Dexmedetomidine Use in Infants undergoing Cooling due to Neonatal Encephalopathy (DICE trial)

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Background and Introduction

Neonatal encephalopathy (NE) due hypoxia-ischemia encephalopathy (HIE) remains the leading cause of neonatal mortality and long term poor neurodevelopmental outcomes worldwide (1-3). The incidence of HIE ranges from 1 to 7 per 1000 births, with most estimates ranging between 1 to 3 per 1000. Up to 12,000 infants are affected each year in the US (2). Therapeutic Hypothermia (TH) initiated within 6 hours of life has become standard of care in developed countries and has been shown to mitigate brain damage (4-6). However, current data from the more recent cooling trials demonstrate that up to 30% of treated infants either died or had moderate to severe neurologic disabilities including long-term motor and cognitive dysfunction (7). Disabilities include cerebral palsy, mental retardation, epilepsy, and visual impairment among others adverse outcomes. No additional therapies have yet proven to be efficacious in further reducing brain injury and impairment for these high-risk infants.

Newborns with moderate-to-severe NE may also present with multiorgan failure and may develop cardiovascular instability, renal and liver insufficiency, seizures, and respiratory failure requiring mechanical ventilation. Due to their critical condition in the Neonatal Intensive Care Unit (NICU) they commonly receive drugs such as morphine for pain and sedation management and to prevent shivering (8, 9). A recent study showed that up to 64% neonates treated with TH at 125 NICUs received opioids (10). However, the efficacy of morphine for sedation during TH has not been evaluated in clinical trials and has raised concerns for short and long-term safety issues including depressed ventilation, hypotension, gastrointestinal dysmotility and importantly, possible adverse effects on neurodevelopmental outcomes (11-13). For instance, morphine has been shown to increase apoptosis in both human microglial cells and neuronal like cells of neonatal rats (14-16). Furthermore, animal studies support long-term negative effects on behavior and brain function following the administration of morphine to neonatal rats. Pups that received morphine from day 1-7 of life were smaller than saline-treated littermates and showed retarded motor development. As adults, the morphine-treated rats had impaired motor coordination, altered gait, and altered patterns of activity in an open field (17).

In summary, there is insufficient evidence to recommend routine use of morphine during TH. Further research into the treatment of pain and stress during TH and its effects on the injured brain is essential to guide the development of clinical treatment protocols.

Dexmedetomidine is a potent α_2-adrenergic receptor agonist that may be a better alternative to morphine for newborns with neonatal encephalopathy treated with TH (18). dexmedetomidine provides sedation, analgesia, and prevents shivering but does not suppress ventilation (19, 20). Importantly, there is increasing evidence that dexmedetomidine has neuroprotective properties in several injury models including ischemia-reperfusion, inflammation, anesthesia, and traumatic brain injury (21-25). In other preclinical studies of HIE insult, dexmedetomidine was shown to act as potent neuroprotector via stimulation of the α-2A adrenoreceptors (26). Exposure to dexmedetomidine following perinatal HIE reduced cortical and white matter lesion sizes and was associated with improved neurologic functional deficit (27).

Even though there are limited data on pharmacokinetics (PK), safety and efficacy of dexmedetomidine in infants with neonatal encephalopathy it has been increasingly administered in many centers (28-30).

A recent phase I, single-center, open-label study evaluated the PK and safety of dexmedetomidine administered to 7 neonates ≥36 weeks gestational age with moderate-to-severe HIE, who received a continuous dexmedetomidine infusion during TH and during the 6 h rewarming period. In cooled newborns with HIE, dexmedetomidine clearance was either comparable or lower, distribution volume was larger, and elimination half-life was longer compared to corresponding values reported for normothermic newborns without HIE. No acute adverse events were associated with dexmedetomidine treatment in this small group of infants (18).

Rationale and Justification

Management of neonatal pain and sedation often includes opioid therapy. A growing body of evidence suggests long-term harm associated with neonatal opioid exposure. Providing optimal sedation while neonates are undergoing TH may be beneficial but also presents therapeutic challenges. While there is evidence from animal models of brain injury and clinical trials in adults to support the safety and neuroprotective properties of dexmedetomidine, there are no published large clinical trials demonstrating safety and efficacy of dexmedetomidine use in neonates with HIE during treatment with TH.

This study is innovative in proposing a Phase II, 2-arm trial providing the opportunity to evaluate the use dexmedetomidine as compared to the use of morphine for sedation and pain management. We propose to confirm optimal dexmedetomidine dosing by collecting opportunistic PK data and determine safety of dexmedetomidine in this population. These data will inform a larger phase III efficacy trial.

The proposed study is also innovative in implementing the use of a battery of tests that have been shown to aid in the early detection of cerebral palsy (CP). These validated tests include the Generalized Movement Assessment (GMA) performed 1 week (±2 days) after last dose of morphine or dexmedetomidine is given even if beyond treatment period is weaned off or around discharge, whichever happens first and at 3-4 months (±1 month) of life complemented with the Hammersmith Infant Neurological Exam (HINE) performed at 3-4 months (±1 month) and at 6-9 months (±1 month) of age. Additional testing to assess motor function includes the Test of Infant Motor Performance (TIMP) performed at 3-4 months (±1 month) and Peabody Developmental Motor Skills (PDMS-2) performed at 6-9 months (±1 month). The Ages and Stages Questionnaires (ASQ-3) will also be completed by parents at the 6–9-month visit (±1 month) to screen infant in areas of communication, gross motor, fine motor, problem solving, and personal-social.

Potential Impact

Neonatal encephalopathy remains an important cause of death and adverse neurological outcome. TH has been shown to provide only partial neurodevelopmental benefit in these neonates. Because of the vulnerability of the brain following hypoxia ischemia, avoiding the use of potentially harmful drugs for

sedation such as morphine and replacing it with a potentially safer drug such as dexmedetomidine may further reduce brain injury. In the present study, we anticipate that dexmedetomidine administered for sedation during TH will be safe. In addition, we will further ascertain dexmedetomidine PK characteristics in these critically ill infants. Finally, we propose that the use of dexmedetomidine in this group of patients will demonstrate improved short and long-term outcomes. **If we establish that dexmedetomidine administration during TH in neonates with encephalopathy is safe and is associated with improved outcomes, we plan to design a Phase III research proposal using the PK data obtained from this trial to further determine efficacy of this intervention on long-term neurodevelopment at 2 years of age.**

Supportive Preliminary Data

Morphine is commonly used during therapeutic hypothermia

The use of opiates such as morphine for management of pain, sedation and shivering during TH is quite common due to the critical condition of neonates with HIE (8, 9). A recent study showed that up to 64% neonates treated with TH at 125 NICUs received opioids (10). Unpublished data from the ongoing High Dose Erythropoietin for Asphyxia and Encephalopathy (HEAL) trial demonstrates that out of 501 infants with HIE and undergoing TH entered in the study at 17 large NICU centers, 328 (65%) received morphine in the first 4 days of life (ClinicalTrials.gov NCT02811263).

However, the use of morphine for sedation during TH has raised concerns for short and long-term safety issues including depressed ventilation, hypotension, gastrointestinal dysmotility and importantly, possible adverse effects on neurodevelopmental outcomes (11-13). In neonatal rats, morphine increased apoptosis and lead to long-term negative effects on behavior and brain function including impaired motor coordination gait (14-17).

Dexmedetomidine is neuroprotective in preclinical studies

Dexmedetomidine is a highly protein-bound drug. In plasma, 94% of dexmedetomidine is bound to albumin and α_1-glycoprotein. In pre-clinical animal studies, it was found that dexmedetomidine readily crosses the blood–brain and placenta barriers (31, 32).

Wang et al studied the neuroprotective action of dexmedetomidine in a mouse traumatic brain injury model. Following controlled cortical impact, adult mice received 3 days of consecutive dexmedetomidine therapy (25 μg/kg per day). dexmedetomidine treatment decreased neurological dysfunction and brain edema, decreased post-traumatic inflammation, up-regulated tight junction protein expression, and reduced secondary blood-brain barrier damage and apoptosis. Mechanisms implicated included down-regulation of the NF-κB and NLRP3 inflammasome pathways (31).

Yin et al. assessed dexmedetomidine neuroprotective functions in early brain injury following subarachnoid hemorrhage in adult Sprague-Dawley rats. dexmedetomidine (25 μg/kg) or vehicle was administered intraperitoneally 2 h after injury.

Dexmedetomidine treatment improved neurological scores, alleviated brain edema, reduced the permeability of the blood-brain barrier and up-regulated the expression of tight junction proteins. In addition, dexmedetomidine decreased cell apoptosis at 24 h after injury. Mechanisms included suppression of the activation of the TLR4/NF-κB pathway and the NLRP3 inflammasome (33).

HIE pathophysiology in the newborn shares several of these apoptotic and inflammatory pathways, therefore dexmedetomidine may have a role for neuroprotection following HIE.

Dexmedetomidine use in a newborn piglet model of HIE undergoing hypothermia

Ezzati et al reported the PK of dexmedetomidine administered to 9 newborn piglets following HIE, in a de-escalation dose study. dexmedetomidine was administered with a loading dose of 1 μg/kg and maintenance infusion at doses from 10 down to 0.6 μg/kg/h. In piglets with plasma concentrations greater than 1 μg /l there were periods of bradycardia, hypotension, hypertension, and cardiac arrest. Clearance was reduced by 32.7% at a temperature of 33.5 degrees C and by 55.8% following hypoxia-ischemia. In this animal model dexmedetomidine clearance was reduced almost tenfold compared with adult values. Importantly, high plasma levels of dexmedetomidine were associated with cardiovascular complications (34).

Clinical studies: dexmedetomidine is neuroprotective in adult population

A recent meta-analysis assessed the **neuroprotective effects of dexmedetomidine** on ischemic brain injury in adults. Nineteen RCTs including 879 patients were included. Results showed that compared with placebo, dexmedetomidine reduced the surge of TNF-alpha, neuron-specific enolase, cortisol, and glucose as well as decreased the rise in CRP level at postoperative day one. In response to stress reaction, dexmedetomidine attenuated the stress-related increase of blood pressure, heart rate and intracranial pressure without significant effects on cerebral oxygen metabolism. The authors concluded that the use of dexmedetomidine could reduce the release of inflammatory mediators and neuroendocrine hormones as well as maintain intracranial homoeostasis, alleviating ischemic brain injury and exerting an effect on brain protection (35).

Dexmedetomidine studies in the pediatric and neonatal intensive care unit populations. Although there is no approved indication in the pediatric population, literature reports on pediatric applications of dexmedetomidine have increased in number. In 2013 the Dexdor® section on pediatric pharmacology was updated to include the use of dexmedetomidine in post-operative pediatric ICU patients (>1 month and <17 years) for up to 24 h (36). In children, dexmedetomidine appears to exhibit a level of efficacy similar to that seen in adults and to be well tolerated.

The PK of dexmedetomidine in pediatric intensive care patients was evaluated by Potts et al. (37), Wiczling et al.(38) and Greenberg et al (39). In these studies, dexmedetomidine was delivered via a combination of a short (5 or 10 min) loading dose (0.25 to 6 μg/kg/h) followed by a maintenance dose that ranged from 0.2 to

1.4 µg/kg/h. This range in dosing provided adequate sedation without adverse effects. However, the authors concluded that immature clearance in the first year of life and a higher clearance in small children would require infusion rates that change with age.

In neonates, body composition, fat distribution, and lower protein and albumin levels may contribute to a larger volume of distribution and an increased elimination half-life. Also, the immaturity of hepatic metabolism can affect dexmedetomidine PK. Particularly important to the present study, an immature or dysfunctional blood–brain barrier may cause higher cerebrospinal fluid concentrations with increased sedative and analgesic effects (40).

Dexmedetomidine efficacy and pharmacokinetics were determined in 24 term (36–44 weeks) and 18 preterm (28–36 weeks) neonates. Preterm neonates had lower weight-adjusted plasma clearance and an increased elimination half-life than term neonates. In terms of safety, 56 adverse events (AE) were reported in 26 patients (62%) but only 3 (5%) were related to dexmedetomidine. There were no serious AEs and no AEs or hemodynamic changes requiring dexmedetomidine discontinuation (28). Safety and PK of dexmedetomidine was also studied in cardiac post-operative neonatal patients (41). The authors concluded that dexmedetomidine clearance was significantly diminished in full-term newborns and increased rapidly in the first few weeks of life. In terms of safety, continuous infusions of up to 0.3 mug/kg/h in neonates and 0.75 mug/kg/h in infants were well tolerated. More recently, Zuppa et al completed a multicenter investigation of dexmedetomidine bolus and infusion in 122 neonates undergoing corrective infant cardiac surgery (42). The authors concluded that using a careful dosing strategy, dexmedetomidine resulted in low incidence and severity of adverse safety events.

Dexmedetomidine PK in neonates with HIE receiving hypothermia

Recently McAdams et al evaluated PK and safety of dexmedetomidine in a phase I, single-center, open-label study in 7 neonates ≥36 weeks' gestational age (GA) diagnosed with moderate-to-severe HIE. Infants received a continuous dexmedetomidine infusion during TH and the 6 h rewarming period. In cooled infants with HIE clearance was either comparable or lower, distribution volume was larger, and elimination half-life was longer compared to corresponding estimates previously reported for normothermic newborns without HIE as shown in table 1. Plasma concentrations in cooled newborns with HIE rose at a slower rate in the initial hours of infusion while similar steady-state levels were achieved. There were no acute adverse events associated with dexmedetomidine treatment. The authors concluded that dexmedetomidine appeared safe for neonates with HIE during TH at infusion doses up to 0.4 mug/kg/h.

However, to overcome the initial lag in rise of plasma dexmedetomidine concentration a loading dose strategy is strongly suggested and therefore included in the present study (18).

TABLE 1	McAdams (18)	Chrysostomou (28)	Greenberg (39)
Gestational Age (Wks)	39.6 ± 1.4	39.1 ± 1.6	39 (27–40)
Postnatal Age (days, wks)	1.7 ± 0.5 d	2.23 ± 1.60 wks	6.14 (0.57–29) wks
Weight	3.51 ± 0.54	3.40 ± 0.60	4.02 (2.00–6.00)
dexmedetomidine infusion rate (µg/k/h)	0.4	0.2	0.5–2.5 (max)
C_{max} (pg/mL)	537 ± 180	968 ± 1011[a]	304 ± 49
T_{max} (h)	31.0 ± 16.8	NR	End of infusion
AUC $_{0-\infty}$ (ng/mL.h)	28.4 ± 8.6	NR	NR
CL (L/h/Kg)	0.761 ± 0.155	0.907 ± 0.502	1.23 ± 0.08
MRT (h)	6.84 ± 3.20	4.26 ± 3.90	1.24 ± 0.09 *

Mean ± SD or median- NR, not reported; Cmax, maximum observed plasma concentration; Tmax, time of Cmax; AUC0–∞, area under the plasma concentration-time curve from time 0 to infinity; CL, clearance; MRT, mean residence time; ∗p < 0.05 for a 2-tailed, 2-sample t-test between reported values for normothermic, non-HIE newborns and observed values for cooled newborns with HIE.

In conclusion, studies performed in newborn infants demonstrate inter-individual variability due to factors such as body weight as well as significant maturation effects with dexmedetomidine clearance. Thus, dexmedetomidine dosing in infants with HIE undergoing TH needs further investigation. We propose to determine optimal dosing by collecting 2 opportunistic PK samples and PRN PK samples any time there is an adverse event.

Dexmedetomidine safety in the newborn

Side effects of dexmedetomidine are mainly restricted to hemodynamic alterations including hypertension, bradycardia, and hypotension secondary to pre- and postsynaptic α_2-receptor activation, which causes vasoconstriction, vasodilatation, and reflex bradycardia (43, 44). Importantly, most side effects of dexmedetomidine are related to sympatholytic effects and appear to be dose dependent and predictable in neonates (45).

In a retrospective study, O'Mara et al evaluated the effectiveness and short-term safety of dexmedetomidine infusion for sedation in 19 term neonates undergoing TH for HIE. Initiation of dexmedetomidine infusion did not negatively impact HR, mean arterial blood pressures, or cerebral saturations. dexmedetomidine use was not associated with any extubation failures or increase need for vasopressors (29).

Of importance to the present proposal is that dexmedetomidine can affect thermoregulation by affecting vasoconstriction and non-shivering thermogenesis by lipolysis, both key mechanisms present in the newborn infant. Therefore, neonates with HIE undergoing TH and receiving dexmedetomidine may be vulnerable to abnormal temperature regulation (46, 47).

In conclusion, safety of dexmedetomidine administration in this particular population of critically ill infants with multiorgan failure needs further evaluation. We will determine safety during the first 4 days of life by documenting potential adverse events such as hypotension, acute renal failure, liver failure, poor cardiac function, cardiac arrhythmias, respiratory failure, hypothermia, and seizures outside of normal range for the study population.

Potential benefits of Dexmedetomidine use for sedation

Surkov examined the impact of dexmedetomidine and other sedatives (morphine, sodium oxybutyrate, and diazepam) on the cerebral blood flow and clinical outcomes in 205 term neonates with HIE. Infants were randomized to receive dexmedetomidine (n=46) versus the control group (n=159). Patients in the dexmedetomidine cohort were extubated sooner, required lower dose of inotropes, had decreased seizure burden and lower incidence of abnormal neuroimaging (48).

Other potential benefits of using dexmedetomidine versus morphine include time to full oral feedings following cessation of TH and need for nasogastric (NG) feedings or Gastric Tube (G Tube) at time of discharge. O'Mara et al observed shorter duration of parenteral nutrition and time to full oral feedings in babies receiving dexmedetomidine for sedation during TH when compared to previously published historical patients in their NICU (29).

In summary, data from clinical dexmedetomidine studies in newborn infants is scarce but it indicates that dexmedetomidine used at the appropriate dose seems to be a safe sedative agent with a stable hemodynamics profile, no adverse cerebral influence, and possible neuroprotective effects in term infants with HIE. In the present study we aim to validate this safety profile and establish short and long term benefits by assessing neurodevelopmental outcomes for this distinct NICU population.

Purpose and Objectives

Neonatal hypoxia-ischemia encephalopathy (HIE) is the leading cause of neonatal death and poor neurodevelopmental outcomes worldwide. Despite early intervention using therapeutic hypothermia (TH), current data from recent trials demonstrate 29% of treated infants either died or had moderate to severe neurologic disabilities including long-term motor and cognitive dysfunction. No additional therapies have yet proven to be efficacious in further reducing brain injury and impairment for these high-risk infants. Furthermore, additional brain injury may be caused by concomitant use of drugs such as morphine to treat pain and sedation in this population. Morphine use in animal models has been shown to induce neuronal

apoptosis. Developing adjunctive therapies that improve outcomes in infants with HIE is an urgent, unmet public health need.

Dexmedetomidine is a potent α_2-adrenergic receptor agonist that may be a better alternative to morphine for newborns with neonatal encephalopathy treated with TH. Dexmedetomidine provides sedation, analgesia, and prevents shivering but does not suppress ventilation. Importantly, there is increasing evidence that dexmedetomidine has neuroprotective properties in several injury models including ischemia-reperfusion, inflammation, and traumatic brain injury. Even though there are limited data on pharmacokinetics (PK), safety and efficacy of dexmedetomidine in infants with neonatal encephalopathy it has been increasingly administered in many centers.

We **hypothesize** that dexmedetomidine administered as sedative therapy to infants ≥36 weeks gestational age with moderate to severe HIE undergoing TH will be safe and will be associated with improved short and long-term outcomes.

To test this hypothesis, we have designed a Phase II multicenter, randomized, safety and pharmacokinetics (PK) trial. Fifty infants (n=25 in each arm) ≥36 weeks gestational age with HIE will be randomized to receive either dexmedetomidine (1 μg/kg for loading dose followed by 0.2 to 0.5 μg/kg/h continuous infusion) or morphine (0.02-0.05 mg/kg/dose intermittent dosing q 3 hours IV PRN or as continuous infusion dose of 0.005- 0.01 mg/kg/hr). Patients will be stratified by severity of encephalopathy (moderate vs. severe).

We propose the following specific aims:

Specific Aim 1. To examine ***safety measures*** comparing infants receiving dexmedetomidine to those administered morphine. The goal of this aim is to determine whether there are risks to dexmedetomidine administration in this population of infants with neonatal encephalopathy undergoing TH. Safety will be evaluated during the first 4 days of life by documenting potential adverse events such as hypotension, hypertension, bradycardia, cardiac arrhythmias, hypothermia, and seizures outside of normal range for the study population. Other safety measures will be recorded include monitoring standard of care (SOC) labs for events such as renal failure or liver failure.

Specific Aim 2. To further determine ***dexmedetomidine pharmacokinetics*** in this population of newborns with HIE during treatment with TH. Two to four opportunistic PK samples will be obtained for each participant at time of routine laboratories. Further PRN PK samples will also be collected any time there is a serious or severe adverse event that is at least possibly related to dexmedetomidine resulting in holding or discontinuing the dexmedetomidine infusion. For this study, only dexmedetomidine levels will be measured. An additional 0.5mL blood sample will be taken in conjunction with standard of care labs during the first 96-120 hours of life; this will be 2 to 4 samples (total of 1 to 2 mL) depending on when SOC labs are drawn; and total blood volume drawn at any one time and overall will not exceed clinical limits.

Specific Aim 3. To determine whether dexmedetomidine use for sedation versus morphine is associated with ***improved short-term*** outcomes during initial hospitalization (shivering, respiratory support, time to full PO feedings, G tube/NG

feedings at discharge, seizure burden, hearing impairment, MRI); and on ***long-term neurodevelopmental outcomes***: 1) Generalized Movement Assessment (cerebral palsy assessment) performed 1 week (±2 days) after last dose of morphine or dexmedetomidine is given even if beyond treatment period is weaned off or at time of hospital discharge whichever happens sooner and at 3-4 months of life; 2) Hammersmith Infant Neurological Exam (HINE) performed at 3-4 and at 6-9 months of life; 3) Test of Infant Motor Performance (TIMP) performed at 3-4 months of life; and 4) Peabody Developmental Motor Skills (PDMS-2) performed at 6-9 months of life. The Ages and Stages Questionnaires (ASQ-3) will also be completed by parents at the 6-9 months of life visit to screen infants in areas of communication, gross motor, fine motor, problem solving, and personal-social.

Promising preliminary data show that dexmedetomidine might improve outcomes, but optimal dosing, safety, and efficacy still need to be established. We propose to confirm dexmedetomidine optimal dosing by collecting opportunistic PK data and determine safety of dexmedetomidine in this population in a phase II safety trial. These data will inform a larger phase III efficacy trial.

Study Populations

Age of Participants: Newborns at >36 weeks gestation

Sample Size:

At All Sites in Utah: 50 (received at least one dose of study drug)

Inclusion Criteria:

- Neonates ≥36 weeks' gestational age diagnosed with moderate-to-severe neonatal encephalopathy and treated with TH (target temperature 33.5°C) per unit standard of care.
- Infants requiring sedation and/or treatment to prevent discomfort or shivering during TH, shown by one of the following criteria:
    - As assessed by the Neonatal Pain, Agitation, and Sedation Scale (N-PASS) scores and/or a modified Bedside Shivering Assessment Scale; OR
    - Continuous infusion of morphine, dexmedetomidine, or another analgesic is currently being given as standard care.
- Signed informed consent document approved by the Institutional Review Board (IRB) obtained prior to randomization.

Exclusion Criteria:

- Known chromosomal anomalies
- Cyanotic congenital heart defects
- Redirection of care being considered because of moribund condition, or a decision made to withhold full support
- Maternal use of opioids or opiates (illegal use and or mothers under treatment for opioid use)

Design

Approach

This is a Phase II multicenter, randomized, safety and pharmacokinetics (PK) trial. Figure 1 depicts study design and interventions. Therapeutic hypothermia (TH) and brain MRI will be performed per standard of care, and neurodevelopmental impairment (NDI) follow up is also done as considered standard of care. Fifty infants (n=25 in each arm) ≥36 weeks GA with HIE will be randomized to receive either dexmedetomidine (1 μg/kg for loading dose followed by 0.2 to 0.5 μg/kg/h continuous infusion); a continuous morphine infusion of 0.005-0.01mg/kg/hr; or intermittent dosed morphine 0.02 mg/kg/dose PRN IV q 3 hours up to 0.05mg/kg/dose all titrated per NPASS score.

Initiation and titration of the study drugs should follow the guidelines detailed in figures 2 and 3. However, it is always up to the clinical team as to when study drug should be started and discontinued based on real-time clinical assessments. Escalation or weaning of study sedation/pain medication and/or use of other sedatives/analgesics is always at the discretion of the attending neonatologist and clinical care team.

Optional Dexmedetomidine Dose Titration

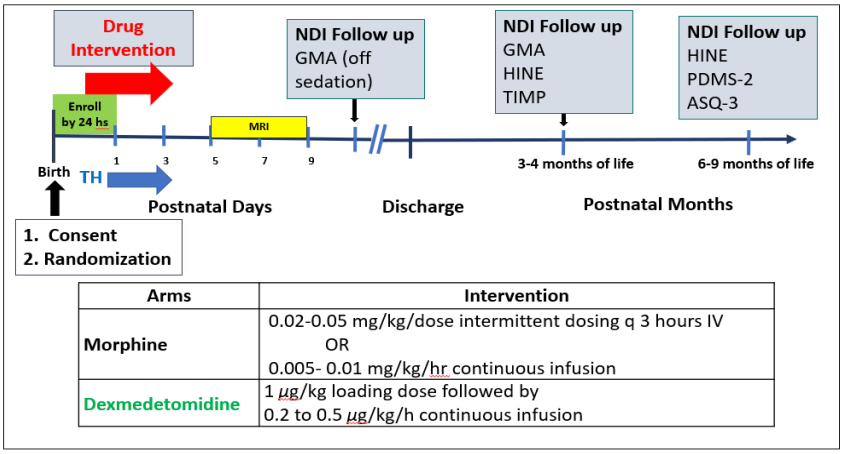
If dexmedetomidine has reached the maximum of 0.5 µg/kg/h per protocol and optimal pain/agitation scores have not been met, the protocol does NOT dictate how the infant should be treated and the attending neonatologist can choose to treat the baby per his/her discretion. Potential options for patients requiring more than 0.5 ug/kg/hr of dexmedetomidine include: ongoing dose escalation of dexmedetomidine up to a maximum of 1.0ug/kg/h continuous infusion or the neonatologist may choose to give standard of care morphine/alternative analgesic. Whatever option is chosen, it must be approved by the attending. It is important to note that the infant can continue to use study dexmedetomidine from the investigational pharmacy through the 96-hour period as needed.

Optional Morphine Dose Titration

If intermittent, PRN morphine has reached the maximum of 0.05 mg/kg/dose per protocol OR the continuous morphine has reached the maximum of 0.01 mg/kg/hr AND optimal pain/agitation scores have not been met, the protocol does NOT dictate how the infant should be treated and the attending neonatologist can choose to treat the baby per his/her discretion. Options for patients requiring beyond protocol maximum doses include SOC intermittent, PRN morphine doses OR escalation of continuous morphine per SOC.

The active treatment period for this study is from randomization through 96 hours of life. Once the infant is 96 hours of life, all subsequent treatment for pain/agitation/sedation will be given as standard of care.

FIGURE 1



Arms	Intervention
Morphine	0.02-0.05 mg/kg/dose intermittent dosing q 3 hours IV OR 0.005- 0.01 mg/kg/hr continuous infusion
Dexmedetomidine	1 µg/kg loading dose followed by 0.2 to 0.5 µg/kg/h continuous infusion

NDI (neurodevelopmental impairment); MRI (Magnetic Resonance Imaging); GMA (Generalized Movement Assessment); HINE (Hammersmith Infant Neurological Exam); TIMP (Test of Infant Motor Performance); PDMS-2 (Peabody Developmental Motor Skills); ASQ-3 (The Ages and Stages Questionnaires)

Study Sites

The study will be carried out at four hospitals in Utah:

1. University of Utah Hospital and Clinics (UUHC), Salt Lake City, Utah
2. Intermountain Medical Center (IMED), Salt Lake City, Utah
3. Primary Children's Hospital (PCH), Salt Lake City, Utah
4. Utah Valley Hospital (UVH), Provo, Utah

Study Procedures

Recruitment/Participant Identification Process:

The study team will be notified by either the charge nurse or attending neonatologist of the admitting NICUs when an eligible infant is being transported in or has been born into the facility. The study team will review with the attending neonatologist all of the inclusion/exclusion criteria prior to approaching parents for consent.

Informed Consent:

1) In the Newborn ICU where the study is being carried out or
2) In the event of a transport, consent may take place in the referring hospital/ delivery center via the transpot team and/or via econsent/facsimile/phone.

Description of the consent process(es), including the timing of consent:

There is no obligatory waiting period for the consent to be obtained (other than allowing the parents time to ask questions and discuss the study between themselves and/or other family members). There is a time frame for consent (within 24 hours of birth) for the infant to receive the investigational drug as early as possible.

NOTE: Infant is not enrolled into the study until infant is randomized.

Procedures:
After eligibility is confirmed, with approval from the Attending Care Provider, the parent or legal guardian will be approached for consent. All patients eligible for this study will be managed in the NICU.

Telephone/Electronic Consent

Newborn patients are frequently transfered to the NICUs at the University of Utah, Primary Children's Medical Center, Intermountain Medical Center, or Utah Valley Hospital, for treatment with TH. In an effort to approach for the study as soon as possible, the transport team may give the potential eligible parents or legal guardians a brochure and/or a full hard copy of the consent form, explaining that if their baby meets criteria for the DICE trial, the research team may contact them with more information.

If the transport team does not have a hard copy of the consent, the research team will send a copy of the consent via facsimile to the hospital/ delivery center where the parents/legal guardians are physically located or electronically directly to the parents if the parents have given the research team verbal permission to send it.

Phone consent for the study will be done by the research team or neonatologist on service or on call in the NICU. The study and its options will be explained to the parent(s) ensuring that they know this is a voluntary study and they are in no way obligated to participate. They will be allowed time to ask questions over the phone and will be able to read the consent document as it is discussed. If they give their consent, one parent can provide written consent either by signing the paper consent or by e-consent. Study procedures will begin only after signed consent has been received by the study team. This can be a paper consent given to the transport team, e-consent, or a copy of the signed paper consent provided to the study team by email, text, fax, etc.

Interventions

All infants admitted to the NICU at a study site with a diagnosis of HIE and undergoing TH will be screened for the study. If the infant meets eligibility criteria and the attending physician agrees to the patient's entry into the study, the parents will be approached for informed consent. Time for obtaining consent is up to 24 hours of age. TH should be done as standard of care per unit guidelines via whole body hypothermia using a servo mechanism.

Randomization Procedures

Eligible infants with informed consent will be randomized to receive either dexmedetomidine (1 μg/kg for loading dose followed by 0.2 to 0.5 μg/kg/h continuous infusion); a continuous morphine infusion of 0.005-0.01mg/kg/hr; or intermittent dosed morphine 0.02 mg/kg/dose PRN IV q 3 hours up to 0.05mg/kg/dose.

Randomization will be stratified by severity of encephalopathy. A variable permuted, block size will be used. Centralized randomization will be managed by the Data Coordinating Center at the University of Utah.

NOTE: Infant is not enrolled into the study until infant is randomized. If an infant is randomized and then does not receive at least one dose of study drug by 96 hours of life or prior to death (if occurs < 96 hours of life), this infant is not considered evaluable and will be withdrawn from the study and no further interventions or data will be collected. Infant will be replaced with another subject. We will replace subjects until we have at least 50 evaluable subjects.

Study Drug Administration

Dexmedetomidine hydrochloride injection (4 mcg/mL base) or continuous morphine sulfate (at NICU concentrations per standard of care institutional practices mg/mL) will be administered in a primed intravenous line via a computer-controlled infusion device programmed by trained NICU nurses. Morphine sulfate (at NICU concentrations per standard of care institutional practices) may also be given as bolus intermittent dosing. The initial study drug dose should be given once NPASS or BSAS scores are met after randomization and no later than 96 hours of age. There is no defined time interval between a qualifying NPASS or BSAS score and initiation of study drug. Therefore, for those infants not receiving any drug/s for pain/sedation management prior to randomization, the most recent score(s) obtained prior to the study drug initiation must be a qualifying score (NPASS ≥4 and/or BSAS ≥2). If morphine, dexmedetomidine, or another analgesic drug is already infusing continously prior to study drug initiation, no qualifying NPASS or BSAS is required in order to start study drug. Transition from ongoing continuous infusions to study drug infusion or bolus dosing will be done per attending and PI preference and can begin any time after randomization. Any open-label analgesic given during transition period will not be considered a deviation.

NOTE: Continuous dosing of either dexmedetomidine or morphine will require an IV that is not also being used for intermittent medications that require flushing before or after infusion of the intermittent medication.

Initiation and adjustment of the dexmedetomidine and morphine doses (i.e., increasing, decreasing, or holding the dose) during TH will be based on the algorithm in figures 2 and 3. To standardize clinical management during the trial, **the Neonatal Pain, Agitation, and Sedation Scale (N-PASS)** and the Bedside Shivering Assessment Scale (BSAS) scores will used prior to and during dexmedetomidine and morphine exposure to determine sedation and analgesia effectiveness of both drugs (49, 50).

The N-PASS tool uses 5 assessment criteria (crying/irritability, behavior/state, facial expression, extremities/tone, and vital signs); each criterion is graded 0, –1, or –2 for sedation and 0, 1, or 2 for pain/agitation and these two scores are calculated independently. A pain/agitation score >3 is considered to reflect significant pain or agitation, at which point supplemental sedation or analgesia therapy will be administered/escalated following the algorithm in figure 2. A sedation score of less than -2 indicates light sedation and warrants consideration for therapy weaning, a score less than -5 warrants weaning. This is shown in Table 2.

TABLE 2

N-PASS: Neonatal Pain, Agitation, & Sedation Scale

Pat Hummel MA, RNC, NNP, PNP, APN/CNP & Mary Puchalski MS, RNC, APN/CNS *(Modified for UU Research)*

Assessment	Sedation		Normal Sedation/Pain	Pain / Agitation	
Criteria	-2	-1	0/0	1	2
Crying Irritability	No cry with painful stimuli	Moans or cries minimally with painful stimuli	No sedation / No pain signs	Irritable or crying at intervals Consolable	High-pitched or silent-continuous cry Inconsolable
Behavior State	No arousal to any stimuli No spontaneous movement	Arouses minimally to stimuli Little spontaneous movement	No sedation / No pain signs Appropriate for gestational age	Restless, squirming Awakens frequently	Arching, kicking Constantly awake or Arouses minimally / no movement (not sedated)
Facial Expression	Mouth is lax No expression	Minimal expression with stimuli	No sedation / No pain signs Relaxed, Appropriate	Any pain expression intermittent	Any pain expression continual
Extremities Tone	No grasp reflex Flaccid tone	Weak grasp reflex ↓ muscle tone	No sedation / No pain signs Relaxed hands and feet, Normal tone	Intermittent clenched toes, fists or finger splay Body is not tense	Continual clenched toes, fists, or finger splay Body is tense
Vital Signs HR, RR, BP, SaO_2	No variability with stimuli Hypoventilation or apnea	< 10% variability from baseline with stimuli	No sedation / No pain signs Within baseline or normal for gestational age	↑ 10-20% from baseline SaO_2 76-85% with stimulation – quick ↑	↑ > 20% from baseline SaO_2 ≤ 75% with stimulation – slow ↑ Out of sync with vent

Pain and sedation will be assessed a minimum of every 3-4 hours ±30 min (at the time of vital signs assessments) as well as 30-60 minutes post escalation or de-escalation of medication. Escalation or weaning of study sedation/pain medication and/or use of other sedatives/analgesics is always at the discretion of the attending neonatologist. Open-label morphine will be available for rescue analgesia or to prevent shivering as per the discretion of the medical team (incidence, dose, and duration of treatment with adjunctive sedation medications will be recorded).

The BSAS is a 4-point scale that rates the severity of shivering, shown in Table 3.

TABLE 3

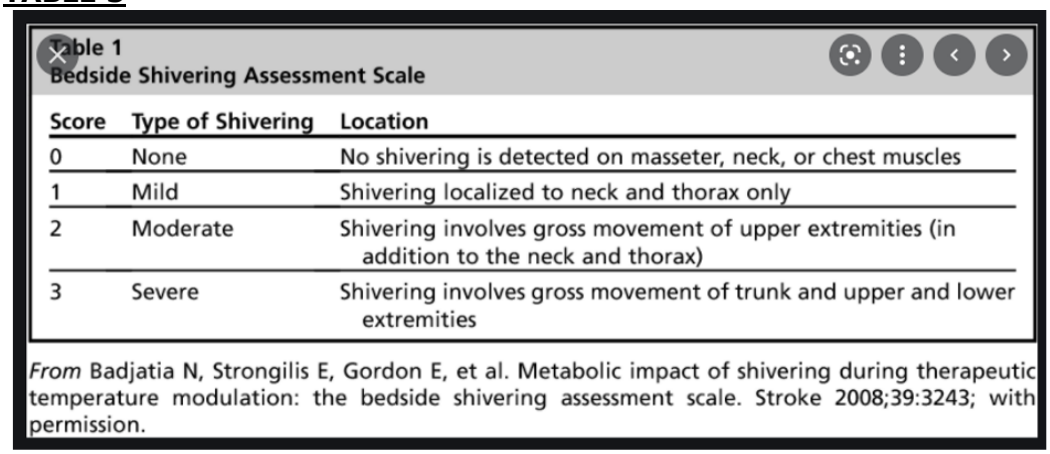


Table 1
Bedside Shivering Assessment Scale

Score	Type of Shivering	Location
0	None	No shivering is detected on masseter, neck, or chest muscles
1	Mild	Shivering localized to neck and thorax only
2	Moderate	Shivering involves gross movement of upper extremities (in addition to the neck and thorax)
3	Severe	Shivering involves gross movement of trunk and upper and lower extremities

From Badjatia N, Strongilis E, Gordon E, et al. Metabolic impact of shivering during therapeutic temperature modulation: the bedside shivering assessment scale. Stroke 2008;39:3243; with permission.

Both dexmedetomidine and morphine can be used to treat shivering and study doses can be adjusted based on the shivering score. It is important to note that non-pharmacological interventions are not used to treat shivering, so in the Figures 2 and 3, this step is skipped when treating shivering.

Figures 2 and 3 are the guidelines that will be used to determine the dosing of the study drug. Although it is highly preferred for the clinical team to use the guidelines as presented, it is always up to the clinical team as to when study drug should be started and discontinued based on real-time clinical assessments. Escalation or weaning of study sedation/pain medication and/or use of other sedatives/analgesics is always at the discretion of the attending neonatologist and clinical care team. Advancing or decreasing the rate/dosage of the study drug in different increments from the guidelines **is allowed** and not considered a deviation. Additionally, administration or escalation of a study drug dose prior to a painful/uncomfortable procedure/intervention **is allowed** and not considered a deviation. Documentation of all study drug administration and discussions by the clinical team regarding study drug administration are charted in the electronic healthcare records per unit protocol.

Study drug may be allowed to infuse longer than 96 hours of age if needed for short term clinical purposes, such as conducting the SOC MRI. Study drug can continue infusing until clinical dexmedetomidine or morphine can be ordered or, if needed short-term for a procedure, procedure is completed (without a clinical order). A clinical order will be required if the continuous study drug is needed for longer than 6 hours after the 96 hours of life (study treatment period).

FIGURE 2

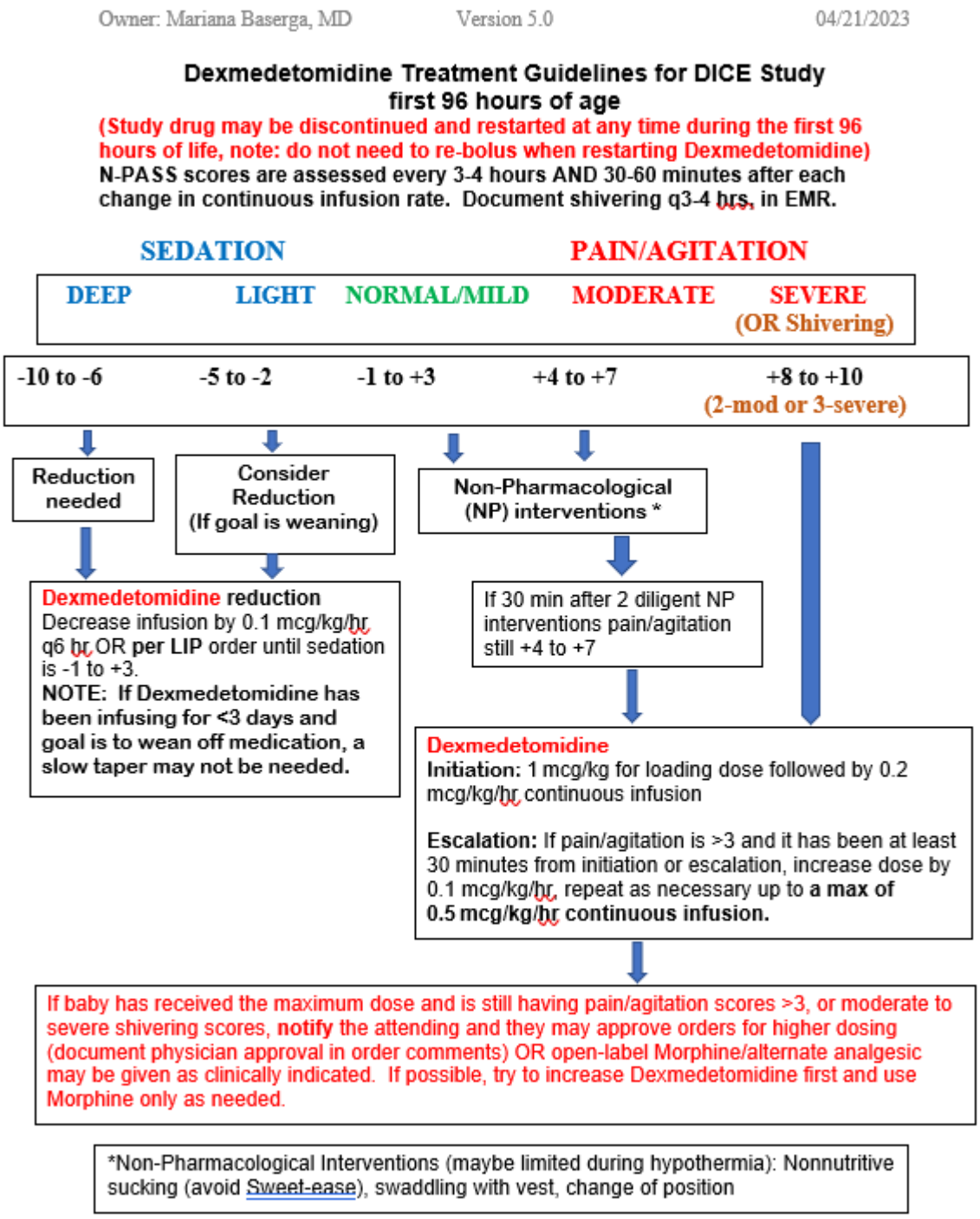

Owner: Mariana Baserga, MD
Version 5.0
04/21/2023
Dexmedetomidine Treatment Guidelines for DICE Study
first 96 hours of age
(Study drug may be discontinued and restarted at any time during the first 96 hours of life, note: do not need to re-bolus when restarting Dexmedetomidine)
N-PASS scores are assessed every 3-4 hours AND 30-60 minutes after each change in continuous infusion rate. Document shivering q3-4 hrs, in EMR.
SEDATION
PAIN/AGITATION
DEEP
LIGHT
NORMAL/MILD
MODERATE
SEVERE
(OR Shivering)
-10 to -6
-5 to -2
-1 to +3
+4 to +7
+8 to +10
(2-mod or 3-severe)
Reduction needed
Consider Reduction
(If goal is weaning)
Non-Pharmacological (NP) interventions *
Dexmedetomidine reduction
Decrease infusion by 0.1 mcg/kg/hr q6 hr OR per LIP order until sedation is -1 to +3.
NOTE: If Dexmedetomidine has been infusing for <3 days and goal is to wean off medication, a slow taper may not be needed.
If 30 min after 2 diligent NP interventions pain/agitation still +4 to +7
Dexmedetomidine
Initiation: 1 mcg/kg for loading dose followed by 0.2 mcg/kg/hr continuous infusion
Escalation: If pain/agitation is >3 and it has been at least 30 minutes from initiation or escalation, increase dose by 0.1 mcg/kg/hr, repeat as necessary up to a max of 0.5 mcg/kg/hr continuous infusion.
If baby has received the maximum dose and is still having pain/agitation scores >3, or moderate to severe shivering scores, notify the attending and they may approve orders for higher dosing (document physician approval in order comments) OR open-label Morphine/alternate analgesic may be given as clinically indicated. If possible, try to increase Dexmedetomidine first and use Morphine only as needed.
*Non-Pharmacological Interventions (maybe limited during hypothermia): Nonnutritive sucking (avoid Sweet-ease), swaddling with vest, change of position

FIGURE 3

Owner: Mariana Baserga, MD Version 5.0 04/21/2023

Morphine Treatment Guidelines for DICE Study first 96 hours of age

(Study drug may be discontinued/restarted at any time during the first 96 hours of life)

N-PASS scores are assessed every 3-4 hours AND 30-60 minutes after each dose change or with continuous infusion rate change. Document shivering q3-4 hrs in EMR.

SEDATION		PAIN/AGITATION		
DEEP	LIGHT	NORMAL/MILD	MODERATE	SEVERE (OR Shivering)
-10 to -6	-5 to -2	-1 to +3	+4 to +7	8 to +10 (2-mod or 3-severe)

Reduction needed

Consider Reduction (If goal is weaning)

Non- Pharmacological (NP) interventions*

Reduction in % of max. dose Morphine given. Please notify LIP/PI for any dose changes needed.

If after 2 diligent NP interventions and pain/agitation still +4 to +7

Morphine

IV Push Intermittent Dosing Initiation: 0.02 mg/kg/dose intermittent dosing q3 h IV PRN (may be scheduled q3 h if needed)

Escalation: If pain/agitation is >3, and it has been at least 30 minutes to 3 hours from last dose, may give another increased dose by 0.01 mg/kg/dose, repeat as necessary up to a max dose of **0.05 mg/kg/dose.**

CONTINUOUS INFUSION Initiation: 0.005 mg/kg/hr.

Escalation: If pain/agitation is >3 and it has been at least 30 minutes from initiation or escalation, increase dose by 0.002 mg/kg/hr, repeat as necessary up to a max 0.01 mg/kg/hr continuous infusion.

If baby has received the maximum PRN dose/infusion rate and is still scoring >3 with pain/agitation scores, notify the LIP/attending so an order for SOC higher PRN dosing/rate OR alternate analgesic can be written.

*Non-Pharmacological Interventions (maybe limited during hypothermia): Nonnutritive sucking (avoid Sweet-ease), swaddling with vest, change of position.

Dose Justification

Based on previous studies (see preliminary data section; table 1) in this population and standard of care practices, infants will be randomized to receive either dexmedetomidine (1 μg/kg for loading dose followed by 0.2 to 0.5 μg/kg/h continuous infusion); a continuous morphine infusion of 0.005-0.01mg/kg/hr; or intermittent dosed morphine 0.02 mg/kg/dose PRN IV q 3 hours up to 0.05mg/kg/dose (18). All are titrated based on NPASS or BSAS scores.

Outcomes and data to be collected

Primary outcome for this proposal will be safety.

Primary Safety Endpoints:

1) Cardiovascular

Adverse events will be defined as follows:

-Severe bradycardia (heart rate <60 sustained for >30 minutes)
-Severe tachycardia or other cardiac arrythmias (arrhythmia requiring treatment)
-Severe hypotension (mean blood pressure (BP) <30 or decrement in mean BP >20-30 OR receiving volume therapy >40-80 ml/kg during a 24 h period OR escalation of vasoactive inotropic score by > 30 compared to baseline)
-Severe hypertension (>2SD normal mean blood pressure and requiring treatment)

2) Temperature

-Severe hypothermia will be defined as esophageal temperature <31 C for >30 minutes

3) Increased seizure burden (>4 hours of electrographic seizure burden in the first 24 hours after birth)

Secondary outcomes will include short and long-term clinical aspects during initial hospitalization as well as during neurodevelopmental follow up.

*These **efficacy outcomes** will include:*

- **Respiratory support** (need for nasal cannula, CPAP, invasive mechanical ventilation/modality) and time to extubation
- **Incidence of shivering**
- **Time to full PO feedings** (days)
- **G tube/NG feedings at hospital discharge**
- **Duration of central venous and arterial access**
- **Neurologic exam at 7 days of life**
- **Hearing screen results**
- **Brain MRI, Head US**
- **aEEG and Video EEG**
- **Need for other medications** (antibiotics, steroids, other sedatives, or opiates) will be systematically collected

For long-term neurodevelopmental outcomes, follow up will include:

1) Generalized Movement Assessment (GMA) performed 1 week (±2 days) after sedation is weaned off or before discharge if less than 7 days: and at 3-4 months (±1 month) of life.

2) Hammersmith Infant Neurological Exam (HINE) performed at 3-4 months (±1 month) and at 6 to 9months (±1 month) of life.

3) Test of Infant Motor Performance (TIMP) performed at 3-4 months (±1 month) of life.

4) Peabody Developmental Motor Skills (PDMS-2) at 6-9 months (±1 month) of life.

5) The Ages and Stages Questionnaires (ASQ-3) will also be completed by parents at the 6-9 months (±1 month) visit.

Case report forms will be developed to collect maternal characteristics (including labor & delivery details), neonatal characteristics including NICU assessments, and primary and secondary outcomes. Neonatal data will include gestational age, birth weight, gender, Apgar scores, delivery room resuscitation, and pertinent details of the hospital course.

Procedures performed for research purposes only:

Study drug (dexmedetomidine)

PK levels of blood **(for infants randomized to and have received dexmedetomidine only)**

Statistical Methods, Data Analysis, and Interpretation

Sample size and resulting power
The primary outcome in this proposal will be safety as described on page 10. Secondary outcomes will include short and long-term clinical aspects including initial hospitalization (shivering, respiratory support, time to full oral feedings, G tube or tube feedings at discharge, seizure burden; neurologic exam at 7 days of life, MRI) as well as neurodevelopmental follow up.

For the **sample size calculation**, 50 evaluable subjects (25 in each arm) would yield 80% power to compare dexmedetomidine relative to Morphine for key safety measures using tolerance limits of 0.5 SDs for quantitative measures of organ function. This sample size is also considered adequate to evaluate neurodevelopmental outcomes explored in this proposal as secondary outcomes. Lastly, the sample size of 50 is adequate to evaluate feasibility for a potential phase III trial. For the **analysis of safety effect**, we will use an intention-to treat strategy. To compare adverse event and serious adverse event counts between treatment groups we will use a Poisson regression model with robust SEs adjusting for the severity of encephalopathy. To calculate the effect of dexmedetomidine versus morphine on developmental outcome measures, we will use 95% confidence intervals, and corresponding P values (significance, $P < .05$) using a linear regression with robust SEs, with and without adjusting for age at testing and for severity of encephalopathy at baseline. All statistical analyses will be performed by an independent biostatistician.

Methodology

Specific Aim 1- Safety

To examine safety measures comparing infants receiving dexmedetomidine with infant administered morphine. The goal of this aim is to determine whether there are risks to dexmedetomidine administration in this population of infants with HIE undergoing hypothermia.

An **adverse event** (AE) will be defined as any adverse change from the patient's baseline condition that occurred following the first administration of the study drug through the end of the study period. Safety will be evaluated during the first 4 days of life by documenting

potential adverse events such as (but not limited to) severe hypotension, severe hypertension, severe hypothermia, acute renal failure, liver failure, cardiac arrhythmias, and seizures outside of normal range for the study population.

Serious adverse events (SAE) will be defined as any event that results in: death, a life-threatening event (severe cardiac arrythmia), persistent or significant disability/ incapacity, and/or prolongs inpatient hospitalization. SAEs will be reported until 7 days following final dose of study drug during the treatment period or until hospital discharge, whichever comes first.

Sample size and resulting power

For the sample size calculation, 50 evaluable subjects (25 in each arm) would yield 80% power to compare dexmedetomidine relative to Morphine for key safety measures using tolerance limits of 0.5 SDs for quantitative measures of organ function. The sample size of 50 would be adequate to evaluate feasibility for a potential phase III trial. To compare AEs and SAEs counts between treatment groups we will use a Poisson regression model with robust SEs adjusting for the severity of encephalopathy.

Data Safety Monitoring Committee (DSMC)

An independent Data Safety and Monitoring Committee (DSMC) will monitor study progress, safety, and efficacy. The DSMC is composed by study monitors qualified by training and experience to monitor the progress of the investigation. An interim safety analysis will be performed after a total of 10 infants are enrolled.

The trial will be temporarily suspended for the initial safety look and as deemed necessary by the DSMC for any subsequent safety concerns.

Serious Adverse Events (SAEs) reporting

All SAEs will be reported to the site PI within 24 hours of learning of the event. The PI will be responsible for reporting the unanticipated adverse events 1) to the local IRB in accordance with the local IRB policies; 2) to the DSMC chair within 24 hours of receiving the report; 3) to the FDA in an IND safety report in accordance with FDA regulations; and 4) to all participating site PI's. The DSMC will determine if the adverse event changes the risk to study subjects. If the information changes the known risk to subjects, the DSMC report of this event will be released to all participating investigators by the PI.

Criteria for withholding or stopping the study drug-Dexmedetomidine only

Criteria for withholding or stopping dexmedetomidine will be the occurrence of an adverse reaction requiring intervention (one or more of the following):

1) Severe bradycardia (heart rate <60 sustained for >30 minutes)

2) Severe tachycardia or other cardiac arrythmias (atrial fibrillation) requiring treatment
3) Severe hypotension (<30mm Hg mean arterial pressure) OR decrement in mean BP >20 to 30 mmHg compared to baseline OR receiving volume therapy > 40 to 80 ml/kg during each 24 h period of the study monitoring period: OR escalation of vasoactive inotropic score by > 20 to 30 compared to baseline
4) Severe hypertension (sustained SBP >95th centile) requiring treatment.
5) Severe hypothermia (esophageal temperature <31 C) for > 30 min
6) Infant placed on ECMO
7) And at the discretion of the attending physician after consultation with the study/site PI.

Following holding or stopping, study dexmedetomidine may be restarted per PI and attending physician discretion while the infant is within the study treatment period (within 96 hours of age). When restarting dexmedetomidine, a loading dose will not be repeated, and the starting infusion rate will be at the discretion of the attending as long as it is within protocol limits.

If the decision is made not to restart dexmedetomidine, further management will proceed per usual care at the site.

NOTE: Withholding or discontinuing morphine for adverse events is at the discretion of the attending neonatologist per unit standard of care.

Study Halting Rules

Enrollment and study drug administration will be halted for DSMC review, but PK sampling and follow-up will continue, if any of the following are reported:

- Death of a subject receiving Dexmedetomidine infusion and prior to the subject's hospital discharge that was not the result of moderate or severe hypoxia ischemia encephalopathy (HIE) and associated multiorgan disfunction, regardless of relatedness to study product. The incidence of intrahospital death for patients with HIE is 10% in the most recent reports.
- Occurrence of an allergic/hypersensitivity reaction (anaphylaxis) in any subject during the drug infusion manifested by bronchospasm with or without urticaria or angioedema requiring hemodynamic support with pressor medications or mechanical ventilation.
- Two or more subjects with a new Grade 3 or higher AE that is at least possibly related to study drug.

Laboratory: Data from clinical laboratory studies that are routinely assessed in infants undergoing cooling (first 72 hours) will be collected as part of the present study, including: liver and renal function, troponin, hematocrit level, white cell count, platelet count, PT, PTT, fibrinogen, and d-dimer. This data will be collected through discharge.

Other data to be collected include brain imaging studies (Cranial US, MRI) as SOC during hospital stay.

Specific Aim 2

Pharmacokinetics

The objective of this specific aim will be to determine dexmedetomidine PK in this population of newborns with HIE during treatment with TH and rewarming periods (Day 1-4). **PKs will only be drawn and analyzed in babies randomized to AND receive dexmedetomidine.**

Blood Samples-Dexmedetomidine arm only

To avoid excessive phlebotomy losses, a sparse sampling strategy analysis will be employed. Two to four opportunistic blood samples (0.5 mL) will be obtained for each participant at the time of routine laboratories. Further PRN PK samples will also be collected any time there is a serious or severe adverse event that is at least possibly related to dexmedetomidine resulting in holding or discontinuing the dexmedetomidine infusion. An additional 0.5mL blood sample will be taken in conjunction with standard of care labs during the first 96-120 hours of life; this will be 2 to 4 samples (total of 1 to 2 mL) depending on when SOC labs are drawn; and total blood volume drawn at any one time and overall will not exceed clinical limits. Blood samples will be centrifuged and the plasma frozen at -80°C until shipping. Collected samples will be shipped/transported in batches to Frontage Laboratories for analysis.

Dexmedetomidine HPLC-MS/MS analysis for PK samples

Dexmedetomidine concentrations in plasma will be quantified using a validated HPLC-MS/MS assay. Accuracy and precision are within the Food and Drug Administration bioanalytical assay validation criteria (e.g., ±15%). The lower limit of quantification is 5 pg/mL. This method was successfully employed in a PK study of dexmedetomidine in neonates and infants after cardiac surgery (51).

Pharmacokinetic Analysis

Dexmedetomidine plasma PK data will be analyzed with a nonlinear mixed effects modeling approach using NONMEM (version 7.4, Icon Solutions, Ellicott City, MD, USA). One- and two-compartment PK models will be explored. Between-subject variability will be assessed for PK model parameters using an exponential relationship. Both diagonal and block Omega matrices for covariance will be explored. Proportional, additive, and combined (additive plus proportional) residual error models will be explored. Bodyweight (WT) will be included as a covariate for clearance (CL) and volume of distribution (V) in the base model. The relationship between WT and PK parameters will be characterized using a fixed exponent (0.75 and 1) for CL and V parameters, respectively. Other covariates will be tested for model inclusion based on physiological relevance and by visual inspection of scatter and box plots. The relationship between age and CL will be explored using a sigmoidal maximum efficacy (E_{max}) maturation function as shown in equation (1). As a measure of age, PNA and PMA were explored.

(1)

$$F_{age} = \frac{age^{HILL}}{TM_{50}{}^{HILL} + age^{HILL}}$$

where F_{age} denotes the fraction of the adult CL value; TM_{50} represents the value of age (days for PNA and weeks for PMA) when 50% adult CL is reached; and *Hill* (Hill coefficient) is a slope parameter for the sigmoidal maturation model.(52) A forward inclusion ($p<0.05$) and backward elimination ($p<0.01$) approach will be used to evaluate statistical significance.

Models will be evaluated based on successful minimization, diagnostic plots, plausibility, and precision of parameter estimates, as well as objective function value (OFV) and shrinkage values. Parameter precision for the final model will be evaluated using non-parametric bootstrapping (1000 replicates) to generate the 95% confidence intervals for parameter estimates. Visual predictive check (VPC) will be performed whereby the final model will be used to generate 1000 Monte Carlo simulation replicates per time point of dexmedetomidine exposure. The number of observed concentrations outside of the 90% prediction interval for each time point will be quantified. Monte Carlo simulations will be used to evaluate different dosing regimens and calculate the probability of achieving the target exposure.

Specific Aim 3

Clinical and Neurodevelopmental Outcomes

Secondary efficacy outcomes will include short and long-term clinical aspects collected during initial hospitalization as well as during neurodevelopmental follow up.

1) To determine whether dexmedetomidine use for sedation versus morphine is associated with **improved short-term clinical outcomes** during initial hospitalization the following data will be collected:

- **Respiratory support**:

The need for Nasal Cannula, High Flow Nasal Cannula, CPAP, invasive mechanical ventilation/modality; and time to extubation will be recorded.

-**Shivering:**

Shivering will be assessed and recorded using an adapted version of the Bedside Shivering Assessment Scale (Badjatia et al.)(53). This tool has only been validated in brain-injured adults but used in neonates successfully (18). It consists of a 4-point scale which rates shivering as none: no shivering noted (0), mild: shivering localized to the neck and/or chest only (1), moderate: shivering involves upper extremities, plus neck and chest (3), or severe: shivering involves whole body (4). Shivering scores will be recorded along with the date, time, and duration of the episode and whether morphine was given.

- Duration of central venous and arterial access

- **Time to full PO feedings**

- Need for G tube/NG feedings at discharge

- Neurologic exam: Performed at 7 days of life (±2 days) or prior to discharge

- Brain MRI, Head US

- aEEG and Video EEG

- Hearing Screen

- Need for other medications (antibiotics, steroids, other sedatives, or opiates) will be systematically collected)

2) To determine whether dexmedetomidine use for sedation versus morphine is associated with **improved neurodevelopmental outcomes** at time of follow up the following data will be collected:

Neurodevelopmental outcomes are typically assessed as SOC and include the following exams: Generalized Movement Assessment (GMA) at 3-4 months of life (±1 month); the Hammersmith Infant Neurological Examination (HINE) at 3-4 and 6-9 months of life (±1 month); the Test of Infant Motor Performance (TIMP) performed at 3-4 months (± 1 month) of life; and 4) the Peabody Developmental Motor Skills (PDMS-2) at 6-9 months (± 1 month) of life. Data will be collected for the study from these exams. There may be circumstances in which a test listed above is not done as part of the standard of care visit and this will not be considered a deviation. These tests will be used as markers of neurodevelopmental safety in the assessment of dexmedetomidine versus morphine. The ASQ-3 will also be completed by parents at the 6-9 month (±1 month) visit to screen infants in areas of communication, gross motor, fine motor, problem solving, and personal-social.

The GMA, HINE, TIMP, and PDMS-2 are established, validated tests of neurodevelopment in both low and high-risk infants. The combination of GMA, HINE and TIMP are core components of the neurodevelopmental assessment for early diagnosis of cerebral palsy (CP)(54-56). In a systematic review by Novak et al, the most predictive tools for detecting CP risk before 5 months' corrected age were term-age MRI (86%-89% sensitivity), the GMA (98% sensitivity), and the HINE (90% sensitivity). After 5 months' corrected age, the most predictive tools for detecting risk were MRI (86%-89% sensitivity), and the HINE (90% sensitivity)(56).

The HINE optimality score is based on the frequency distribution of neurological findings in a typical infant age group, when an item is found in at least 90% of infants it is considered optimal. Use of the HINE at 3-4 and at 6-9 months of age allows for multiple assessments of motor development. A cut off of 57 is used to identify infants at 3 months who are high risk for CP and those infants are followed with an additional visit at 6 months of age. Total HINE scores as well as asymmetries will be assessed and compared between treatment groups.

The GMA will be assessed as a dichotomous variable (fidgety present or fidgety absent/abnormal fidgety before hospital discharge when off sedation and repeated at 3-4 months of life).

The ASQ-3 screening tool has been validated in several studies and is recommended by the AAP as one of the developmental surveillance and screening tests (57). Psychometric studies based on a normative sample of more than 18,000 questionnaires showed high reliability, internal consistency, sensitivity, and specificity.

All infants will be followed up at the University of Utah Neonatal Follow Up Program. The Neonatal Follow-up Program is well suited to provide follow up for this study. In addition to providing neurodevelopmental assessments for high-risk neonates it is one of 6 universities nationally that has partnered with the Cerebral Palsy Foundation and Nationwide Children's Hospital in the Early Detection of CP and has extensive expertise in the GMA, HINE, TIMP, and PDMS-2.

Statistical Analyses

The sample size of 50 evaluable (25 infants in each study arm) was determined to provide sufficient evidence regarding the equivalence or noninferiority of dexmedetomidine relative to morphine for key safety measures and is also considered adequate to evaluate neurodevelopmental outcomes explored as secondary outcomes. Any infants who are enrolled and randomized, but who do not receive a study drug dose will not be included in the analysis and another infant will be recruited for randomization. To calculate the effect of dexmedetomidine versus morphine on developmental outcome measures, we will use 95% confidence intervals, and corresponding P values (significance, $P < .05$) using an appropriately developed generalized regression with robust SEs, adjusting as appropriate with patient covariates, including, potentially age at testing and severity of encephalopathy at baseline. Variable selection will be based on corrected Akaike Information Criterion (AICc) for an all-subsets selection technique.

For summary statistics, we will compare categorical variables between randomized treatment groups by using a $\chi 2$ test, or Fisher's exact test when the expected count of any category is ≤ 5. To compare baseline continuous variables between treatment groups, a two-sided t test will be used. All statistical analyses will be performed by an independent biostatistician by using statistical software.

Additional Follow-up Assessments

No additional follow-up assessments are planned for this protocol after the 9 months assessment; however, consent for future contact will be sought if additional follow-up of the study participants is conceived. Future follow-up will require informed consent and appropriate institutional and regulatory approval.

Annual Reports

Annual Reports are required on all active IND applications. If the IND is placed in inactive status, annual reports are not required. (21 CFR 312.45(c)). Annual reports are required to be submitted to the FDA within 60 days of the anniversary of the date that the IND went into effect (21 CFR 212.33). Please update the protocol to address annual reporting.

Protocol Amendments

Any amendments or administrative changes to an IRB approved protocol will not be initiated without submission of an amendment for IRB review and approval. Any amendments to the protocol that significantly affect the safety of subjects, the scope of the investigation, or the scientific quality of the study are required to submit the amendment for FDA review.

Record Retention

Records will be maintained at the study site per, 21 CFR 312.57. A sponsor shall retain the study records and reports for 2 years after a marketing application is approved for the study drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified.

Protocol Violations and Deviations

All applicable protocol violations and deviations will be reported within guidelines set forth by the governing Institutional Review Board.

A deviation is any departure from the defined procedures and treatment plans as outlined in the protocol version or application version submitted and previously approved by the Institutional Review Board.

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