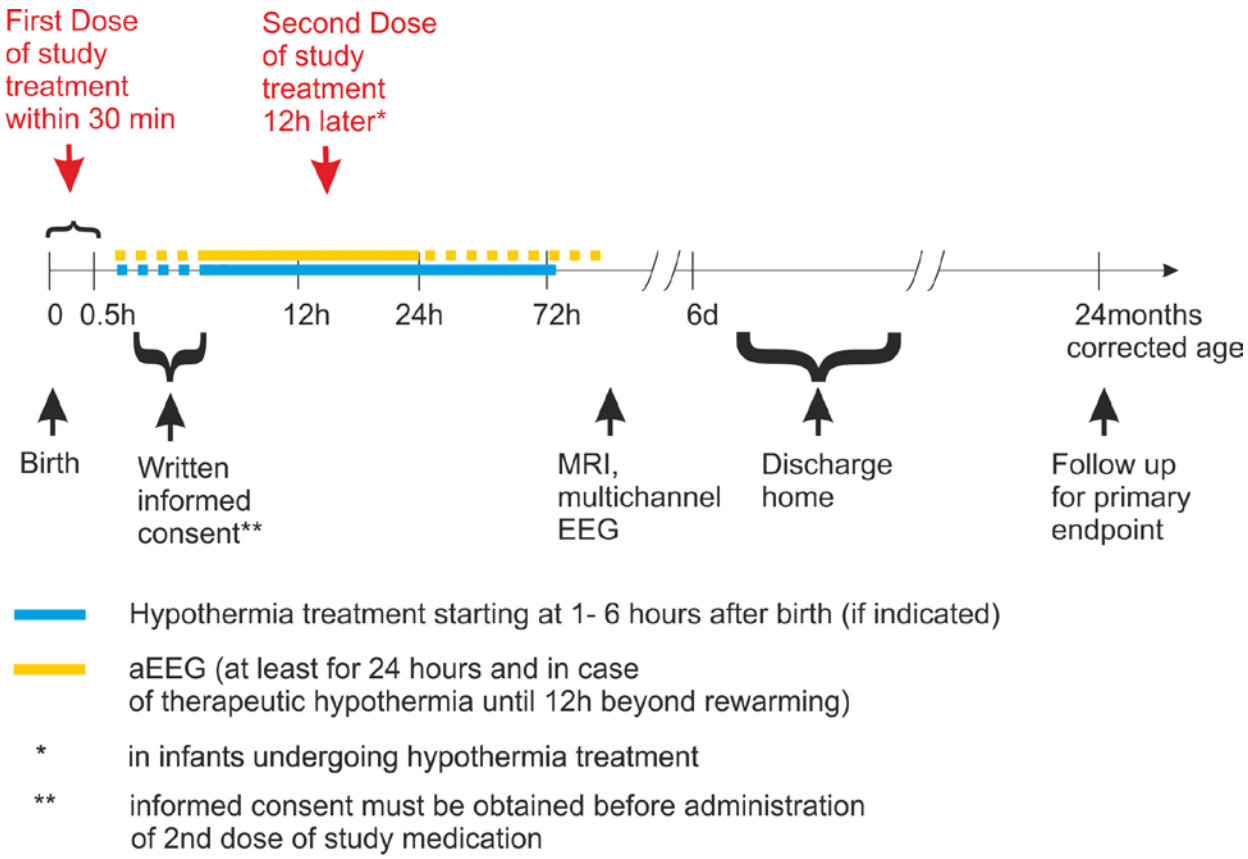


Figure 3: Description of study visits (does not include pharmacokinetics, ultrasound, biomarker sampling, and discharge assessments, etc. which are detailed in section 7.3)

Definition: for the purpose of the ALBINO study, the 'official' date and time of birth documented assigned to the child by the obstetrician/midwife will be documented. (Preferably this should be the moment of complete delivery of the child out of the womb).

7.2 Description of study process for individual patient

The following flow chart describes the study process for the individual patient.

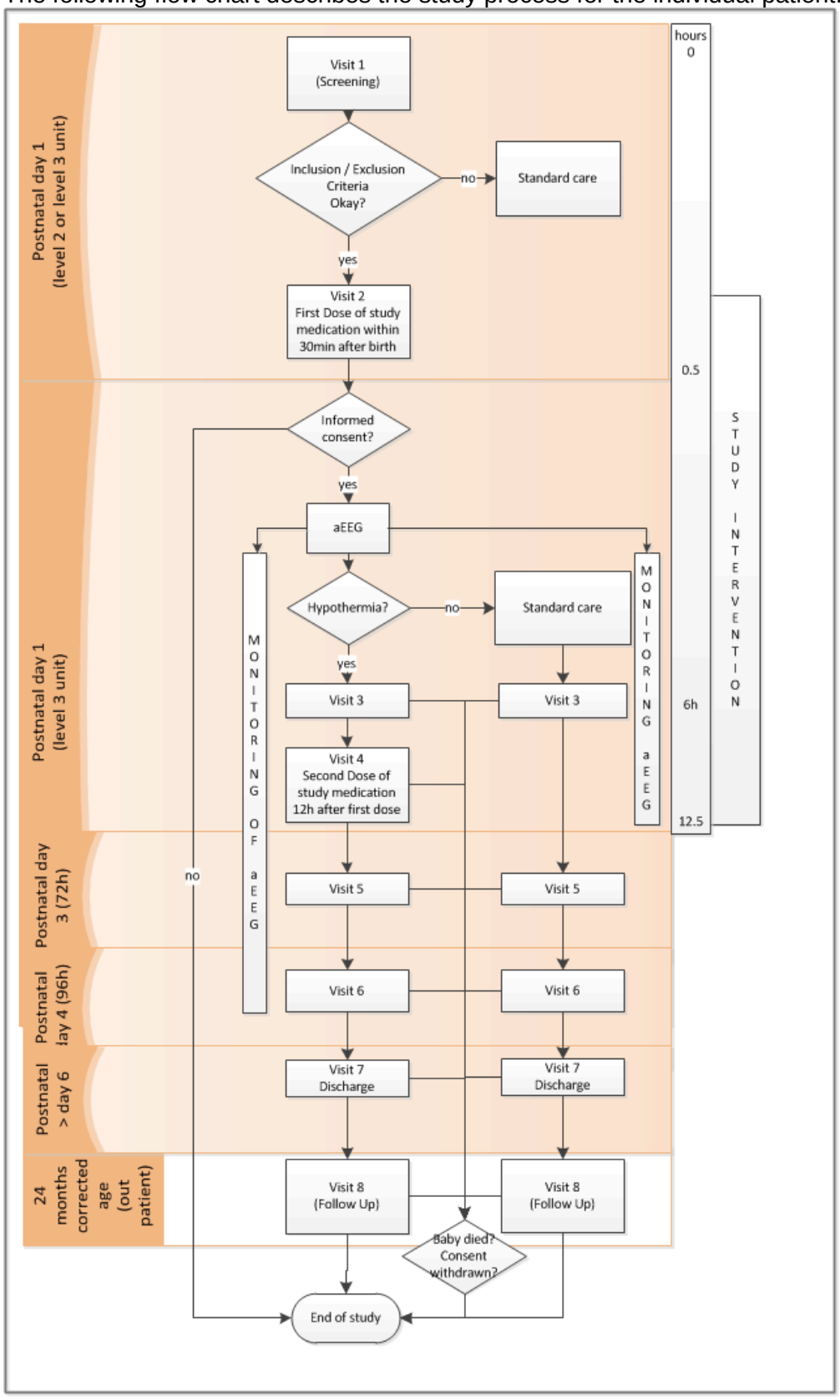


Figure 4: Flow chart of individual study process.

7.3 Study-related tests and examinations

Examinations will be done and documented according to the scheme in table 1.

Table 1: Study-related tests and examinations

Point(s) of time / Action	Screening	In the study						Follow-up	End of study
Visit	1	2	3	4	5	6	7	8	
Postnatal Hour / Time Point	0.1-0.25	0.1-0.5	1-6	12	72	96-168 (24-240)	Discharge	24 m	
Patient characteristics									
Inclusion criteria	•								
Exclusion criteria	•								
Informed consent			•						
Maternal Data			•						
Infant Data			•						
Baseline Data			•						
Medical history since discharge / family and socioeconomic data								•	
Standard Clinical Intervention									
Therapeutic hypothermia (33.5°C) (if clinically indicated, starts at <6h, for 72h, with slow rewarming)			continuously						
Study Intervention									
Randomisation		•							
Study Medication (Allopurinol/Placebo) *2nd dose only if on hypothermia tx.		• 1st dose		• 2nd dose*					
Assessment of Surrogate Markers									
Thompson Score a) before hypothermia (i.e., <6h) and b) at 84-108h (or before discharge – whichever comes first) in those not undergoing hypothermia, and at 12-36 h post re-warming in those undergoing hypothermia			•			•			
aEEG* continuously to start as soon as possible after birth until 24h in those not undergoing hypothermia, and until 12h post re-warming in those undergoing hypothermia			continuously						
Magnetic Resonance Imaging* - conventional - diffusion imaging with ADC map - if possible DTI, proton-MRS, SWI, PCA and ASL						•			
Multichannel EEG*						•			
Brain injury (by ultrasound)*			• (day 1)		• (day 3)	• (day 5)			

Point(s) of time / Action	Screening	In the study						Follow-up	End of study
Visit	1	2	3	4	5	6	7	8	
Postnatal Hour / Time Point	0.1-0.25	0.1-0.5	1-6	12	72	96-168 (24-240)	Discharge	24 m	
Assessment of Surrogate Markers		*(continued from previous page)*							
Peroxidation products * † (Biochemical Biomarkers 1)		• (Cord blood)	• (plasma 2h±0.5h & 1st urine)	• (urine at ~24h)		• (urine at ~84h)			
S100B and inflammasome mediated cytokines * † (Biochemical Biomarkers 2)			• (at 4h ±2h)	• (at 24h ±6h)		•			
Outcome Assessment									
Bayley III (cognitive, language, and motor composite scores)								•	
Cerebral Palsy and GMFCS (according to SCPE)								•	
Pharmacokinetics and Safety Assessment									
Serum concentrations of Allopurinol/Mannitol* † (individualized sampling at pre-defined intervals – always at time of clinically indicated blood sampling)			•	•	•	•			
Adverse events		throughout the study							
Concomitant Medications			•	•	•	•	•	•	
End of hospitalisation / study									
date of discharge							•		
multi-organ dysfunction							•		
Date and reason for end of study (no consent, consent withdrawn, follow-up completed, lost to follow-up, death)									• •

* *Centralized assessment and data documentation for all study centers*

† *Selected centers only*

Table 2: Centralized assessment and data documentation

Form	**documented by**
Magnetic resonance Imaging and Cerebral Ultrasound	Utrecht
aEEG	Wien
Multichannel EEG	Helsinki
Peroxidation Products	Valencia
S100B and Inflammasome	Dresden
Pharmacokinetics (Serum concentrations Allo- and Oxypurinol as well as Xanthine and Hypoxanthine)	NN

7.3.1 Assessment of Thompson Score for classification of HIE severity

Background:
The Thompson score is an internationally used scoring system to classify HIE severity [Thompson Acta Paediatr 1997].

Indication:
The assessment of the Thompson score (or at least components thereof) is clinically indicated for HIE severity classification and for assessment of need for therapeutic hypothermia.

Timing:
For the ALBINO trial a Thompson score should be documented:
a) at 2-6 h after birth and before initiation of therapeutic hypothermia.
and
b) at 84-108 h after birth (or before discharge – whichever comes first) in those not undergoing hypothermia, and at 12-36 h post re-warming in those undergoing hypothermia

Procedure:
A clinical examination will be performed by a physician, which will elicit the 9 clinical signs described in the following table. Each finding will be documented in the study database for later classification of HIE severity.

Clinical Signs	0	1	2	3
Tone	normal	hypertone	hypotone	flaccid
LOC	normal	hyperalert	lethargic	comatose
Fits/Seizures	none	< 3 per day	> 2 per day	
Posture	normal	fisting, cylcing	strong distal flexion	decerebrate
Moro	normal	partial	absent	
Grasp	normal	poor	absent	
Suck	normal	poor	absent ± bites	
Respiration	normal	hyperventilation	brief apnea	IPPV (apnea)
Fontanelle	normal	bulging*, not tense	tense	

[Thompson Acta Paediatr 1997]
(*for the purpose of this study “full” was replaced by “bulging” for clarity)

7.3.2 Magnetic Resonance Imaging (MRI)

Background:
Cerebral MRI will be performed for additional outcome assessment and for identification of non-HIE abnormalities.

Indication:
Cerebral MRIs in the ALBINO study population are considered ‘clinically indicated’ by international standards.
If, according to local standards, a clinical indication for a cerebral MRI scan is not given, particularly in infants who recover very quickly and do not fulfill criteria for hypothermia treatment, local investigators may decide not to perform a MRI scan because of lack of clinical indication.

If a clinical indication for the MRI scan is not given – parents must be informed about the study-driven nature of the MRI scan including the dilemma of unexpected findings and asked for written informed consent prior to the examination.

Timing:
Cerebral MRI should be performed in all ALBINO patients preferably on day of life 4-6. MRI-scans from day of life 2-10 will be evaluated and documented. In the rare case of two MRI scans only the one with the better quality regarding the degree of severity of HIE will be documented in the database.

Procedure:
Preparation:
Infants should be prepared according to local standards. The lead institution for cerebral MRI scans in the ALBINO study (UMC Utrecht) recommends considering the following:

-Feeding 30-60min before MRI
-Use of ear muffs (e.g., http://earmuffsforkids.com)
-Use of MRI compatible electrodes / pulse oximetry
-Wrap a blanket tightly around the baby, preferably a vacuum fixation pillow (e.g., http://cfimedical.com/))

Sedation:
Infants should be sedated according to local protocols. The lead institution for cerebral MRI scans in the ALBINO study (UMC Utrecht) recommends to consider the following:

-50-60 mg/kg chloralhydrate orally

MRI-Sequences:
A detailed Exam card / SOP (separate document, not part of this protocol) will be provided by the lead institution for cerebral MRI scans in the ALBINO study (UMC Utrecht) to local contacts for MRI scans.
Sequences will include (as a minimum standard) the following:

-Axial scan protocol including:
-T1 (<2 mm slice thickness, no gap)
-T2 (<2 mm slice thickness, no gap)
-Diffusion Weighted Imaging (DWI) with ADC map

If available highly recommended additional sequences are:

- Proton Magnetic Resonance Spectroscopy (proton MRS) (long TE i.e. 272/288)
- Diffusion Tensor Imaging (DTI) (45 directions)
- Phase Contrast Angiography (PCA)
- Susceptibility Weighted Imaging (SWI)
- Arterial Spin Labeling (ASL)

Local Readings:
A) ADC-map-measurement of 4 regions of interest (left and right basal ganglia (nucleus lentiformis) and left and right thalamus) must be measured at the console where MRI is done, directly while performing the MRI (i.e., in each local center with MRI). UMC Utrecht will provide a SOP for these ADC-map-measurements (separate document – not part of this protocol). A screen capture of these measurements has to be saved together with the entire MRI examination. Following pseudonymization, the DICOM-file of the entire MRI examination will be uploaded to the central server located at UMC Utrecht (see below). ADC-map-measurements will be assessed for quality and entered into the study database by staff at UMC Utrecht.

B) If available: proton MRS should be done in a region of interest located in the central grey matter (left sided basal ganglia) and raw data as well as graphs should be transmitted as part of the DICOM-file. As above: proton MRS data will be assessed for quality and entered into the study database by staff at UMC Utrecht.
C) Local readings should be recorded by each site in the patient's file (per local standard).

MRI pseudonymisation and upload for central reading:
An ALBINO-SOP for MRI Pseudonymisation and Upload will be provided by UMC Utrecht (separate document – not part of this protocol). In short: a DICOM-file of the MRI examination will be pseudonymized with appropriate software provided by UMC Utrecht on a local computer, removing all personal identifiers (i.e., name, first name, date of birth, …) and inserting the **patient identification number** (**PIN**, referred to in section 5.1). Only the pseudonymized DICOM files will be uploaded from local PC to a server located at UMC Utrecht (http://www.xnat.org/).

Central Reading:
At the UMC Utrecht, at least two trained MRI examiners will assign the adapted Barkovich score and will assess the presence of "definite ischemia based on DWI" in parts of the brain not assessed by the modified Barkovitch score. Cerebral perfusion will be assessed on PCA and ASL sequences. Hemorrhages will be identified on T1/T2 but also on SWI sequences. Automated software will calculate unmyelinated white matter volume, myelinated white matter volume, cortical grey matter volume, central grey matter volume, brainstem volume, cerebellar volume, intracranial extracerebral cerebro-spinal fluid volume, and ventricle cerebro-spinal fluid volume. Likewise other assessments of white matter micro-structure analysis derived by diffusion tensor imaging and brain morphology will be done centrally.
An overall assessment of

7.3.3 Cerebral Ultrasound (CUS) Examination

Background:
CUS examinations will be performed for additional outcome assessment based on the resistive index (Ri) and for identification of non-HIE abnormalities.

Indication:
CUS examinations in the ALBINO study population are considered 'clinically indicated' by international standards.

Timing:
CUS examinations should be performed on day 1 (<24h after birth, mandatory), day 3 (mandatory), day 5 (not mandatory in case of early discharge home).

Procedure:
Performance of CUS:
A detailed SOP (separate document, not part of this protocol) will be provided by the lead institution for CUS in the ALBINO study (UMC Utrecht).
The following should be documented on each CUS examination:

- six coronal (C1-C6),
- five sagittal (S1-S5),
- one mastoid image and
- a Doppler examination of the anterior cerebral artery (ACA) indicating the peak-systolic and end-diastolic flow velocity in the ACA to enable calculation of the resistive index (Ri).

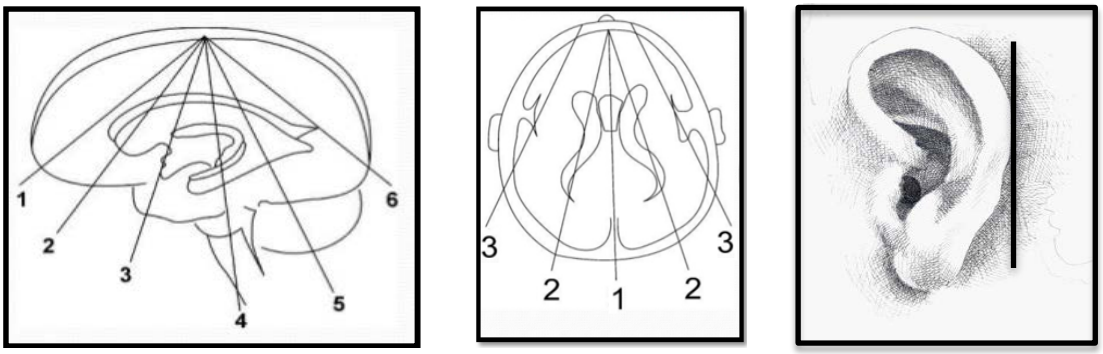


Figure 5: Demonstration of 6 coronal, 5 sagital and 1 mastoid image to be uploaded (graphs kindly provided by M. Benders)

Local Reading:
Local readings should be recorded by each site in the patient's file (per local standard).

CUS pseudonymisation and upload for central reading:
An ALBINO-SOP for CUS Pseudonymisation and Upload will be provided by UMC Utrecht (separate document – not part of this protocol). In short: Only the pseudonymized files with the **patient identification number** (**PIN**, referred to in section 5.1) as pseudonym will be uploaded from local PC to a server located at UMC Utrecht (http://www.xnat.org/).

Central Reading:
At the UMC Utrecht, at least two trained CUS examiners, after reaching consensus, will document the following in the study database:
a) peak-systolic and end-diastolic flow velocity in the ACA to calculate the Ri as potential predictor of severity of brain injury
b) screening for pathology (findings related to asphyxia such as slit-like ventricles and hyperechogenicity of the basal ganglia, of the thalami, and of the white matter; structural pathology such as ventriculomegaly, gyration abnormalities, porencephaly and cysts; and other pathology such as IVH, thalamic hemorrhage, cerebellar hemorrhage, sinus vein thrombosis, perinatal arterial ischemic stroke, and post hemorrhagic ventricular dilatation),
c) verification of normal brain anatomy

7.3.4 Amplitude integrate Electroencephalogramm (aEEG)

Background:
aEEG will be performed for additional outcome assessment.

Indication:
aEEG examinations in the ALBINO study population are considered 'clinically indicated' by international standards.

Timing:
An aEEG examinations should be performed as follows:

a) *infants eventually undergoing hypothermia:* start as soon as possible (mandatory within 6h after birth), preferably continuous recording until 12 hours post complete re-warming. (Interruptions of recordings and storing as several files possible if clinically/technically required)
b) *infants recovering quickly:* start as soon as possible (mandatory within 6h after birth), preferably continuous recording for 24 hours or until normal background pattern and sleep-wake cycling have been documented (whichever comes later). (Minimum length of aEEG-

recording 90minutes, short recordings must show normal background pattern (continuous normal voltage) and sleep/wake cycling)

Procedure:
Performance and documentation of aEEG:
A detailed SOP (separate document, not part of this protocol) will be provided by the lead institutions for aEEG/mchEEG in the ALBINO study (MU Vienna and Helsiniki University). Essentially, aEEG will be performed according to local standards. An eCRF form has to be filled for every recording to enable central interpretation of the file. This eCRF-form requests file name, start/stop-dates and times, sedation / opioid analgesia / anticonvulsive therapy (tick-list), type of aEEG device). This eCRF-form must be completed by centers at the time of upload of each pseudonymized aEEG file to MU Vienna (see below).

Local Reading:
Local readings should be recorded by each centre in the patient's file (per local standard).

aEEG pseudonymisation and upload for central reading:
Preferably aEEG files should be exported as pseudonymized .edf-files (OBM as edf-export, Nicone), and, in pseudonymized fashion only, uploaded to "lifelines iEEG server" (server located in Europe) according to details provided in the ALBINO-SOP for aEEG/mchEEG-Pseudonymisation and Upload.
'Old' aEEG technology (Brainz/Olympic) will require to email/mail pseudonymized proprietary download-files to MU Vienna according to details provided in the ALBINO-SOP for aEEG/mchEEG-Pseudonymisation and Upload.

Central Reading:
At the MU Vienna, at least two trained aEEG examiners will document the following:

aEEG will be analysed for the following epochs: 0-12h, 12-24h, 24-48h, 48-72h, and 72-96h; i.e., ensuring assessment until 12h after re-warming in infants undergoing therapeutic hypothermia.
The following a) and b) will be documented:
a) %-time of an epoch without analyzable aEEG recording
b) %-time of analyzable recording with each of the following background patterns: flat trace / burst-suppression / discontinuous low voltage / discontinuous normal voltage / continuous normal voltage)

Further outcomes to be documented:
- Most severe seizure activity in all recordings (selection of: absent / single / repetitive / status)
- Begin (date and time) and end (date and time) of all single seizure activities recorded
- Date and time of first normalization of aEEG trace: continuous normal voltage
- Date and time of first immature sleep-wake-cycling (if applicable)
- Date and time of first fully developed sleep-wake-cycling

7.3.5 multichannel Electroencephalogramm (mchEEG)

Background:
mchEEG will be performed for additional outcome assessment.

Indication:
mchEEG examinations in the ALBINO study population are considered 'clinically indicated' by international standards, they are not mandatory.

If, according to local standards, a clinical indication for a mchEEG is not given, particularly in infants who recover very quickly and do not fulfill criteria for hypothermia treatment, local investigators may decide not to perform a mchEEG because of lack of clinical indication.
If a clinical indication for mchEEG is not given – parents must be informed about the study-driven nature of the MRI scan including the dilemma of unexpected findings and asked for written informed consent prior to the examination.

Timing:
mchEEG should be performed at (3)-4-5 days postnatally but at least 12 h after rewarming. Recording should include at least one ”good” quiet sleep cycle and hence should typically last 50-90min.

Procedure:
Performance of mchEEG:
mchEEG should be performed as 20ch EEG if possible.
A detailed SOP (separate document, not part of this protocol) will be provided by the lead institution for mchEEG in the ALBINO study (University of Helsinki).
Extensive teaching material for performance of mchEEG is available at the nemo-homepage (http://www.nemo-europe.com/) – this should be highlighted to personal involved in recording.

Local Reading:
Local readings should be recorded in eCRF by each site as:
a) normal / moderately abnormal / severely abnormal background activity – and
b) seizure activity (selection 1 of 4: absent / single / repetitive / status).

mchEEG pseudonymisation and upload for central reading:
Preferably mchEEG files should be exported as pseudonymized .edf-files (OBM as edf-export, Nicone), and, in pseudonymized fashion only, uploaded to “lifelines iEEG server” (server located in Europe) according to details provided in the ALBINO-SOP for aEEG/mchEEG-Pseudonymisation and Upload.

Central Reading of mchEEG:
Activation synchrony index (ASI; Raesaenen, Neuroimage 2013) will be used for quantifying interhemispheric coordination (a.k.a. ‘asynchrony’).
Phase synchrony at oscillation and event level [Tokariev, Neuroimage 2012] as well as *amplitude correlations* [Omidvarnia A, Cerebral Cortex 2014] will be used for further graph theoretical metrics of global networking.

7.3.6 Peroxidation Products (Biomarker 1)

Background:
Peroxidation products will be measured in blood samples from selected centers as proof of principle to verify whether early allopurinol results in reduced concentrations of peroxidation products. Urine and plasma concentrations of peroxidation products reflect *oxidative damage* which is the sum of oxidative stress and anti-oxidant defence mechanisms. These examinations will be limited to special study centers – a limited number of only 100 patients should be enrolled.

Indication:
Study-driven blood and urine sample. Because of the study-driven nature – blood sampling must be coordinated with clinically indicated blood samples to avoid study-driven needle

sticks. Urine will be collected from cotton pads that are placed in the diaper – or in case there is a clinically indicated urethral catheter from this catheter.

Timing of blood and urine samples:
Plasma1: 0.5ml (if possible arterial) **cord** blood (Delivery room) (no blood loss for infant), type of blood arterial or venous or unknown and time of sampling to be documented in eCRF
Plasma2: 0.5ml arterial or venous blood (NICU) at 2h±0.5h (i.e., >1h after resuscitation and before active Hypothermia treatment starts).
Urine1: 1.5ml urine – upon arrival to the NICU (1st urine to be collected if possible before onset hypothermia – but therapeutic hypothermia should not be delayed)
Urine2: 1.5ml urine – at approx. 24 hours
Urine3: 1.5ml urine – after rewarming

Procedure:
Handling of blood samples (A detailed **ALBINO SOP for Peroxidation Products** will be provided (separate document – not part of this protocol) by the lead institution for the analysis (University Hospital LaFe, Valencia):

1. A) arterial cord blood samples (0.5 ml) are collected from double clamped cord and transferred to EDTA containing tubes.
   B) peripheral venous blood samples (0.5 ml) are collected at 2h±0.5h also in EDTA tubes. Blood samples must be coordinated with clinically indicated blood samples.
2. Samples are immediately (within <10min) centrifuged at 1500g for 10 min at room temperature to separate plasma fraction. Centrifuge should be suitable for EDTA tubes. If not, EDTA-blood has to be pipetted into Eppendorf tubes for centrifugation.
3. Pipette supernatant plasma into a clean Eppendorf containing 20 µL BHT solution (0.25 mg/ml in ethanol). These Eppendorf tubes with 20µl BHT will be provided to the sites collecting blood samples by the (University Hospital LaFe, Valencia). These will be labelled appropriately and can be shipped and kept at room temperature.
4. Centrifuged at 2500g for 15 min at room temperature to remove platelets from the plasma samples.
5. Pipette supernatant (platelet free plasma) removed and stored in labeled 1.5 ml tubes
6. Ultra-freeze at -80ºC immediately if possible – but can be kept at -20ºC for up to 72 hours and then ultra-freeze at -80ºC. (samples obtained on the week-end)

Handling of Urine samples (see also detailed **ALBINO SOP for Peroxidation Products**):

1. Place cotton pad in diaper, changed every hour and manually express or centrifuge cotton pad, as many times as necessary, until collect 1.5-2 ml (during this time preserve at 4 ºC).
2. Urine is stable and does not undergo auto-oxidation
3. Separate in 2 aliquots of 800 µl and 200 µl and pipette in adequately labelled dry tubes (standard 1,5 mL tubes)
4. No processing is needed.
5. Ultra-freeze at -80ºC immediately if possible – but can be kept at -20ºC for up to 72 hours and then ultra-freeze at -80ºC (samples obtained on the week-end)

Sample Labelling
Sample Labels should include the following

- Patient identification number (PIN, as described in section 5.1)
- Date and time of collection
- Type of sample (urine / plasma)
- (Sample purpose:) Peroxidation Products

Sample Storage

Aliquots of plasma treated with BHT solution and of urine are stored at -80ºC at site for a maximum of 4-6 months.

Sample Shipment

1. Samples are shipped in dry ice with a reputed international courier.
2. Shipment should preferably be done on Mondays!
3. Contact lab personal always before shipment:
   Angel Sánchez: +34 637 673 932 (asanchezillana@gmail.com)
   Julia Kuligowski: +34 600 201 153 (julia.kuligowski@uv.es)

Address for shipment:

GRUPO DE INVESTIGACIÓN EN PERINATOLOGÍA
TORRE A; PISO 6º
INSTITUTO DE INVESTIGACIÓN SANITARIA LA FE
AVENIDA FERNANDO ABRIL MARTORELL 106
46026 VALENCIA; SPAIN.

Concentrations determined in each sample:

1. F2-Isoprostanes (arachidonic acid)
2. Isofurans (arachidonic acid)
3. Neuroprostanes (docosahexaenoic acid)
4. Neurofurans (docosahexaenoic acid)
5. Dihomo-isoprostanes (adrenic acid)
6. Meta and Ortho – tyrosines (proteins)
7. 3-Chlor-tyrosine (inflammation)
8. 8-oxodG (damage to DNA)
9. Nitro-tyrosine (Nitrosative damage to proteins)

7.3.7 S100B and Inflammasome-mediated Cytokines (Biomarker 2)

Background:
S100B measurements in serum will be performed for additional outcome assessment. S100B is characterized well as a biomarker of brain injury. Analysis of the inflammasome-mediated cytokines IL-1a, IL-1b, IL-1Ra, IL-18, IL-18BP and IL-33 will be performed additionally to address the question if early allopurinol treatment affects inflammasome activation in HIE. Inflammasome activation is known to contribute to the development of acute brain injury.

Indication:
Study-driven blood sample. Because of the study-driven nature – blood sampling must be coordinated with clinically indicated blood samples to avoid study-driven needle stick.

Timing of blood sampling:
Serum 1: 4h±2h after birth, 0.5ml arterial or venous blood
Serum 2: 24h±6h after birth, 0.5ml arterial or venous blood

Procedure:
Handling of blood samples
(A detailed ALBINO-SOP on **Blood sampling for S100B and inflammasome-mediated cytokines** will be provided (separate document – not part of this protocol) by the lead institution for the analysis (TU Dresden):

1. Arterial or venous blood samples (0.5 ml) are collected at 4h±2h and 24h±6h in **SERUM** tubes or Eppendorf tubes without anticoagulant (**not** into EDTA !). Blood samples must be coordinated with clinically indicated blood samples.
2. Document time after birth for each serum sample
3. Allow the blood to clot at room temperature (approx. 15min).
4. Centrifuge samples at 1500g for 10 min at room temperature to separate serum fraction.
5. Pipette supernatant serum into a clean, labelled Eppendorf 1.5 ml tube
6. Ultra-freeze at -80ºC immediately if possible – but can be kept at -20ºC for up to 72 hours and then ultra-freeze at -80ºC (for samples obtained on the weekend).
7. Do **not allow freeze-thawing** cycles!

Sample Labelling
Sample Labels should include the following

- Patient identification number (PIN, as described in section 5.1)
- Date and time of collection
- (Sample purpose:) S100B/Inflammsome

Sample Storage
Serum samples are stored at -80ºC at site for a maximum of 4-6 months.

Sample Shipment
1. Samples are shipped on dry ice (-75°C) with a reputed international courier.
2. Shipment should preferably be done on Mondays!
3. Contact lab always before shipment (via phone or eMail):
   Stefan Winkler: +49-351-45818552, stefan.winkler@uniklinikum-dresden.de
4. Give courier tracking number to Stefan Winkler: +49-351-45818552,
   stefan.winkler@uniklinikum-dresden.de

Sample Measurement
Biomarkers are analyzed using a multiplex immunoassay. To avoid analyte degradation, samples will be analyzed within 12 months.

Address for shipment:
Uniklinikum Dresden
Kinderklinik Haus 21
Labor Klinische Forschung
z.Hd. Stefan Winkler
Fetscherstr. 74
01307 Dresden
Germany
stefan.winkler@uniklinikum-dresden.de

Concentrations determined in each sample:

1. S100B
2. IL-1a
3. IL1b
4. IL-1RA
5. IL-18
6. IL-18BP
7. IL-33

7.3.8 Pharmacokinetics

Background:

Preliminary pharmacokinetic evaluation in previous trials of allopurinol (all from the pre-hypothermia era) have to be complemented by additional data, particularly in infants undergoing hypothermic treatment for HIE. Sparse sampling and Population-PK modelling will be applied to reduce the patient burden to a minimum.
20-25 verum and 20-25 mannitol-placebo samples per sampling interval will be sufficient. To investigate the effect of cooling on the PK of allopurinol and oxipurinol a minimum of 10 patients exposed to hypothermia are required. To allow for missed sampling, insufficient sample size, potential problems with transport of samples etc., 100 patients will be allocated to have 4-6 blood samples to allow for 20 verum samples in each time interval.

Indication:
Study-driven blood samples. Because of the study-driven nature – blood sampling must be coordinated with clinically indicated blood samples to avoid study-driven needle stick. This is possible because infants are only allocated to time intervals for sampling (instead of exact time points).

Procedure:
(A detailed ALBINO-SOP on **Pharmacokinetics** will be provided (separate document – not part of this protocol) by the lead institution for the pharmacokinetics (ACEpharm and KU Leuven)
PK blood sampling will be limited to selected study centers which do not take part in biomarker sampling for peroxidation products.

Selection of centers involved in PK blood sampling:
A selection of centers involved in PK blood sampling will be done based on availability of staff and equipment.

Sampling strategy and handling
Samples are to be collected during 'time intervals' (in relation to the onset of administration of study medication) *with exact registration* of the time of sampling.

Notification of sampling intervals
Notification whether a given patient belongs to Group A or B will be provided within the study medication package.

Non-hypothermia, 5 samples/patient
Group A_{NH} = 15-60 min, 1.5-4 h, 8-12 h, 18-24 h, 60-72 h
Group B_{NH} = 15-60 min, 1.5-4 h, 8-12 h, 36-48 h, 96-168 h

Hypothermia, 6 samples/patient
Group A_H = 15-60 min, 1.5-4 h, troughlevel t=12 h, *13-14h*, 18-24 h, 60-72 h
Group B_H = 15-60 min, 1.5-4 h, troughlevel t=12 h, *13-14h*, 36-48 h, 96-168 h

Sample handling:
1. Heparinized tubes,
2. each blood sample should contain 0.25 ml to ensure that at least 0.1 ml of plasma is available for analysis
3. arterial samples are encouraged (if an arterial line is available)
4. sample collected should be on ice immediately afterwards,
5. centrifuge (3000 rpm for 10 minutes),
6. Pipette supernatant serum into a clean, labelled Eppendorf 1.5 ml tube
7. Ultra-freeze at -80ºC immediately if possible – but can be kept at -20ºC for up to 72 hours and then ultra-freeze at -80ºC (for samples obtained on the weekend).

Sample Labelling

Sample Labels should include the following

- Patient identification number (PIN, as described in section 5.1)
- Date and time of collection
- (Sample purpose:) PK-analysis
- Sample Number: '1' - '5' in non-hypothermia infants / '1' - '6' in hypothermia infants

Sample storage:
All samples should be stored at -80°C

Sample shipment:
Shipment should be structured and organized in line with GCP/GLP guidelines to ensure quality throughout the process.
Ship on dry ice according to ALBINO-SOP on **Pharmacokinetics** (separate document not part of this protocol).

Address for Shipment:
Address for Shipment according to ALBINO-SOP on **Pharmacokinetics**.

Sample analysis:
The analytical facility will be un-blinded to treatment allocation (after signing an appropriate confidentiality agreement), while maintaining blinding of treatment allocation in all clinical centers.
Sample analysis (measurement of allopurinol, oxypurinol, xanthine, hypoxanthine, and uric acid concentrations in patients allocated to verum, and mannitol concentrations in patients allocated to placebo) will be done in line with GLP-guidelines according to internal SOPs and standards after appropriate validation procedures in a GLP-certified laboratory (name and address to be determined - according to ALBINO-SOP on **Pharmacokinetics)**.

PK analysis:
The population PK model will be calculated in collaboration of KU Leuven and the pharmacy at UMC Utrecht. Earlier publications on allopurinol pharmacokinetics in neonates will be taken into account [van Bel, Pediatrics 1998 and van Kesteren, Ther Drug Monit 2006]. This approach will also facilitate the use of a TDM 2006 dataset to cross validate the model.

Pre-defined plasma concentration target range:
To turn the PK analysis into a validation of the dosing regimen used, or to suggest changes in the dosing regimen, the following minimum target area under the concentration curve (AUC) for both allopurinol and oxipurinol should be reached in more than two thirds (>66%) of patients analysed:

Based on the lowest individual values observed during previous studies of allopurinol in neonates, i.e., for allopurinol ~5mg/L at 2h and ~2mg/L at 12h [van Bel Pediatrics 1998] and of ~7mg/L at 2h and <1mg/L at 12hours [van Kesteren TDM 2006] the target minimum allopurinol concentration AUC should be 43.5mg/L*h between 0-12hours. Based on the lowest individual values observed for oxyprinol [van Kesteren, Ther Drug Monit 2006], i.e., ~0.5mg/L at 2h and ~3.5mg/L at 12h, the target minimum oxypurinol concentration AUC should be 26.5mg/L*h. (See Figure 5, below)

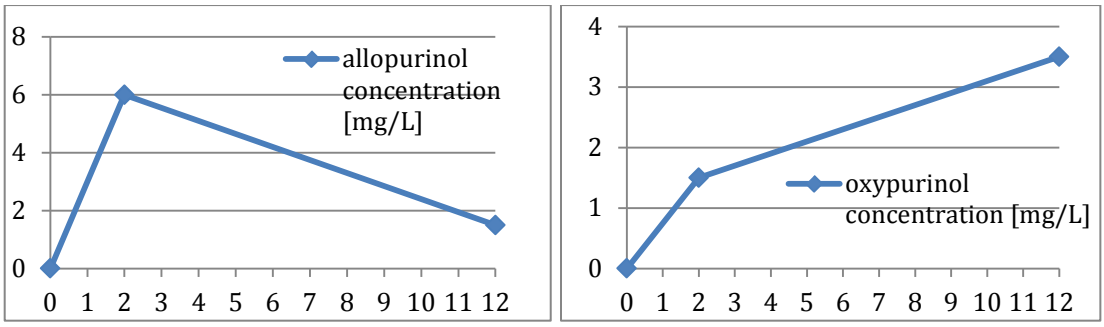


Figure 5: expected minimum concentrations of allopurinol and oxypurinol in plasma based on previous studies [van Bel Pediatrics 1998; van Kesteren, Ther Drug Monit 2006].

7.3.9 Standardized assessment of long-term outcome at 24 months of age (required for the primary outcome)

Background:
The long-term neurocognitive development of infants following perinatal asphyxia and hypoxic-ischemic encephalopathy determines their ability to lead their lives independently without assistance. This outcome was considered to be most meaningful for the patients and their parents and was hence selected as primary outcome of the ALBINO study. See also section *9.2.1 Primary Endpoint* for detailed justification and description of analysis.

Indication:
In the context of the ALBINO study this will be a study-driven examination in most settings.

Timing:
The standardized assessment should be performed at 24 months of age with a permissible window of 23-25 months of age. Because only term and near-term infants will be enrolled – correction for prematurity is not required for scheduling the examination. However, as per Bayley test instructions, the 'corrected' age will be determined before embarking into the test for correct assignment of start and stop item sets and conversion of raw scores into index scores.

Procedure:
Details will be provided in a separate ALBINO Follow-up Manual which is not part of this protocol. In short, the standardized assessment at 24 months of age shall include the following:

1) Anthropometric measures
- Weight
- Head circumference
- Length or height

2) A neurological examination by an experienced paediatric neurologist
A general history and a physical and neurological examination shall be used to determine the presence of cerebral palsy. Cerebral palsy will be diagnosed if the child has a non-progressive motor impairment characterized by abnormal muscle tone and impaired range or control of movements, according to the criteria defined by the European network 'Surveillance of CP in Europe'.
The neurological status of the child shall be classified as follows:
- Normal
- Unspecific mild abnormalities

- Severely abnormal, e.g., cerebral palsy

If 'severely abnormal' applies, the form of severe neurological impairment should be further classified as:

- Unilateral spastic CP
- Bilateral spastic CP
- Ataxic CP
- Dyskinetic CP
- No CP, other severe abnormality
  If other severe abnormality, please specify: _____

Missing values for assessment of CP

Missing values for presence or absence of cerebral palsy because parents refuse the assessment at the study site will be imputed for assessment of the primary outcome as follows:

CP will be imputed as present if

- the family paediatrician/doctor/health professional caring for the child or the parents reports the diagnosis of CP or describe a non-progressive motor impairment characterized by abnormal muscle tone and impaired range or control of movements

CP will be imputed as absent if

- the family paediatrician/doctor/health professional caring for the child or the parents denies the diagnosis of CP and report 'normal' motor development

Any such imputation will be reported in the final report and the scientific publication.

3) Classification of the Gross Motor Function using the Gross Motor Function Classification System (developed by Palisano et al.)

Following more detailed instructions in the ALBINO Follow-up manual, the most appropriate GMFCS level, that best describes the motor achievements of the child should be assigned (even if the child does not have CP).

0 Walks 10 steps independently
I Sits, hands free for play and creeps/crawls on hands and knees, pulls to stand; cruises or walks with hands held
II Uses hands for sitting support; creeps on stomach or crawls; may cruise/pull to stand
III Sits with external support for lower trunk; rolls; creeps on stomach
IV Good head control in supported sitting; can roll to supine; may roll to prone (but does not creep)
V Unable to maintain anti-gravity head and trunk postures in prone or sitting; little

A score sheet for GMFCS-assessment is provided in the Investigator Site File, this score sheet must be completed and signed and becomes source data that should be entered into the patient's files.

4) Short assessment of motor milestones

For right hand:

- None of the below apply (e.g., no hand control (e.g., because of severe CP))
- Grasp with fist
- Sissors grasp
- Pincer grasp
- Not assessed

For left hand:

- None of the below apply (e.g., no hand control (e.g., because of severe CP))
- Grasp with fist
- Sissors grasp

- Pincer grasp
- Not assessed

Stand/Walk/Run

- None of the below apply (e.g., no leg control (e.g., because of severe CP))
- Pulls up to stand, and stands while being held
- Walks freely without problems
- Runs without problems and free standing up from squat
- Not assessed

Speech

- None of the below apply (e.g., no speech (e.g., because of tracheostomy))
- Vocalizes
- Chains of syllables ('wawawa')
- Doubles syllables ('mamam')
- Uses Mum, Dad and one more word correctly
- 2-word-sentences, points to several parts of the body, and recognizes pictures
- Not assessed

5) The Bayley Scales of Infant and Toddler Development (3rd edition) in the following referred to as Bayley III

To determine neurodevelopmental outcome in a standardized fashion, all components of the Bayley Scales of Infant Development (3rd edition) will be applied from which the composite scores of the cognitive and the language subtests are selected for evaluation of cognitive / language function and the motor-composite-score is selected for assessment of motor function.

A cognitive / language / motor delay will be defined as cognitive-composite-score, a language-composite-score, or a motor-composite-score <85, respectively, for classification with respect to the primary or secondary outcomes.

The Bayley Scales of Infant and Toddler Development (3rd edition) should be applied by trained and certified personnel according to the relevant handbook.

The following data will be documented for three composite scores:

- Lowest item set applied (based on basal/entry rules)
- Raw score
- Scaled Score

Because of the importance of uniform application of the rules for performance of the Bayley-III test, pseudonymized Bayley-III-score sheets (after removal blackening of all personal identifiers and insertion of the **patient identification number** (**PIN**, described in section 5.1)) have to be scanned and sent to the Sponsor for verification at:

kialbino@med.uni-tuebingen.de

Language barriers:

Children whose primary language differs from the local official language should be assessed in the presence of a suitable interpreter, unless the examiner is fluent in the child's primary language. If an interpreter is needed, inform the interpreter to translate instructions as closely as possible, and not to repeat instructions unless permitted by the examiner.

Missing values:

- Children who are tested with the cognitive or language subtests of the Bayley test but reach a score below the lower margin, should have a cognitive-composite and a language-composite-score assigned below the lower margin as detailed in the ALBINO Follow-up Manual.

- If a child's severe impairments preclude testing, the child should be categorized as "not successfully tested because of severe neurodevelopmental impairments", the cognitive-composite-score and the language-composite score should be imputed according to a pre-defined scheme (please refer to the ALBINO Follow-up Manual).
- For children who cannot be tested because of severe visual impairment or because of profound hearing loss that cannot be corrected with a hearing aid. These children should be categorized as "not successfully tested because of visual / heraring impairment" according to the ALBINO Follow-up Manual and the visual/hearing impairment should be documented (see below).
- For children who cannot be tested because of behavioural problems, Bayley testing should be attempted again on another visit. If persistent behavioural problems preclude Bayley testing, these children should be categorized as "not successfully tested because of behavioural problems" according to the ALBINO Follow-up Manual.
- If the parents refuse the assessment at the study center, any other assessment of neurocognitive and motor development has to be documented in the patient's charts and in the eCRF (please refer to the ALBINO Follow-Up Manual). Cognitive- and language-composite-scores will be imputed as follows:

  A score ">85" will be imputed if
  - a different cognitive test has been performed elsewhere and scored higher than 1SD below the mean
  - the family paediatrician/doctor/health professional caring for the child or the parents rate the infant as "normal"

  A score "<85" will be imputed if
  - a different cognitive test has been performed elsewhere and scored lower than 1SD below the mean
  - the family paediatrician/doctor/health professional caring for the child or the parents rate the infant as "delayed" or "impaired".

  Any such imputation will be described in the final report and the scientific publication.

6) Assessment for Severe Visual and Severe hearing Impairments

"*Severe visual impairment*" should be documented if the best corrected vision in the better eye yields a visual acuity less than 6/60 m (20/200 ft) according to relevant doctor's reports / discharge summary.
"*Severe hearing impairment*" is defined as need for hearing aid or cochlear implant.

Any clinical suspicion of previously undiagnosed visual or hearing problems during the ALBINO follow-up visit requires a referral to an eye specialist or an ENT-specialists / paedaudiologists.

7) Assessment of persisting seizures or persisting need for anticonvulsive therapy

Seizure activity and need for anticonvulsive therapy should be documented as a selection of the following:

- "Seizure during the last 3 months despite anticonvulsive therapy."
- "No seizures during the last 6 months but on anticonvulsive therapy for any time during the last 6 months."
- "No seizures during the last 6 months and no anticonvulsive therapy for the last 6 months (but child had received anticonvulsive therapy any time after discharge from neonatal hospital admission)."
- "Child never received anticonvulsive therapy since discharge from neonatal hospital admission."

8) Documentation of the family situation and the socioeconomic status

To enable the verification that unintended differences in family situation or socioeconomic status may impact on results of the long-term follow-up assessment, the family situation should be classified as follows:

- Child lives with at least 1 biological parent in a two parent family
- Child lives with 1 biological parent in a single parent family
- Child lives with foster / adoptive parents or a relative
- Child lives in institutional care
- Other: _____

The socioeconomic status of the child's family should be classified as follows according to the highest level of the primary caregiver's education / formal schooling:

- 9 years or less
- At least 10 years (high school graduate)
- College or university graduate or skilled craftsmanship or similar

Measures for Cohort Maintenance:

A. Obtain the following information prior to patient discharge:
- Home address, email address, and phone numbers, in particular cell phone numbers of parents.
- Verbal consent (to be documented in the ISF) to be contacted through social media – e.g., facebook.
- Work address and phone number of parents. Name and contact information of 2 relatives (e.g., grandparents) or close friends of the family.
- Name, address and phone number of the family physician or pediatrician

B. **Maintain periodic contact** with the family, e.g. by making 1-year and 2-year birthday telephone calls. Some centres may have other scheduled clinic visits between discharge and 23 - 25 months corrected age. Verify the above contact information with the parents at each visit.

C. **Remind the parents at each interim clinic visit of the importance of the 23 - 25 months follow-up assessment.**

D. Send the ALBINO birthday card in the respective national language.

Continued efforts to assess long-term development

If, despite best efforts, a centre cannot complete an assessment by the end of the 23-25 month window, attempts to locate and test the child should continue. From a methodological viewpoint it is much better to have completed a late assessment than to have no assessment at all, consequently investigators should not stop the effort once the 25 month time point is reached. Note that the Bayley III test can be administered until the child has reached 42 months of age.

Home visits:

If parents are unable to attend the ALBINO follow-up in the clinic, an attempt should be made to assess the child in her/his home environment.

Telephone Interview:

If parents are unable to bring the child for a follow-up visit to a suitable clinic, and home visits are not possible, an attempt should be made to obtain as much information as possible through a telephone interview with the parents and if possible the local pediatrician.

Verification of doctor's reports and discharge summaries

Parents should be asked to bring doctor's reports and discharge summaries to the follow-up appointment and these should be screened for important findings related to the primary and secondary outcome measures.

Documentation:
For all examinations and tests, the actual date of assessment and the source of information (local Pediatric Neurology Department, external Pediatric Neurology Department, Family Pediatrician, Parents, other) have to be recorded separately in the eCRF.

7.4 Individual end of study

Study will be completed for each patient after follow-up at 2 years of age or following death before 2 years of age.

7.5 Individual preterm end of study

For the individual patient: Parents can withdraw their baby at any time from the study. These parents must then be approached and asked for permission for follow-up and analysis of safety data. If this permission is given, these latter patients will be maintained in the study and their outcome will be included in the intention-to-treat analysis, however, no further dose of study medication is administered and no further blood sample will be taken. If permission for follow-up and analysis of safety data is not given, documentation must end at the time of withdrawal of consent. Information collected up to this point must be maintained in the database.

7.6 Individual premature discontinuation of treatment

For the individual patient: Treatment may be terminated for safety concerns by the attending physician together with the local principal investigator or according to the wish of the patient's parents at any time. The reasons for premature discontinuation of treatment have to be documented in the study data base. These patients will be maintained in the study and their outcome will be included in the intention-to-treat analysis.

7.7 Premature discontinuation of the study

For a study centre: A study centre not following the protocol or failing to recruit patients may be closed prematurely. All infants already recruited at that centre will be maintained in the study and their outcome will be included in the intention-to-treat analysis.

For the whole study: (a) for efficacy: an interim analysis of efficacy is not intended because the first follow-up data will become available after the end of the recruitment period of 24 months. (b) for safety: any complication occurring during the care of an infant enrolled in this trial will have to be reported immediately to the coordinating investigator according to ICH-GCP guidelines and national and European regulations. These will be included in a) regular safety reports to the data monitoring and safety committee, who will continuously keep track of the incidence of such events in both study groups, and also in b) the annual safety reports to the regulatory authorities and ethics committees.
The trial will be stopped by the coordinating investigator on the advice of the data monitoring and safety committee if the risk-benefit ratio of the intervention (i.e., allopurinol) is significantly changed based on new published data becoming available. The trial will also be stopped in case major complications at least potentially related to the intervention occur more frequently in the treatment group as assessed by the DMC according to the DMC charter.

7.8 Treatment and follow-up beyond the end of the study intervention

All medical therapy and the timing of discharge home will be determined by the local attending neonatologist according to standard care guidelines of the respective center.

After discharge home, all patients will be treated with standard treatment (if any treatment is required at all) and continued care is in the responsibility of the family pediatrician in cooperation with the local study center.
Follow-up is provided by the local study center according to the local standards, e.g., in the hospital of the Sponsor the first visit in the follow-up clinic is usually scheduled at four

months corrected age and further follow-up is provided according to the needs of the patient and family.
The follow-up visit at 24 months corrected age with standardized neurological examination and neuro-developmental assessment is scheduled according to European standards.

Further standard follow-up will be provided by the Pediatrician according to national and local standards and additional specialized neurodevelopmental / pulmonary / cardiac / ... follow-up will be provided by the local study center if required.

8 Safety

8.1 Possible undesirable effects of treatment

Because of the high pH of Allopurinol PFI after reconstitution, administration requires a secure intravenous access.

Intra-arterial administration is strictly prohibited !

Study medication can be administered via umbilical **venous** catheter (UVC) – but care has to be taken that:

1) the UVC is in the "emergency position" (i.e. in the "venous duct", i.e. introduced only to 5-6cm below the skin) and
2) blood can be easily aspirated from the UVC.

Early replacement of the UVC is strongly recommended (e.g. by a peripherally inserted central venous catheter) – and reasons for leaving UVC in place have to be documented (i.e., poor peripheral perfusion / very sick baby) in the patient's charts.

In case study medication was administered via an UVC, an abdominal ultrasound verifying normal perfusion through the portal vein has to be documented after UVC was retrieved (e.g. at latest at day 3). If correct placement of the UVC in the right atrium had been documented prior to study drug administration by ultrasound or X-ray of - this abdominal ultrasound examination is at the discretion of the clinical team.

As far as reported in the previous trials of antenatal [Torrance, Pediatrics 2009, Kaandorp, Arch Dis. Childhood F&N 2014] and postnatal [vanBel, Pediatrics 1998, Gunes, Pediatr Neurol 2007, Benders, Arch Dis Child 2006] allopurinol in HIE and high dose allopurinol in other clinical settings in neonates and infants [Clancy, Pediatrics 2001], there is no evidence for severe adverse effects of allopurinol in newborn infants even at high doses.

Irritation of the vascular and peri-vascular tissue (transitional redness, swelling and tenderness) may occasionally occur and have uniformly been transient in previous studies [vanBel, Pediatrics 1998, Gunes, Pediatr Neurol 2007, Benders, Arch Dis Child 2006], but could theoretically have long-lasting cosmetic consequences.

A Cochrane review concluded: „The available data have not raised major safety concerns related to use [of allopurinol] in newborn infants." [Chaudhari, Cochrane Database Syst Rev 2008].

8.2 Ongoing safety evaluation throughout the clinical trial

An independent data monitoring and safety committee (DMC) (consisting of independent experts: paediatricians, neonatal MR-specialists and a biostatistician not involved in this trial) will be implemented to assess the progress of the clinical trial and cumulative safety data for evidence of treatment harm and benefit.
The DMC will convene by telephone conference or in person regularly during the recruitment period (as will be detailed in the **DMC Charter**). The DMC may give advice to modify or terminate the trial at any time before complete recruitment of patients if (a) new data become available that suggest that the risk/benefit ratio for the patients is significantly changed and the pursuit of the trial may harm patients, or (b) successful termination of the study becomes unfeasible because of poor recruitment.

Most importantly, the DMC will be provided with the rates of adverse events (i.e., seizures, discharge on mechanical ventilation, discharge with gavage feeds, unforeseen adverse events, etc.) in both groups for comparison. The DMC may give advice to modify or terminate the trial if analyses show a higher rate of adverse events in any of the treatment groups.
The DMC is not responsible to assess critical efficacy endpoints because first measurements of these endpoints will be performed when recruitment and study treatment have been terminated.
A detailed DMC Manual will be written before patient recruitment in agreement with all DMC members.
For time points of safety reports to DMC please refer to 9.4. safety analyses.

8.3 Adverse events and pharmacovigilance

Adverse events may occur during every clinical trial. All adverse events have to be managed with appropriate diagnostic work-up and causal and supportive treatment, ensuring that the source of harm is removed.
After stabilization of the patient's condition, documentation, assessment, classification and reporting are the next steps.

A pharmacovigilance system will be implemented governing documentation, assessment, classification and reporting of adverse events until the end of the study. Reported adverse events will appropriately be provided to the Sponsor's representative, the Principal Investigator, the Data Monitoring Committee and the relevant Ethics Committees and Regulatory authorities at latest in annual safety reports or earlier according to the rules set forth by ICH-GCP.
Details on the pharmacovigilance related definitions, classifications, and reporting procedures are described in detail in the **pharmacovigilance manual** to this study (which is a separate document, and not part of this protocol).

In the context of the ALBINO-trial, ACE Pharmaceuticals will take the responsibility for collecting and reporting of Adverse Events.

The collection and reporting of Adverse Events is described herein and in the Pharmacovigilance Manual (PVM). It is in conformity with GVP guidance on reporting of 'Adverse Reactions' in clinical trials.

Definitions

An **Adverse Event** is defined as *any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.*

An **Adverse (Drug) Reaction**, also known as the old term 'side-effect', is *any unintended response to a medicinal product related to any dose*.

An Adverse Event or an Adverse Reaction in the context of the ALBINO trial is considered ***Serious*** if the event:
- results in death,
- is life threatening (which refers to an event in which the patient was at risk of death at the time of the event),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or

- jeopardises the patient or requires intervention to prevent one of the other outcomes listed in the definition above.

A description / list of known/expected Adverse Reactions and Serious Adverse Reactions (SARs) of allopurinol is presented in the PVM.

A description / list of known/expected Adverse Events and Serious Adverse Events to be expected in asphyxiated neonates is also presented in the PVM.

Such expected AE/SAE or AR/SAR will be recorded in the eCRF, either in the "expected AE/AR form" or in the "discharge and safety form", are exempted from expedited reporting and do not require reporting in additional AE/AR-eCRF-forms.

Justification of such exemption of expedited reporting:
AEs and SAEs are extremely common in the study population and will not be related to the administration of study medication in the vast majority of AEs/SAEs (see also section 2.2.8 "No evidence of harm from allopurinol …"). Given the limited public funding of this trial and the expected very high workload of processing expedited reports for both investigators and the coordinating clinical trial it seems to be justified to record all AEs/SAEs in the eCRF (and appropriately report them to the DMC, the regulatory authorities, and the relevant ethics committees as described herein and in section 9.4 "Safety analyses") and limit expedited reporting to events described below under A)-C):

Expedited reporting is required for the following A) – C):
A) SUSAE:
A SUSAE (*Suspected Unexpected Serious Adverse Event*) is a SAE which occurs unexpectedly and for which an assessment for a potential relationship to the study medication results in the classification "probably or certainly unrelated" (i.e. if there are other explanations for the occurrence of the event that are considered more likely than the administration of study medication (allopurinol or mannitol-placebo)).
B) SUSAR:
A SUSAR is a *Suspected Unexpected Serious Adverse Reaction* which is unknown. For classification of an SAE as a SUSAR the assessment for a potential relationship to the study medication *must* have resulted in the classification "probably or certainly related" (i.e. if there aren't any other (better) explanations for the occurrence of the event than the administration of study medication (allopurinol or mannitol-placebo)).
C) Expected SAR of which the outcome is unexpected
Example: although hypersensitivity reactions to allopurinol have been reported, the outcome of these reactions is usually "resolved" after stopping of the medication and appropriate supportive treatment. Should - for example - a child die *because of* a hypersensitivity reaction, this would require expedited reporting.

Timeline of Expedited Reporting:
a) SUSAEs, SUSARs, and SARs with unexpected outcome must be reported
by the investigator within 24 hours to ACE Pharmaceuticals (contact details see PVM).

b) SUSARs and SARs with unexpected outcome must be reported
by ACE Pharmaceuticals on behalf of the Sponsor within 15 days (in case of fatal or life-threatening SUSARs within 7 days) to the clinical trial module of EudraVigilance, to all Ethics Committees involved, to the DMC and to all Investigators.

Execution of Expedited Reporting:

SUSAEs, SUSARs and SARs with unexpected outcomes must be reported by the investigator, both
a) on paper form for expedited reporting adapted from the CIOMS I form. One form is required for every single event and patient (the identity of the patient will be pseudonymized by use of the **patient identification number** (**PIN**; see section 5.1 "Screening and Recruitment" of this protocol). A completed form must be signed by the investigator or a physician member of the investigator group. The signed forms can be scanned and sent electronically. The report form is present in the Pharmacovigilance Manual.
and
b) on an additional 'unexpected-AE-form' of the eCRF

Regular Reporting
All other Adverse Reactions and Adverse Events must be recorded in the eCRF (either in the expected AE/AR-form or in additional unexpected AE-forms.
For these an **annual safety report (ASR)** will be made and sent to all competent Regulatory Authorities and all Ethics Committees involved by the Sponsor. The first report will be drafted one year after first approval of the study.
Parts of these annual safety reports will be included into the **periodic safety update report (PSUR)** for ACEPURIN by ACE.

Reporting to the DMC
Furthermore, all Adverse Reactions and all Adverse Events will be reported to the DMC at pre-defined intervals detailed in the DMC charter.

Shared pharmacovigilance obligations
To be able to meet the shared pharmacovigilance obligations, safety data have to be exchanged between ACE Pharmaceuticals, the Sponsor and the investigators of the ALBINO-trial.

8.4 Emergency Code Breaking

The **medication identification number (MIN, e.g., 1001)** enables identification of medication type (verum or placebo) as detailed under 6.2.1. This MIN will hence enable emergency code breaking.

Code Breaking must be limited to circumstances where knowledge about the treatment group is necessary for appropriate treatment of a (serious) adverse event – although such circumstances are not conceivable at the time of drafting the study protocol. Particularly there is no antidote for allopurinol.

Investigators should always make every effort for best supportive treatment of any adverse event, and in case an adverse event is thought to be at least possibly related to study medication (potential 'adverse reaction') should consider withholding a second dose of study medication (as detailed under section 7.6 'Individual preterm end of treatment'). Withholding a second dose of study medication for safety concerns as detailed under section 7.6 does ***not*** require code breaking.

Code Breaking Procedure:
Emergency envelopes will be produced by ACE pharmaceuticals and provided with study medication package.

At the study site, the emergency envelopes have to be kept separately from the study medication in the Investigator Site File (ISF) in a sealed plastic cover along with appropriate instructions.
These instructions for emergency code breaking should specify, that both the relevant National Coordinator and the coordinating investigator or his deputy coordinating investigator should be contacted before code breaking to discuss the need for unblinding the patient's treatment group assignment. Additionally, the procedures for documentation of emergency code breaking in the ISF as well as the eCRF should be described.

9 Information on statistics, evaluation

9.1 Sample Size

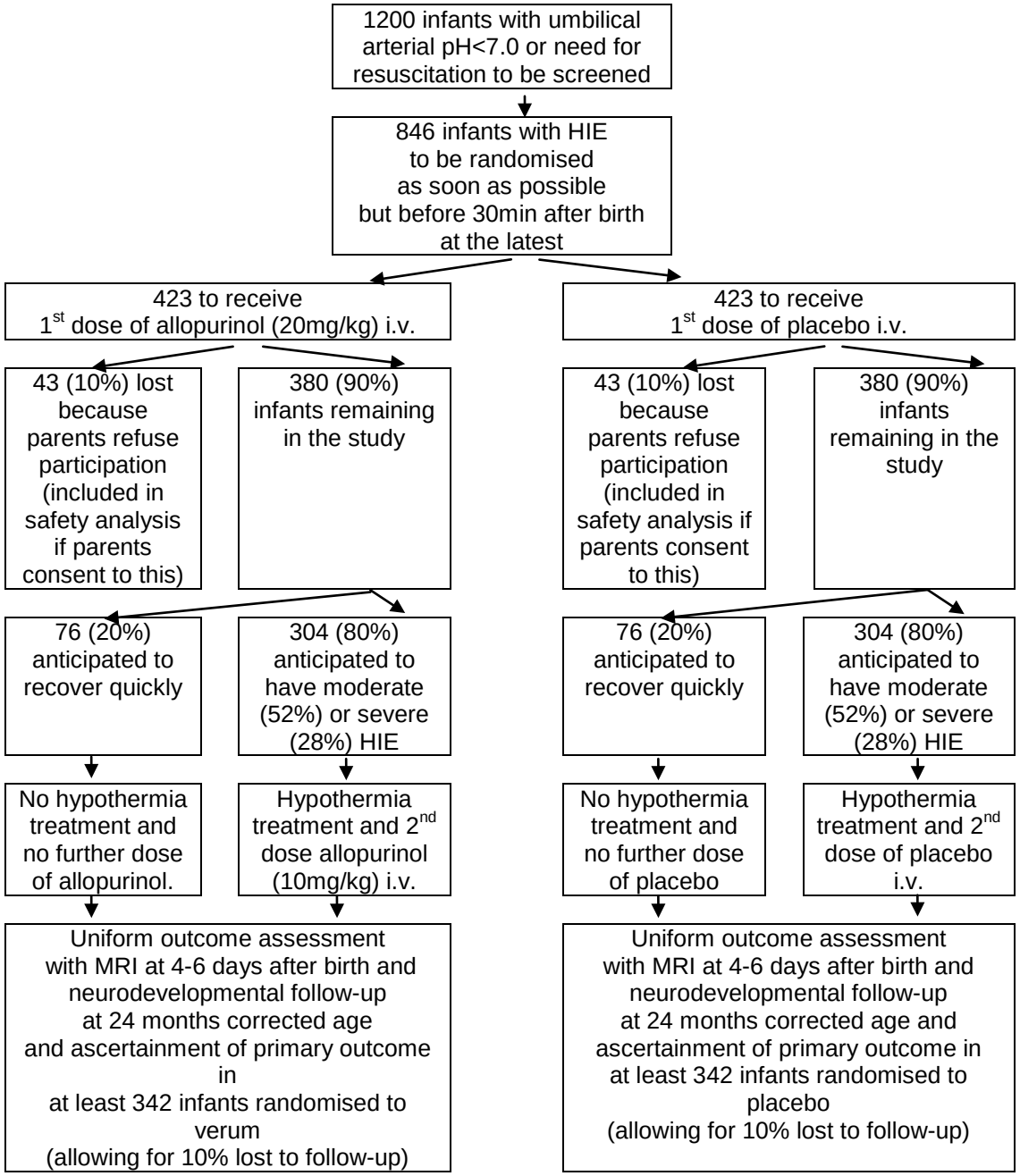
For sample size calculations the following assumptions have been made:
A) from preliminary clinical studies from the pre-therapeutic hypothermia era: a reported incidence of the combined outcome of death or severe neuro-developmental impairment (NDI) of 65% (control) and 25% (allopurinol) (i.e., RR of 2.6) for patients with moderate HIE, and no benefit of allopurinol in patients with severe HIE [Kaandorp, Arch Dis Child 2012] and in infants with mild or no HIE,
B) from clinical studies on hypothermic treatment: a reported incidence of death or severe NDI after hypothermia treatment of 32% for patients with moderate, and of 72% for those with severe HIE [Shankaran, NEJM 2005] (i.e., this incidence has to be expected in the control group),
C) a distribution of 20% mild or no HIE, 52% moderate versus 28% severe HIE in the study population (corresponding to 65% moderate versus 35% severe HIE as described by [Shankaran, NEJM 2005])
D) resulting in an expected incidence of death or severe NDI of 37% in the control group and of 27% in the allopurinol group (expecting a RR of 2.6 for moderate HIE, no effect for no, mild, and severe HIE, and a distribution of 20%, 52%, and 28% of no/mild vs. moderate vs. severe HIE),
E) alpha=0.05, power 80%, two-sided X^2-test.

Based on these assumptions, a total of 682 infants (341 per treatment group) will be required in whom the primary outcome can be ascertained. Assuming a drop-out rate of 10% for loss to follow-up, a total of 760 infants need to be enrolled with formal written consent. Assuming that 10% of parents will refuse participation after initial dose of study drug (see section 1.1.1.6.8 feasibility of recruitment and section 4. ethical considerations for details of consent procedure) 846 infants have to be randomized immediately after birth.

Reassessment of the sample size calculation:
After one third of the whole sample has been included (i.e., 280 patients) and documentation until postnatal day 5 is available, an assessment of the reliability of the expected distribution of the severity (no-mild / moderate / severe) of HIE in the study participants (refering to 9.1.c) will be made by calculating the so far reached frequencies of HIE-severity degrees and their 95%-confidence limits. These data will be reported to the steering committee.
If reasonable, a second assessment will be made when 50% (i.e., 423 patients) have been included and documented until postnatal day 5.
These data will be the basis to decide whether the sample size has to be adjusted because of discrepancies between observed and expected frequencies of the HIE-severity degrees.

Furthermore, at both time points, there will be a re-evaluation of additional new publications related to outcome following hypothermia treatment which may necessitate a change of the expected incidence of the primary outcome in the control group.

Figure 3: Anticipated Trial Flow



The apparently small proportion of children lost because of final refusal by the parents to have their child participate is taking into account the complex procedure for obtaining consent described in detail under ethical considerations (section 10.4.5).

9.2 Data Analyses

9.2.1 Primary endpoint

The **primary endpoint** will be: death or severe neurodevelopmental impairment versus survival without severe neurodevelopmental impairment at the age of two years.
Where severe neurodevelopmental impairment is defined as any of the following: cognitive or language delay defined as a cognitive-composite-score or a language-composite-score on the Bayley Scales of Infant and Toddler Development (3rd edition) < 85 and/or cerebral palsy according to SCPE criteria [SCPE Dev Med Child Neurol 2000].

Analysis of primary endpoint

Primary endpoint will be analyzed in the two treatment groups by chi-square omnibus test with three possible exclusive outcomes (healthy, death, composite outcome for impairment) and post-hoc testing in case of revealing a p-value < 0.05 within the omnibus test [Engel and Franz IJSMR 2016, accepted].

Due to the fact that there are so many centers included into this study the analysis will not be stratified for centers. This is in accordance with ICH E9 for multicenter trials if it is recognized from the start that the limited numbers of subjects per center will make it impracticable to include the center effects in the statistical model.

9.2.2 Secondary endpoints

Secondary endpoints will be analysed at 24 months corrected age between the two treatment groups.

Secondary endpoints will be:

1) Death or neurodevelopmental impairment (NDI)
   The primary endpoint will be reconstituted as dichotomised composite secondary endpoint (survival without NDI versus Death or language-composite-score < 85 or cognitive-composite-score <85 or cerebral palsy present). This will be analyzed by Cochrane-Mantel-Haenzel- X^2-Test.
2) Incidence of CP
   Incidence of CP will be analyzed by Cochrane-Mantel-Haenzel- X^2-Test.
3) GMFCS-score
   GMFCS-Score for quantification of the effects of cerebral palsy and other motor impairments (adapted from Palisano et al. [Palisano Med Child Neurol 1997]) using the ALBINO-GMFCS-score sheet (separate document not part of this protocol) will be analysed. GMFCS-score consists of six categories. Analysis will be done by using Wilcoxon-Mann-Whitney test.
4) Motor-Composite-Score (Bayley III)
   The nummerical data of the motor-composite-score will be analysed using Wilcoxon-Mann-Whitney test. The use of this test accounts for the fact that data will be cut due to lack sensitivity below 50 points.
5) Motor-Composite-Score dichotomised (Bayley III)
   The motor-composite-score will be dichotomised at the cut-off <85 versus ≥85 and analysed by Cochrane-Mantel-Haenzel- X^2-Test.
6) Cognitive-Composite-Score (cognitive subscale, Bayley III)
   The nummerical data of the cognitive-composite-score will be analysed using Wilcoxon-Mann-Whitney test. The use of this test accounts for the fact that data will be cut due to lack sensitivity below 50 points.

7) Cognitive-Composite-Score dichotomised (cognitive subscale, Bayley III)
The cognitive-composite-score will be dichotomised at the cut-off <85 versus ≥85 and analysed by Cochrane-Mantel-Haenzel- X^2-Test.
8) Language-Composite-Score (language subscale, Bayley III)
The raw nummerical data of the language-composite-score will be analysed using Wilcoxon-Mann-Whitney test. The use of this test accounts for the fact that data will be cut due to lack sensitivity below 50 points.
9) Language-Composite-Score dichotomised (language subscale, Bayley III)
The language-composite-score will be dichotomised at the cut-off <85 versus ≥85 and analysed by Cochrane-Mantel-Haenzel- X^2-Test.
10) Single Components of primary endpoint - Graph
Single components and observed combinations of the primary endpoint (healthy, death, CP, language-composite-score <85, cognitive-composite-score <85) will be displayed graphically stratified for the two treatment groups.

9.2.3 Further relevant endpoints

Concerning anthropometric measures, neurological status, milestones, seizure activity, as well as visual and hearing impairments at 2-year follow-up:

- *Progress concerning weight at follow-up*
Progress concerning weight at follow-up will be assessed as SDS-difference ($SDS_{follow\text{-}up} - SDS_{birth}$) and will be analysed using parametric or non-parametric methods as appropriate.
- *Progress concerning head circumference at follow-up*
Progress concerning head circumference at follow-up will be assessed as SDS-difference ($SDS_{follow\text{-}up} - SDS_{birth}$) and will be analyzed using parametric or non-parametric methods as appropriate.
- *Progress concerning height/length at follow-up*
Progress concerning height/length at follow-up will be assessed as SDS-difference ($SDS_{follow\text{-}up} - SDS_{birth}$) and will be analyzed using parametric or non-parametric methods as appropriate.
- *Incidence of severe visual impairment at follow-up*
The incidence of severe visual impairment, as defined in section 7.3.9 (6), will be analyzed using Cochrane Mantel Haenzel test.
- *Incidence of severe hearing impairment at follow-up*
The incidence of severe hearing impairment, as defined in section 7.3.9 (6), will be analyzed using Cochrane Mantel Haenzel test.
- Neurological Status at follow-up
The neurological status at follow-up will be assessed as selection of 1 of 3 as described in section 7.3.9 (2) and will be analysed using Proportional Odds Model.
- Milestones at follow-up
The milestones at follow-up will be assessed as selection of 1 of 5 (or 1 of 7) as described in section 7.3.9 (4) and will be analysed using Proportional Odds Model.
- The incidence of persisting seizure activity and need for anticonvulsive therapy at follow-up as described in section 7.3.9 (7) will be analyzed using using Proportional Odds Model.

Concerning MRI

- Adjusted Barkovich score (Barkovich AJNR 1995, Coskun Am J Neuroradiol 2001, van Rooij Pediatrics 2010, Alderliesten Radiology 2011)
  1. Score for basal ganglia and thalamus (0-4)
  2. Score for watershed areas (0-5)
  3. Score for PLIC (posterior limb of the internal capsule, 0-2)

4. Sum Score

All these analyses will be done using Wilcoxon-Mann-Whitney test accounting for the nature of the data as score data.

- ADC-map-measurement

  The signal intensity will be determined in 4 regions of interest (left and right basal ganglia and left and right thalamus), basal ganglia and thalamic signal intensities will be assessed as means of both sides, and, additionally the mean of all 4 ROIs will be assessed. All these analyses will be done using parametric or non-parametric methods as appropriate.

Additionally the following analyses will be performed, depending on the availability and quality of the acquisition of the scans. Parametric or non-parametric methods will be applied as appropriate.

- Network analysis by the DTI sequences: fractional anisotropy, relative anisotropy, axial diffusitivity and radial diffusitivity values.
- Hyperperfusion will be estimated by measuring the cerebral blood flow on ASL sequences.
- Levels of NAA, choline, creatinine, lactate and Lac/NAA and NAA/choline ratios, measured by proton MRS in the left basal ganglia.
- Volumes will be measured for the following structures: unmyelinated white matter volume, myelinated white matter volume, cortical grey matter volume, central grey matter volume, brainstem volume, cerebellar volume, intracranial extracerebral cerebro-spinal fluid volume, and ventricle cerebro-spinal fluid volume.

Concerning Ultrasound

- Brain injury by cerebral ultrasound including evidence of vasodilation (indicated by a resistive index <0.55 in the (preferably) anterior cerebral artery (ACA) assessed by Doppler ultrasound).

  Incidence of RI<0.55 versus ≥0.55 will be analysed by Cochrane-Mantel-Haenzel- X^2-Test.

  Additionally, absolute values of the Ri will be analysed using parametric or non-parametric methods as appropriate.

Concerning EEG

aEEG will be analysed for the following epochs: 0-12, 12-24h, 24-48h, 48-72h, and 72-96

- The most abnormal background pattern identified in an infant will be compared between treatment groups using Proportional Odds Model.
- aEEG: the dominant background pattern (selection 1 of 5: flat trace / burst-suppression / discontinuous low voltage / discontinuous normal voltage / continuous normal voltage) will be determined for each epoch as the background pattern with the highest %-time value of total analyzable recording time per epoch. The distribution of dominant background patterns per epoch will be compared between treatment groups using Proportional Odds Model.
- aEEG: seizure activity (absent, single, repetitive, status epilepticus) will be assessed . The most severe seizure activity observed throughout all recordings will be compared between treatment groups using Proportional Odds model.
- aEEG: duration from birth until onset of any appreciable sleep wake cycling on aEEG. This will be determined from date/time of birth and date/time of onset of sleep wake cycling on aEEG and analysed using parametric or non-parametric methods as appropriate.
- aEEG: duration from birth until onset of fully developed sleep wake cycling on aEEG. This will be determined from date/time of birth and date/time of onset of sleep wake cycling on aEEG and analysed using parametric or non-parametric methods as appropriate.

- aEEG: duration from birth until onset of first normalization of aEEG trace (continuous normal voltage background pattern). This will be determined from date/time of birth and date/time of onset of continuous normal voltage background pattern and analysed using parametric or non-parametric methods as appropriate..

Concerning peroxidation products (biomarkers 1)

- Concentrations of F2-Isoprostanes
- Concentration of Isofuranes
- Concentrations of neuroprostanes
- Concentrations of neurofurans
- Concentration of Dihomo-isoprostanes
- Concentrations of meta-tyrosine
- Concentrations of ortho-tyrosine
- Concentrations of chloro-tyrosine
- Concentrations of 8-oxoDG
- Concentrations of nitro-tyrosine

in plasma (cord blood and at 2h) and urine (upon arrival to NICU, at 24h and after re-warming) will be analysed using parametric or non-parametric methods as appropriate.

Concerning S100B (biomarkers 2)

- Concentrations of S100B [Roka, Acta Paediatr 2012] in plasma as biomarkers of brain injury will be compared between treatment groups at 4h and at 24h using parametric or non-parametric methods as appropriate.

9.2.4 Multivariate Analyses and Subgroup Analyses

Multivariate analyses of the primary endpoint will be done including gender, postnatal age at administration of first dose of study medication (< 15 min after birth vs. 16-30 min after birth vs. >30 min after birth), encephalopathy (mild versus moderate versus severe; where the degree of HIE severity will be derived from the Thompson Score assessed at 3-6h (before hypothermia) and the initial aEEG findings (first epoch / before hypothermia) as detailed in the Statistical Analysis Plan), and need for therapeutic hypothermia (yes versus no).
Appropriate subgroup analyses will be performed if these multivariate analyses suggest interaction between the intervention and one of these items.

9.2.5 Post hoc Analyses

According to GCP tabulation will be done of all documented data.

For the following items post hoc statistical testing will be applied if any tabulation seems to reveal differences between the two treatment groups:

Concerning Balyey III testing

- Raw scores of the Bayley III substests (cognitive subtest, receptive communication subtest, expressive communication subtest, fine motor sub test and gross motor subtest)

Concerning MRI

- Cerebral morphology by conventional MRI ((Isgum, Med Image Anal. 2015, Kooij, AJNR 2012; Kersbergen, Neuro-image 2014; van de Heuvel, Cerebral Cortex 2014,

Alderliesten, PlosOne 2015, Alderliesten Radiology 2011) including the presence and severity of bleedings assessed in the SWI sequence, the presence of thrombosis according to PCA, and other morphological abnormalities.

Additional explorative univariate and multivariate logistic regression on all MR-data may be performed at UMC Utrecht.

Concerning CUS

- Incidence of normal brain anatomy, asphyxia related abnormalities, and other pathology (as defined in section 7.3.3).

Concerning aEEG

- The distribution of background patterns in each epoch
- The most severe seizure in each epoch.
- The duration of seizure activity as assessed form date/time of first documented seizure activity to date/time of last documented seizure activity
- The duration of seizure activity as assessed as %Time of documented seizure activity related to total aEEG recording time.

Concerning mchEEG

- Parameters of spatial function measured as activation synchrony index (ASI; Raesaenen, Neuroimage 2013) will be used for assessment of quantifying interhemispheric coordination (a.k.a. 'asynchrony').
- Phase synchrony at oscillation and event level [Tokariev, Neuroimage 2012] as well as amplitude correlations [Omidvarnia A, Cerebral Cortex 2014] will be used for ssessment of graph theoretical metrics of global networking (e.g., in [Stam, Clin Neurophysiol 2012]).
- Parameters of temporal function: Both aEEG and multichannel EEG data will be taken to measure temporal correlations with detrended fluctuation analysis (DFA; [Hartley, PloS One 2012]) and metrics of power law scaling [Iyer KK, Annals of Clinical and Transnational Neurology 2014], which aim to disclose compromised temporal stability of brain function as a biomarker of adverse developmental trajectory.

Concerning inflammasome-mediated cytokine concentrations

- Concentrations of inflammasome mediated cytokines in serum at 4h and at 24h

9.2.6 Missing values

For both, the language-composite -score and the cognitive-composite-score (as part of the primary outcome variable) in the intention-to-treat analysis, missing values will be imputed by a pre-defined scheme if appropriate other information is available as outlined in section 7.3.9 (5) and finally described in the ALBINO Follow-up Manual and the statistical analysis plan. Analyses with and without imputation will be done separately.

In case of more than 10% missing values after imputation concerning the primary outcome, a worst case / best case analyses for this endpoint will be performed in the intention to treat population as sensitivity analyses and results will be included in the final report.

No imputation will be done for secondary or further relevant endpoints.

9.2.7 Validation of dosing regimen

In infants, in whom allo- / oxypurinol concentrations are available at all three sampling intervals before/ up to 12hours after the first dose, will be used to validate the dosing regimen.

The proportion of infants who reach areas under the concentration curve (AUCs) greater than the cut-off values described in section 7.3.8 Pharmacokinetics will be calcualted along with the 90% confidence intervals. (90% confidence intervals (instead of 95%-CI) will be calculated to take into account a) the uncertainty that the high concentrations observed during previous trials are necessary for the beneficial effect and b) the anticipate small samples size for PK-analysis.)

To prove with 90% certainty that the AUCs are above the cut-offs in at least two thirds (i.e., >66%) of infants, at least 21 of 25 infants analysed (or at least 18 of 20 infants analysed) have to show AUCs above the cut-off.
If it can not be shown with 90% certainty that at least one AUC (for either allo- or oxypurinol) is higher than the cut-offs in at least two thrids of patients, the dose of the study medication will be increased based on the PK-model obtained from the analysis of all samples.

9.3 Analyses Populations

All analyses will be based on the **ITT-population**.
The ITT-population consists of all patients included into the study for whom written parental consent was obtained.

Additionally the following analyses populations will be defined:

Safety population
The safety population consists of all patients included into the study.

Per-Protocol population (this section is still preliminary)
The PP-Population consists of all patients of the ITT-population without major protocol deviations. The following list of major protocol deviations will lead to an exclusion from the PP-population:

- administration of the study medication more than 45min after birth
- deviation of actual dose of study medication by more than 10% from intended dose (20mg/kg for 1st dose and 10mg/kg for second dose)

Pre-defined Subgroups
Multivariate analyses of the primary endpoint will be done including gender, postnatal age at administration of first dose of study medication (< 15 min after birth vs. 16-30 min after birth vs. >30 min after birth), encephalopathy (mild versus moderate versus severe), and need for therapeutic hypothermia (yes versus no). Appropriate subgroup analyses will be performed if these multivariate analyses suggest interaction between the intervention and one of these items.

9.4 Safety Analyses

Annual safety reports will be prepared according to the state of the art and sent to regulatory authorities and ethics committees.

Safety Analyses and reporting to the DMC will be prepared after a certain number of patients have reached 44 weeks postmenstrual age.
Due to the very vulnerable patients included, safety reporting to the DMC will be done very close-meshed in the beginning of the study, becoming wider-meshed later on.
Therefore, safety reports will be prepared and sent to the DMC after 10, 30, 50 patients have reached 44 weeks postmenstrual age and further on after 100, 200, 400 and 600 patients have reached this age.

Safety parameters will be:

- Rate of moderate and severe HIE
- Lowest pH, lowest PCO_2 and lowest blood glucose, as well as highest PCO_2, highest PO_2, highest base deficit and highest lactate documented in clinically indicated, reliable blood gas analyses during the following time intervals: 0.5-6h after birth, 6-12h after birth, 12-24h after birth independent of the source of blood (venous or arterial or capillary).
- Concentrations of creatine-kinase (CK), lactate dehydrogenase (LDH), alanine amino transferase (ALT), aspartate amino transferase (AST) at 24h (+/-6h) after birth
- Patency of and absence of thrombus in the umbilical portal vein at discharge (after removal of a umbilical venous catheter) in case study medication was administered through an umbilical venous catheter.
- Incidence of respiratory failure before discharge (defined as Oxygenation Index (calculated as ((FiO_2 [%]* Mean Airway Pressure [cmH2O))/PaO_2 [mmHg]) > 5).
- Incidence of circulatory failure before discharge (defined as need for inotropes or vasopressors).
- Incidence of transient renal failure before discharge (defined as oligo-/anuria (<1ml/kg/h) for more than 24h).
- Incidence of persistent renal failure before discharge (defined as the need for renal replacement therapy).
- Incidence of hepatic failure before discharge (defined as PTT>50sec or INR>2).
- Incidence of multi-organ dysfunction before discharge (defined as more than 2 of the above defined single organ failures)
- Most severe clinical seizure activity before discharge
- Need for anticonvulsive treatment before discharge
- Discharge on mechanical ventilation
- Discharge with gavage feeding
- Incidence of all AEs and SAEs reported

The analysis of these safety parameters will be described in detail in the DMC-manual, which is a separate document and not part of this protocol.

Details of the reporting will be determined in a **DMC-Charter** and have to be approved by the DMC.

9.5 Interim analysis

An interim analysis of efficacy is not intended because the first follow-up data will become available after the end of the recruitment period of 30 months.

9.6 Final analyses

All analyses will be predefined in a statistical analysis plan (SAP) written before unblinding of the data after end of study and completion of data monitoring.
Only the analysis of the primary outcome variable in the intention-to-treat (ITT) population will be considered confirmatory. All other analyses including the analysis of the primary

outcome variable in the PP-population and all analyses concerning secondary endpoints will be considered exploratory.

10 Ethical issues, data protection, quality assurance, insurance

10.1 ICH/GCP guidelines, the Helsinki Declaration, legal provisions

The Helsinki Declaration shall be applied to the clinical trial, as well as Good Clinical Practice (GCP) for conducting clinical trials of medicinal products within the European Community, in its current version.
This is a scientific clinical study on an investigational medicinal product; the German Medicines Act (AMG) §40 is applicable in Germany and appropriate National laws in other European countries, respectively.

10.2 Assessment and approval of the protocol by the responsible ethics committees and regulatory authorities

Prior to patient enrollment, the protocol must be approved by the leading Ethics Commission of the Universitätsklinikum Dresden which is responsible for the PI.
Prior to patient enrollment at any study site, the protocol and the study site must be approved by the relevant ethics committees responsible for the respective local investigators and study site.

Likewise, the relevant competent national regulatory authorities must approve the consent procedure and the whole study before the first patient can be recruited at a given study centre.

10.3 Handling of additions/changes to the protocol

In order to ensure comparable conditions and faultless data evaluation, changes to the protocol are not planned. In some exceptional cases, however, this could become necessary. Any additions and changes made to the protocol have to be submitted to the appropriate Ethics Committees and regulatory authorities for review. Changes to protocol procedures (amendments) require a specification of reasons and must be signed by an authorised signature for the respective protocol; the amendments are then considered part of the protocol. Substantial changes, in particular with regard to patients' health interests, require a new decision from the appropriate Ethics Committees and regulatory authorities.

10.4 Ethical issues related to ALBINO including informed consent procedures

The ALBINO study is a blinded randomised controlled study of an investigational medicinal product in human newborn infants at high risk of permanent brain injury because of perinatal hypoxia/ischemia.
This research involves the most vulnerable patient population: being unable to give consent by themselves and at risk of a) being exposed to multiple co-medications (and hence potential drug interactions and adverse events) during the study and b) potential long-term consequences from adverse effects.
Finally, the ALBINO trial will be placebo controlled, i.e., half of the study participants do not even have a potential benefit from participation in the study.

10.4.1 Justification of the ALBINO study in newborn infants

The primary objective of the ALBINO project is to evaluate safety and efficacy of allopurinol for the reduction of death or severe disability in newborn infants after perinatal hypoxic ischemic brain injury.

Despite efforts to optimize perinatal care about 1-4/1000 life born term infants suffer from HIE, and despite improvements in care of the affected newborn infant (including hypothermia treatment), still 40% of infants with moderate or severe HIE will die or suffer from severe and life-long neurodevelopmental impairment.
Without doubt, **there is need for additional (pharmacological) neuroprotection.**

Preclinical data described in detail in section 1.3 suggest benefit from allopurinol treatment and preliminary clinical data in human neonates even suggest long-term benefits at very low risk of serious side-effects. Nevertheless, this preclinical and preliminary clinical data must be verified in a large well-designed and adequately powered pragmatic trial to finally prove or disprove safety and efficacy.
A recent Cochrane systematic review on postnatal allopurinol to reduce brain damage came to exactly this conclusion: "*The available data are not sufficient to determine whether allopurinol has clinically important benefits for newborn infants with hypoxic-ischemic encephalopathy and, therefore, larger trials are needed. Such trials could assess allopurinol as an adjunct to therapeutic hypothermia in infants with moderate and severe encephalopathy and should be designed to exclude clinically important effects on mortality and adverse long-term neurodevelopmental outcomes.*" [Chaudhari Cochrane Database Syst Rev 2008]. Furthermore, benefits may become apparent even in infants with mild HIE.
If safety and efficacy can be proven, an additional, very likely cost-effective neuroprotective intervention will help to further reduce the burden of HIE for future infants.

Because the study addresses a disorder that ultimately only occurs in the newborn population, and because the vulnerability of the developing brain, the inadequate antioxidant mechanisms in the newborn and the consequences of injury are **uniquely different from adult brain injury**, the study must be performed in the newborn population.
Likewise, because this newborn population (particularly if treated with hypothermia treatment) has particular features of drug distribution, metabolism, and elimination, the safety of the drug for newborn infants can only be verified by studying newborn infants. Fatal errors have occurred in the past based on simple extrapolations of efficacy, dosage, and application route from adults to infants.

10.4.2 Favourable risk benefit ratio

Both the favourable preclinical and clinical data on allopurinol for perinatal HIE suggesting benefit, and the fact that no relevant side effects had been observed in the initial trials using the same dose of allopurinol in human neonates (see sections 2.2.7 and 2.2.8) suggest a **favourable risk/benefit ratio**, with a considerable **chance for improved long-term outcome** in those study participants who receive active study medication. Most importantly, based on all available data today, the risk from adverse effects caused by the study medication is much smaller than the risk of life-long disability or death due to the underlying condition, HIE.

10.4.3 Justification of placebo

It is well known that inadequate blinding may result in overestimation of a treatment effect. I.e, results of a non-blinded trial may suggest a treatment effect, even if there is no real beneficial effect. Adequate blinding of a pharmaceutical trial requires the use of a placebo, if the drug is administered in the presence of / or by the medical team taking care of the patient.
The benefits of using a placebo for adequate blinding of parents, care-givers and outcome assessors, must be carefully weight against the fact, that the infants in the placebo group

do not have the chance for improved outcome but are exposed to the additional burden of a clinical trial.
In case of the ALBINO study, infants in the control group are not exposed to relevant risks. Most importantly, there is experience with the placebo in the newborn population and it can be considered safe and without risk. Although additional blood samples required for the study (<2ml/kg during the entire study) and close follow-up including MRI examinations at day 4-6 will cause some burden to both, the verum and placebo group, the use of placebo seems to be justifiable given the overall **group benefit (for future newborn infants with HIE)** who will only be exposed to allopurinol if indeed there is a real and relevant treatment effect). Furthermore, empirical data suggests that sick newborn infants may benefit from participation in a randomised controlled trial even if they are in the placebo group (Schmidt, J Peditr 1999).

10.4.4 Minimal burden from study–driven examinations

Care was taken that the study-driven burden for all participants is as small as possible.
Study driven blood loss will be minimal (total amount < 2ml/kg during the initial hospitalization) because **microanalytical techniques will be applied** for pharmacokinetic testing and assessment of biochemical biomarkers and peroxidation products (using mass spectrometry) whenever feasible.
Furthermore, study-driven blood samples will be **coordinated with clinically required blood samples**. preferably collected through an existing arterial or central venous access to avoid additional pain by venipuncture.
The MRI at 4-6 days of age must not be considered study-driven but is standard of care for assessment of degree and location of brain injury after perinatal HIE. Nevertheless, the MRI will be performed using pre-MRI feeding and facilitated tucking and will not use sedative medication if possible or – at the most will use a single dose of 50mg/kg chloralhydrate (a very mild sedative) for sedation with adequate post sedation surveillance before discharge to exclude harm from study participation. If MRI is not standard of care in a given infant by local standards, additional consent must be obtained from the parents (after full information on risk including incidental / unexpected findings). For these study-driven MRI examinations, sedation beyond feeding and tucking is prohibited.
The neurodevelopmental follow-up examination at 24 months of age both, is standard of care and offers the opportunity to detect minor not yet obvious developmental delays and difficulties, which may then be addressed by adequate interventions.
In summary: According to the risk-levels set forth by the European Medicines Agency there is at the most a “minor increase over minimal risk” associated with the study-driven examinations.

10.4.5 Informed consent

The ALBINO study presents the challenge that the investigational medicinal product will most likely show optimal benefit if administered as soon as possible after secure venous access is achieved (see section 2.2). At the same time, the incidence of the condition to be studied is low (1-4/1000) and the condition can not be anticipated until it actually occurs.
If perinatal asphyxia could be anticipated, it would be prevented by the obstetricians by timely caesarean section. The opposite is the case: perinatal asphyxia resulting in HIE is always an emergency, an unforeseen event, requiring immediate attention, frequently emergency caesarean sections and immediate resuscitation of the newborn infant.

In this situation, the two major prerequisites of truly informed consent (here by the parents as the infant’s representatives) are not realised: a) there is not adequate time to explain in detail the risks and benefits of participating in the study (because the study drug should be

administered as soon as possible after i.v. access was established) and b) the parents are not in a state in which they could reflect these risk and benefits and come to a decision in the best interest of their child. The mother in particular may even be intubated and anesthetized (because of a caesarean section). Consequently, receiving fully informed and written consent in the situation of (imminent) asphyxia of the child is not possible.
Early antenatal consent could be a possibility, but because of the low incidence, this approach would require detailed information of 500-1000 mother/father pairs to finally enrol 1 patient. Based on the experience of the SUPPORT study group with antenatal consent reported by Rich (Rich, Pediatrics 2010) and assuming a representative centre with 5000 births annually, this would require roughly 5000 working hours of a medical doctor per year (more than two full time equivalents (FTE)) to enrol (<) 10 patients into the study. For 800 patients to be enrolled, that would require more than 160FTE (or >1920Person-Months or three times the total staff effort assigned to the whole ALBINO project) just for antenatal consent. Obviously, this is not possible given the limited public funding of this trial. Full antenatal information and consent would also carry the risk of frightening mothers/parents unnecessarily potentially even hindering the process of normal vaginal delivery.
However, not performing the study because no meaningful consent can be obtained in the time frame between indicating emergency delivery and study drug administration postnatally is the worst of all alternatives. This would result in potentially depriving future generations of infants with HIE and their families from the potential benefits of a medication that could considerably reduce the incidence of death or life-long neurodevelopmental impairment.

The ALBINO investigators concluded that the following would be the best way to resolve the absolute need for fully informed written consent on the one hand and the earliest possible study drug administration on the other side:

Local investigators will inform the public in the catchment areas around the recruiting hospitals using mass media about the study, inform pregnant women through information distributed in antenatal care clinics about the study, and inform all women coming to the delivery suites of the recruiting hospitals about the ongoing study. At each level of information women are asked to inform their caregivers if they would not want their infant to participate in the study should the unlikely event of perinatal asphyxia occur in their infant.

In the event of perinatal asphyxia the responsible investigator or his delegate would then ask whether the parents understand the local language (so they could be adequately informed later), whether they have been informed about the study and seek verbal consent of both parents, in case the mother is intubated just of the father, in case the mother is intubated and the father is absent by asking the responsible obstetrician/midwife whether the mother adequately understood the national language, and had expressed the wish of non-participation after having received the information.
This procedure along with the result would then be documented on a form in the infants chart, enabling STUDY DRUG ADMINISTRATION BEFORE WRITTEN CONSENT ONLY IF adequate language capabilities, adequate information could be positively verified AND IF parents had not actively declined study participation. A verbal declaration of intent to participate should also be sought from both parents whenever possible.

Full written informed consent has to be obtained after stabilization and initial drug administration in full detail for the child to remain in the study.

Such a procedure is in compliance with §30 of the Declaration of Helsinki (2014) stating: „*Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised*

representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative."

Along with the Declaration of Helsinki, national law of many European countries enable the conduct of pharmaceutical / interventional studies without prior consent in certain emergency situations. Such a consent procedure is still controversially discussed, despite the fact that emergency care societies support the development of adequate guidance for such trials.

The risk associated with this approach is, that children may become enrolled in the study despite the parents, after having reflected the risks and benefits in more detail, have major objections against study participation. This could seriously affect the trust parents need to set into the care-giving medical team to be able to cope with the situation. As a consequence of the parent's objections, the respective infants would not receive a second dose of study medication and would only be followed to the (nevertheless greatest possible) extend to which the parents are willing to consent.

This approach of information about the trial antenatally and full oral information and written informed consent after first drug administration has been **discussed in detail with parent organisations and was supported** as evident from the appended letter of the European Foundation for the Care of the Newborn Infant (EFCNI).

Obviously, as in any pharmaceutical trial in the EU, **both relevant ethics committees and competent national regulatory authorities will have to approve this consent procedure and to approve the whole study before the first patient can be recruited (Milestone 1 referenced above)**.

10.4.6 Inclusion of infants whose parents/guardians are employees of the Sponsor (or in another way dependent from the Sponsor)

Infants whose parents/guardians are employees of the Sponsor (University Hospital Tuebingen) or who are in any other way dependent from the Sponsor (which will in most cases not be apparent to the investigators before administration of the study medication) will *not* a-priori be excluded from the ALBINO study. However, the responsible investigator must ensure that those parents/guardians have the same opportunity to refuse study participation as any other parent.

10.4.7 Investigators may also be involved in patient care

Taking into account the limited availability of investigators and the limited public funding for this investigator-initiated-trial, investigators may also be part of the clinical team caring for infants screened for / enrolled in the ALBINO trial. Investigators who are also involved in clinical care must be aware that the parents/guardians may perceive difficulties in refusing study participation because they fear that this may annoy their attending doctor or the care-giving medical team.

It is the investigator's responsibility to ensure that parents/guardians are reassured that their decision on study participation will not affect the quality of care nor the affection of the care-giving medical team to their child. Furthermore, it is the investigator's responsibility to ensure that the parental decision indeed will not affect the quality of care nor the affection of the care-giving medical team to the child.

10.5 Data protection statement

The written permission for use of the personal and study-related data as well as transfer of this data in pseudonymised fashion into an electronic data base is required before start of data collection – with the exception of those limited data that will be collected from all screened patients referred to below (Section 10.6).

Only study personel bound by patient/physician confidentiality will have access to the pseudonymised personal data. Local principal investigators have to ensure that only study personel bound by patient/physician confidentiality will have access to patient data including patient identification list and screening list, which kept locally in the ISF.

The parent information will explain that for data verification purposes, authorized representatives of the Universitätsklinikum Tübingen (the sponsor), the regulatory authorities, the relevant ethics committee, or an institutional review board may require direct access to parts of the medical records relevant to the study, including the subjects' medical history. Only infants whose parents or guardians consent to these inspections according to current legislation will be enrolled into this study.

Furthermore, parents are informed about and have to consent to the fact that according to the applicable National Law referred to above (section 10.1), consent to the use of collected data is irrevocable under certain circumstances (if needed a) to proof efficacy, b) to protect the interests of the patient, c) to achieve marketing authorization) detailed in the informed consent form.

10.6 Patient identification list

Documentation of study patients in the CRF and the ISF
In the CRFs, all patients are pseudonymised by means of a patient number to be identified via the patient identification list only. The patient identification list will be kept exclusively by the investigating physician in the investigator site file (ISF).

Documentation of screened patients who were not included into the study
All screened patients based on the inclusion criterion (perinatal or umbilical blood pH <7.0 or base excess < -15 mmol/l) are documented in the database and a Screening List in the investigator site file (ISF) in pseudonymised fashion with data restricted to presence or absence of in- and exclusion criteria, other reasons leading to non-inclusion, and (if applicable vial number and dose of study medication administered during resusciatation.
Such documentation is required to enable complete documentation of the Trial Flow according to internationally accepted CONSORT criteria.

10.7 Monitoring, inspections

Monitoring for this study is provided under the supervision of the National Coordinators of the Partners of the HC2020-project ALBINO. Central monitoring of the electronic CRF will be provided by the Center for Pediatric Clinical Studies, certified by ISO9001.
Monitoring is employed primarily for the subjects' safety, as well as for quality assurance of medical procedures. The centres will be visited by the monitor on a regular basis. In accordance with the laws on data protection, the investigator's files, data collection forms, and original documents have to be made available to the monitor.
The investigators will discuss the course of the study with the monitor in an appropriate manner. Trial institutions, facilities, laboratories, and all data (including raw data and electronic CRFs) must always be available for inspection by an authority.

10.8 Insurance

Where required by National Law, insurance will be sought for all study patients:
Appropriate information on Insurance will be provided in the Parent Information Material.

11 Reporting

11.1 Annual safety reports (ASRs) to authorities

ASRs will be provided yearly to all relevant (i.e., national leading) ethics committees and the national competent authorities.

11.2 Final report

The final report for the study will be compiled by the coordinating investigator within a period of 360 days upon completion of the study, and forwarded to the ethics committees and to the national competent authorities. Furthermore, the coordinating investigator shall submit the final report for publication as soon as possible.

11.3 Publications

The scientific publications will be written by the study authors as stated in the Consortium Agreement / Consortium Plan of the ALBINO Consortium.
Publications of study results for lay persons will be written according to the Grant Agreement with the European Union.