Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Kraft WK, Adeniyi-Jones SC, Chervoneva I, et al. Buprenorphine for the treatment of the neonatal abstinence syndrome. N Engl J Med 2017;376:2341-8. DOI: 10.1056/NEJMoa1614835

This supplement contains the following items:
1. initial and final protocol, and a summary of amendments.
2. Data Safety Monitor Plan (DSM).

There was no separate statistical analysis plan, and an outline of the statistical plan was contained within the protocol and the DSM.

Please note that no patients were enrolled under Protocol Version 1.0. After 4 patients were enrolled, Version 3.0 was approved, and the final amendment Version 4.0 was approved after 19 subjects had enrolled.

1 INITIAL PROTOCOL VERSION 1.0

Title	A Randomized, Active-Control, Double-Blind, Double-Dummy Clinical Trial Comparing Sublingual Buprenorphine And Morphine Solution For The Treatment Of Neonatal Opioid Abstinence Syndrome
Clinical Study Site	Thomas Jefferson University Hospital Philadelphia, PA
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Protocol Date	March 24, 2011
IND	68,403
Institutional Review Board	Thomas Jefferson University
Version	1.0

PROTOCOL SYNOPSIS

Primary Objective	To compare length of treatment using sublingual buprenorphine or oral morphine solution in the pharmacologic treatment of the neonatal abstinence syndrome (NAS).	
Secondary Objectives	To compare length of stay using sublingual buprenorphine or morphine solution To compare requirement for use of supplemental phenobarbital using sublingual buprenorphine or morphine solution. To compare the safety of using sublingual buprenorphine or morphine solution. To estimate the pharmacokinetic parameters of buprenorphine in the treatment of NAS. To estimate the pharmacokinetic parameters of morphine in the treatment of NAS.	
Exploratory Objectives	To examine the influence of genetic variants in the ABCB1 and mu opioid receptor genes on the need for treatment in NAS. To examine the influence of genetic variants in the ABCB1 and mu opioid receptor genes on the duration of treatment in NAS. To explore buprenorphine drug disposition by urinary analysis. To compare Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) values in infants treated with buprenorphine vs. those treated with morphine for NAS.	
Design	Randomized, Blinded, Double Blind, Double Dummy Clinical Trial	
Study Treatments	**Buprenorphine Arm** <u>Buprenorphine</u> Sublingual, q8 hours 0.4 mg ml solution *(Buprenex, Reckitt Benckiser, Richmond, VA) , 30% ethanol USP, in simple syrup USP* **AND** <u>Placebo for Morphine</u> Oral, q 4 hours *FD&C Green #3* *water USP*	**Morphine Arm** <u>Morphine Solution</u> Oral, q 4 hours 0.4 mg ml solution *Morphine in sterile water* **AND** <u>Placebo for Buprenorphine</u> Sublingual, q 8 hours *Water USP* *Simple syrup USP in 1:1 ratio*
Number of Subjects	80 infants, (40 in each treatment arm)	
Population: Inclusion Criteria	Patients eligible for participation include: 1. ≥ 37 weeks gestation 2. Exposure to opiates in utero 3. Demonstration of signs and symptoms of neonatal abstinence syndrome requiring treatment	
Population: Exclusion Criteria	Patients ineligible for participation include:	

	1. Major congenital malformations and/or intrauterine growth retardation (Mamelle 2001) 2. Medical illness requiring intensification of medical therapy. This includes, but is not limited to suspected sepsis requiring antibiotic therapy. 3. Hypoglycemia requiring treatment with intravenous dextrose. 4. Bilirubin >20 mg/dL (The need for phototherapy is not exclusionary) 5. Concomitant benzodiazepine or severe alcohol abuse , self-report of regular use of alcohol or of benzodiazepines use in the past 30 days, and/or receipt of benzodiazepines by prescription (as determined by self-report or intake urine) by the mother 30 days prior to birth, 6. Concomitant use of CYP 3A inhibitors (erythromycin, clarithromycin, ketoconazole, itraconazole, HIV protease inhibitors) or inducers (rifampin, carbamazepine, phenobarbital) prior to initiation of NAS treatment 7. Seizure activity or other neurologic abnormality 8. Breast feeding 9. Inability of mother to give informed consent due to co-morbid psychiatric diagnosis

Table 1: List of Abbreviations

ADR	Adverse Drug Reaction
AE	Adverse Event
DSMB	Data Safety Monitoring Board
CMV	Cytomegalovirus
FDA	Food and Drug Administration
LOT	Length of Treatment
LOS	Length of Stay
NAS	Neonatal Abstinence Syndrome
NICU	Neonatal Intensive Care Unit
NNNS	Neonatal Intensive Care Unit Network Neurobehavioral Scale
SAE	Serious Adverse Event

TJUH	Thomas Jefferson University Hospital

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Figure 1: Study Schema

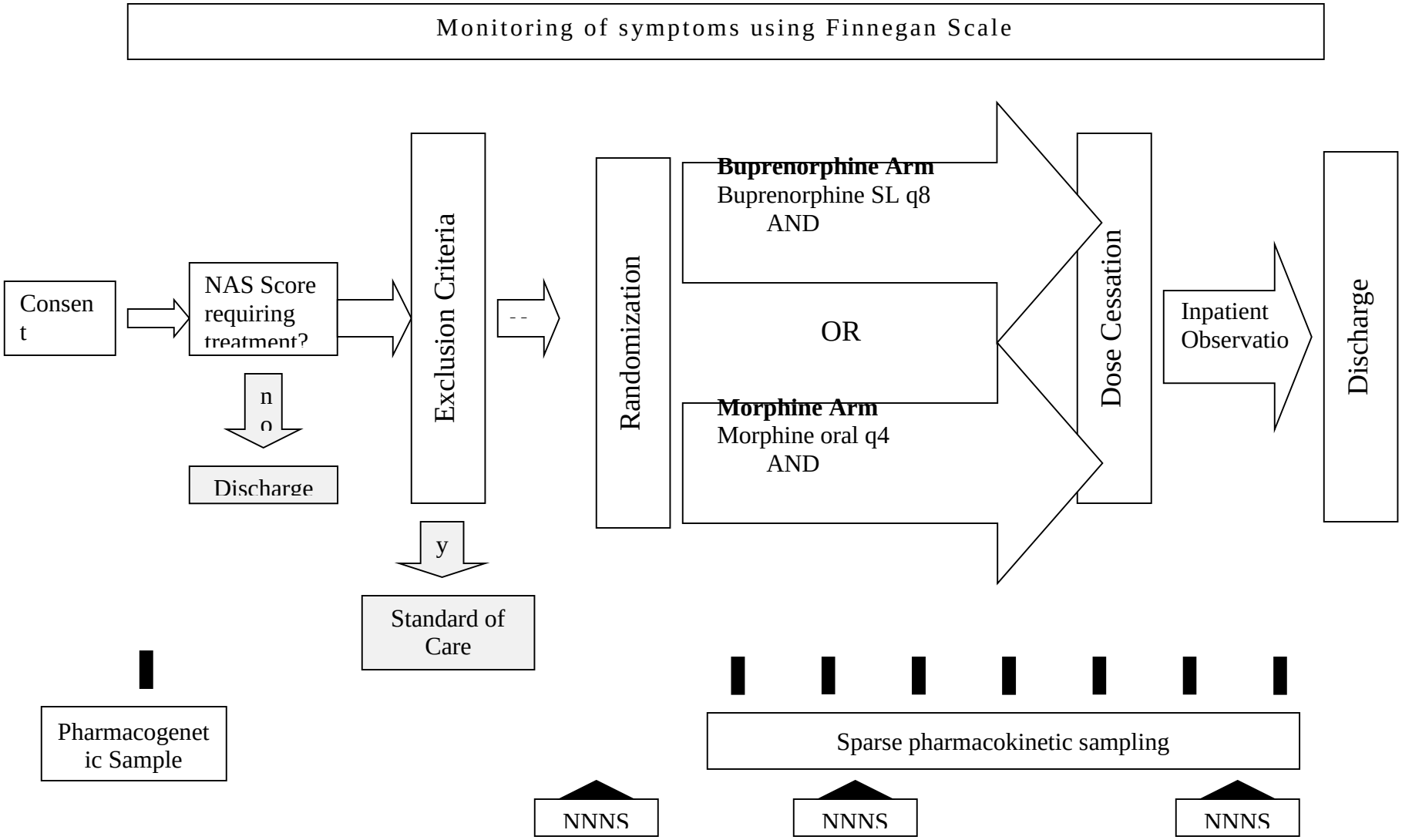


Figure 2: Study Algorithm

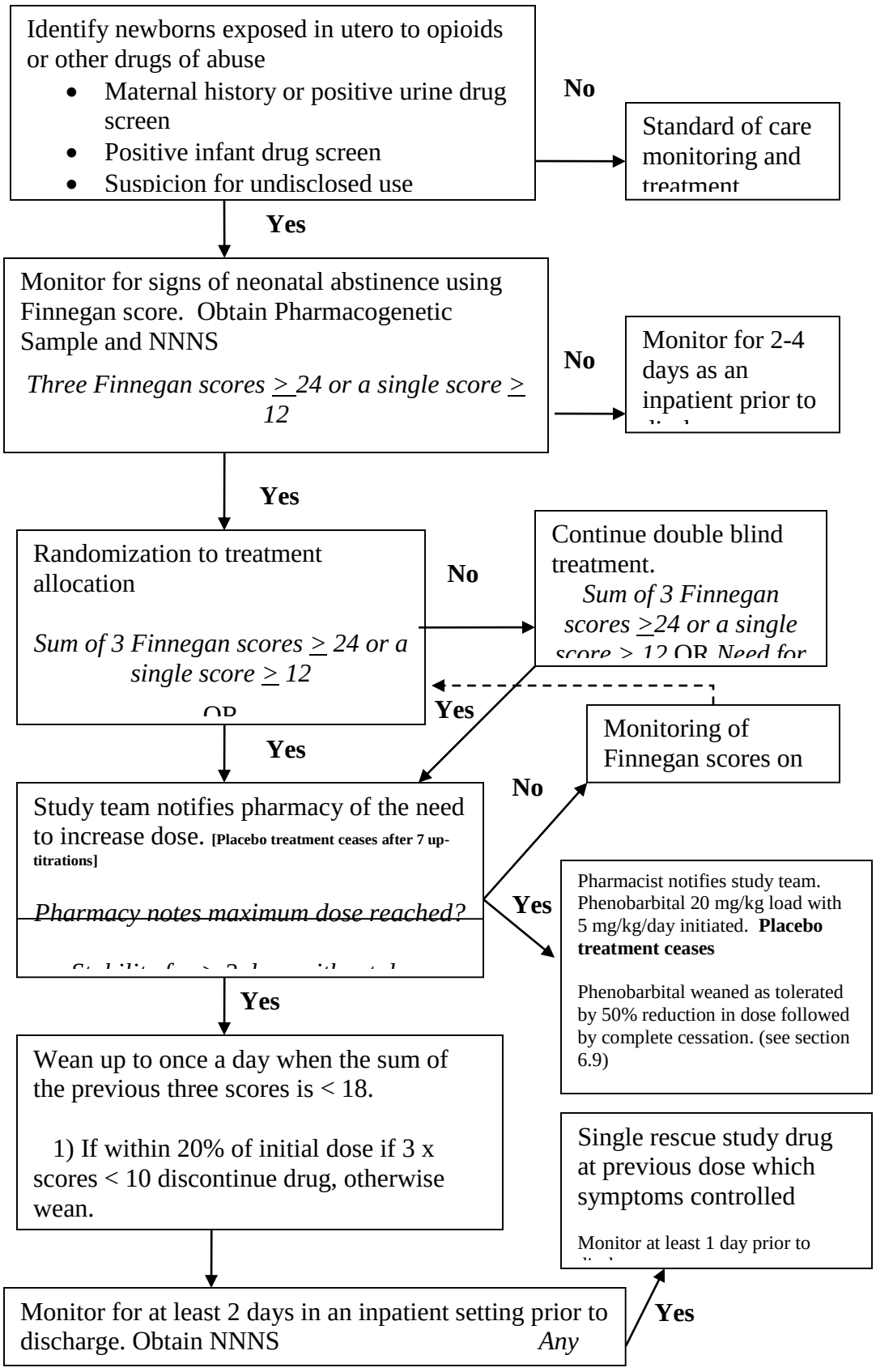


Table 2: Dose Schema for Buprenorphine and Morphine

	Buprenorphine	**Morphine**
Initial daily dose	15.9 mcg/kg/day	0.4 mg/kg/day
Initial unit dose	5.3 mcg/kg q8 hours	0.07 mg/kg q 4 hours
Maximum daily dose	60 mcg/kg/day	1.0 mg/kg/day
Maximum unit dose	20 mcg/kg q8 hours	0.17 mg/kg q 4 hours
Up-titration rate	25%	10%
Maximum # of up-titrations	6	9
Weaning rate	10%	10%
Cessation Dose	Within 10 or 20% of starting dose (section 7.8.1)	0.025 mg/kg q 4 hours
Inpatient observation following cessation of last scheduled dose	At least 2 days	At least 2 days
Inpatient observation following last rescue dose	At least 1 day	At least 1 day

1 INTRODUCTION

a. Background

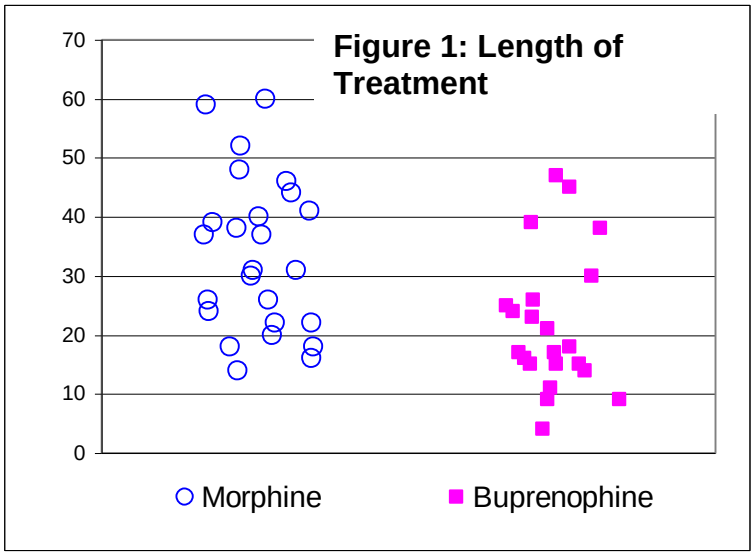
Neonatal withdrawal symptoms have been noted following prenatal exposure to a number of drugs. Examples include opioids, benzodiazepines, mood stabilizing medications, (ACOG Committee on Practice Bulletins--Obstetrics 2008) selective serotonin reuptake inhibitors, (Koren 2005) and nicotine. (Law 2003) For all drug classes except opioids, these symptoms are usually self limited and do not require specific pharmacologic therapy. Neonates exposed to opioids can develop the Neonatal Abstinence Syndrome (NAS), a complex of signs and symptoms in the postnatal period associated with the sudden withdrawal of maternally transferred opioids. Cardinal manifestations include increased muscle tone, autonomic instability, irritability, poor sucking reflex, gastrointestinal symptoms, and impaired weight gain. In epidemiologic studies, maternal opioid abuse is common, with toxicological evidence of use in approximately 1% of births. (Vega 1993) This includes methadone, and increasingly buprenorphine, both of which are used to treat women with physical dependency for opioids. The degree of abstinence symptoms vary in each child. Those with milder symptoms respond well to supportive treatments of swaddling, small calorically dense formula (if not breastfeeding) and minimization of distractive external stimuli. NAS symptoms severe enough to require pharmacologic treatment has been reported to occur in 55-94% of infants born to opioid-dependent mothers, (American Academy of Pediatrics Committee on Drugs 1998) It should be noted that the societal burden of NAS has been difficult to assess based upon limited self report of drug use and underreporting of the condition using ICD classifications. (Burns 2007).

There continues to exist significant heterogeneity in the diagnosis and treatment of neonates at risk for opiate withdrawal. (Crocetti 2007, Sarkar 2006) This is reflected in the variability in length of hospital stay and treatment for such patients. (Jackson 2004, Coyle 2002, Langenfeld 2005, Colombini 2008) Cochrane reviews, (Osborn 2010a, Osborn 2010b) the American Academy of Pediatrics, (American Academy of Pediatrics Committee on Drugs 1998) and expert review identify opioid replacement as the ideal treatment for the withdrawal symptoms associated with *in utero* exposure to opiates. (Johnson 2003)

Buprenorphine is a partial mu opioid receptor antagonist used in the treatment of adult opioid dependency. Cochrane reviews have demonstrated efficacy (retention in treatment programs and suppression of heroin use) of buprenorphine that is comparable to methadone treatment. (Mattick 2008, Gowing 2006) Use of buprenorphine has gained favor due to properties of improved safety, particularly with regard to respiratory depression. Buprenorphine can control abstinence in adults with every other day dosing, due in part to the long residence time at the opioid receptor. (Greenwald 2007) A recent exciting finding is demonstration of improved outcomes in infants with in utero exposure of buprenorphine compared to methadone. (Jones 2010)

Buprenorphine has a number of characteristics that make it an attractive agent in the treatment of NAS and a candidate for the unmet need of improved NAS treatment. As an agonist/antagonist, buprenorphine has a ceiling effect for respiratory depression. There is a lack of the cardiovascular liability associated with methadone as well as an established safety profile in adults. The long half-life and duration of action prevents the rapid change in receptor occupancy that can precipitate withdrawal symptoms. Finally, there is limited abuse liability, which makes consideration of outpatient treatment for NAS a possibility for carefully screened caregivers. Based on these characteristics, the current team of investigators planned and executed the first and only human clinical trial employing buprenorphine and comparing it to oral morphine in the treatment of NAS. The results of the first cohort of 26 randomized infants was reported in a peer reviewed journal. (Kraft 2008) Based upon the experience gained, a second cohort of 24 patients randomized with a revised dose protocol was undertaken. (Kraft 2011) While the goals of the study were demonstration of the feasibility and safety of buprenorphine use in NAS, an analysis of all randomized patients reveals substantial efficacy advantage or buprenorphine over standard of care morphine. Indeed, patients treated with oral morphine had a mean length of treatment of 34 days, whereas those treated with buprenorphine had a mean length of treatment of 23 days. Following log transformation to satisfy normality assumptions, the length of treatment was on average 34% shorter (95% CI: 13%, 50%; p=0.005) in the buprenorphine arm than in those administered oral morphine, and the length of stay was on average 26% shorter (95% CI: 5%, 43%; p=0.020).

Figure 3: Efficacy Outcomes of Buprenorphine compared to Oral Morphine



When a buprenorphine-treated infant with complications and prolonged stay due to an unrelated cytomegalus virus (CMV) is excluded, length of treatment was on average 37% shorter (95% CI: 16%, 52%) on buprenorphine (20 days) than on morphine, and the length of stay was on average 31% shorter (95% CI:

13%, 45%) on buprenorphine. In both analyses, this difference was statistically significant (p=0.002 for both). However, as this was a first human use phase 1 trial in neonates, results must be verified in a double-blind trial.

b. Rationale for Study Design and Dose

1. Standard of Care arm

A consensus does not exist on the use of weight vs. symptom driven dosing regimens of oral morphine. (Jansson 2008a) In addition, there exists considerable heterogeneity in specific morphine-based regimens. (Nandakumar 2006) A symptom-based morphine regimen has been the standard of care treatment at Thomas Jefferson University Hospital (TJUH) for at least 30 years, and was the comparator group used in the phase 1 trial of buprenorphine.

The morphine regimen used as the active control is the standard of care at TJUH. The initial dose is 0.06 mg/kg every 4 hours (0.4 mg/kg/day). Morphine is the most commonly used replacement opioid therapy for NAS. While intravenous morphine pharmacokinetics have been described in neonates, there are no published reports of morphine disposition when administered by the oral route in neonates being treated for NAS or for any other condition. The ontogeny of presystemic morphine metabolism has not been described.

2. Buprenorphine arm

Buprenorphine administered via the sublingual route is efficacious in the treatment of NAS as demonstrated in a pilot study performed at TJUH. (Kraft 2008, Kraft 2011) As the first investigation of sublingual buprenorphine in neonates, the study goals were to explore the safety, feasibility, and pharmacokinetic properties of sublingual buprenorphine. The initial experience was not a formal exploration of efficacy relative to standard of care, but instead a phase 1 trial in a patient population. In the initial cohort, 13 of 26 patients were treated with buprenorphine. Though not statistically significant (p=0.077), buprenorphine was associated with a 22 day length of treatment, compared to 32 for standard of care neonatal opium solution. Observations of need for rapid up-titration and increased need of phenobarbital adjunctive therapy, paired with pharmacokinetic data led to a revised dose schedule for buprenorphine. At the same time, TJUH transitioned from neonatal opium solution to an oral morphine solution, which was equipotent for morphine equivalents. A similar efficacy outcome has been noted in this second cohort of 24 patients. A summary of the doses used in patients thus far is presented in Table 3.

Table 3: Summary of Buprenorphine Dose Cohorts

Dose	Duration	Total subjects enrolled	Buprenorphine

Cohort			Initial dose mcg/kg/day	Maximum dose mcg/kg/day	up-titration rate
1	4/05-1/08	26 *(13 buprenorphine, 13 neonatal opium solution)*	13.2	39	20%
2	3/08- 11/09	24 *(12 buprenorphine,12 morphine)*	15.9	60	25%
Total enrolled		**50** ***(25 buprenorphine, 25 morphine)***			

Of scoring systems available to gauge severity of symptoms and titrate drug dose, the Finnegan score (Finnegan 1992) is most commonly used. (Sarkar 2006) All scoring systems, however, rely on semi-quantitative provider assessments. A double-blinded approach is required to remove any potential for grading bias based upon treatment allocation. The differences in route and frequency of administration between morphine and buprenorphine will require the use of a double dummy design to maintain blinding.

Some clinics treat selected children with NAS on an outpatient basis. This approach decreases inpatient duration of stay, but increases total duration of treatment with an opioid. (Oei 2001) Buprenorphine has less of a potential for diversion than does morphine and less propensity for respiratory depression. For these reasons, buprenorphine may be ideally suited for widening the use of outpatient treatment for NAS.

Maternal use of antidepressants during pregnancy can be associated with neonatal symptoms of the central nervous and respiratory systems. (Koren 2005, Ferreira 2007) The maternal dose to neonatal severity relationship is not established. These symptoms have a temporal onset similar to the abstinence symptoms of methadone abstinence. This exposure is a potential confounder for comparative efficacy examination. However, prospective stratification by self-report of adherence to prescribed psychotropics is not feasible and effective randomization should control for these potential confounders. In addition, as SSRI withdrawal is limited to the first few days of life, it is not expected to significantly impact the primary endpoint of length of stay, a hypothesis supported by lack of effect of SSRI exposure on length of stay at TJUH between 2000-06. (Seligman 2008)

3. Comparative Safety

Treatment of NAS in a highly monitored inpatient setting is very safe. The most concerning side effect of morphine in the treatment of abstinence is the potential for respiratory depression on the basis of an inadvertent overdose. In adults, buprenorphine is associated with a ceiling effect on respiratory depression, with deaths in adults primarily occurring with the co-administration of benzodiazepines. (Megarbane 2006) Preliminary data from TJUH constitutes the majority of what is known about buprenorphine in neonates, and the side effect profile has been favorable. There have been no observed episodes of excessive sedation,

respiratory depression, or aspiration after >1600 doses of sublingual buprenorphine administered to 25 infants. There is a theoretic possibility of precipitation of withdrawal with administration of a partial opioid agonist such as buprenorphine. This has not been an observed worsening of symptoms, which can be hypothesized to because receptor occupancy by methadone is likely already low at the time NAS symptoms necessitate treatment.

Table 4: All Adverse Events Observed in the Clinical Investigation

Subject	Treatment	Adverse Event	Serious AE	Causality
007	buprenorphine	seizure	yes	probably not related
027	buprenorphine	paronychia of finger	no	unrelated
039	morphine	oral thrush	no	unrelated
047	buprenorphine	paronychia of finger	no	unrelated
047	buprenorphine	reflux/poor feeding	yes	probably not related
047	buprenorphine	elevated transaminases	no	probably not related
047	buprenorphine	CMV infection	no	unrelated
047	buprenorphine	aminoaciduria	no	unrelated
049	morphine	conjunctivitis	no	unrelated
050	morphine	oral thrush	no	unrelated
051	buprenorphine	clavicle birth fracture	no	unrelated
052	morphine	reflux	no	unrelated

Two serious AEs took place in the study. The second patient randomized to buprenorphine developed tonic clonic seizures 78 hours after the initial dose. The infant had 4 up titrations prior to this event, but no alteration in dose in the 16 hours immediately preceding the event and had demonstrated improved symptomatic control over this time period with NAS scores between 6 and 8. Buprenorphine was halted and treatment initiated with phenobarbital and oral morphine. Post event evaluation revealed normal serum hematology, chemistry, C-reactive protein, and lumbar puncture indices, and negative cultures. An interictal EEG was negative and MRI of the brain revealed a small amount of dependent subdural hemorrhage within the posterior fossa likely related to the birthing process and deemed unlikely to be symptomatic, with no parenchymal abnormalities. This child's total length of stay was 28 days. At one-year follow up, the child was developmentally normal. A causal link of under-treatment of withdrawal or a dose dependent effect of buprenorphine was not immediately apparent to the investigators. Independent review by the Data Safety Monitoring Board and the Thomas Jefferson University IRB was performed and it was recommended that the trial be resumed using the established protocol.

AN 047, the 22nd patient randomized to buprenorphine, had a number of adverse events, possibly as a result of a cytomegalovirus (CMV) infection acquired late in the inter-uterine period. The infant had hypoglycemia prior to initiation of treatment with buprenorphine, which was considered to be transient. AE's included prolonged reflux/poor feeding, elevated transaminases, aminoaciduria, and paronychia of a finger. Lack of resolution on buprenorphine and phenobarbital, and indeed absence of worsening when these drugs were

removed, led to further medical evaluation. CMV was noted in urine, and a lack of cerebral calcifications or microcephaly at birth suggested a late *in utero* infection. Length of buprenorphine treatment was 45 days and length of stay was 98 days. On the last day of buprenorphine weaning, alanine transaminase was noted to be 30x the upper limit of normal (ULN), while aspartate transaminase (AST) was noted to be 20x ULN. These values remained elevated 6 weeks following the cessation of buprenorphine. Bilirubin was never elevated. Following completion of therapy in this infant, further enrollment was halted and a Data Safety Monitoring Board (DSMB) meeting was called to review the case. The DSMB found causality of the syndrome complex to be unlikely to be related to the administration of buprenorphine and recommended the resumption of the clinical trial with some minor modifications. A revised protocol including these changes was approved by the Thomas Jefferson University Institutional Review Board. These modifications included:

- *addition of liver function tests measured at predose, and 7 (+/- 2) and 21 (+/- 2) days after the first dose of study medication in both buprenorphine and morphine groups*
- *inclusion of hypoglycemia as an exclusion criterion*

Buprenorphine has been associated with elevated liver enzymes, primarily in patients with underlying hepatic dysfunction from viral hepatitis. It should be noted, however, that cohort studies have been reassuring with regard to hepatic safety, (Bogenschutz 2008) and no hepatic toxicity was noted in a 2008 case series of 84 pediatric overdoses of buprenorphine. (Hayes 2008) Buprenorphine has been safely administered to patients with acute hepatitis C and elevated transaminases. In clinical trial experience at TJUH, transaminase levels in the seven other tested infants administered buprenorphine were normal.

c. Pharmacogenetics as a potential diagnostic aid

Individualization of therapy is a primary goal of all of those who prescribe medication. Differential responses to drug therapy based upon heritable factors has been well described in multiple disease states, and there is a great deal of interest in genetic factors leading to a propensity to addiction and/or pharmacodynamic response to various opioids. (Haile 2008) It is important to note that the factors in those two classifications may not be the same. It is highly unlikely that the variability in either infants or adults can be reduced to a monogenic etiology, but several single nucleotide polymorphisms (SNPs) in particular candidate genes have been identified that appear to determine response to opioids for pain or replacement abstinence therapy in adults, and/or for predilection to addiction. (Drakenberg 2006) NAS is a condition with wide variation in the need for pharmacologic therapy, severity of disease, as well as in the duration of therapy. The study of pharmacogenetic effects in newborn patients presents a particular advantage since mood, personality,

socioeconomic and concomitant medical illness covariates are expected to be markedly reduced relative to adults. Toward this end, DNA samples will be collected in those infants at risk and/or treated for NAS. In this fashion, linkages between the propensity to require pharmacologic therapy for NAS, as well as response to existing therapies, can be investigated.

10. STUDY OBJECTIVES

a. Primary Objective

- To compare length of treatment using sublingual buprenorphine or oral morphine solution in the pharmacologic treatment of the NAS.

b. Secondary Objectives

- To compare length of stay using sublingual buprenorphine or morphine solution.
- To compare requirement for use of rescue phenobarbital using sublingual buprenorphine or morphine solution.
- To compare the safety of using sublingual buprenorphine or morphine solution.
- To estimate the pharmacokinetic parameters of buprenorphine in the treatment of NAS.
- To estimate the pharmacokinetic parameters of morphine in the treatment of NAS.

c. Exploratory Objectives

- To examine the influence of pharmacogenetic variants in the ABCB1 and mu opioid receptor genes on the need for treatment in NAS.
- To examine the influence of pharmacogenetic variants in the ABCB1 and mu opioid receptor genes on the duration of treatment in NAS.
- To explore buprenorphine drug disposition by urinary analysis.
- To compare Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) values in infants treated with buprenorphine vs. those treated with morphine for NAS.

11. OVERALL DESIGN AND PLAN OF THE STUDY

a. Overview

This is a single-site, randomized, double-blind, double-dummy, parallel-group clinical trial. Potential patients will be identified in the pre-natal period by staff of the Thomas Jefferson University Family center. Mothers who provide consent will be contacted upon admission to TJUH. Inclusion and exclusion criteria will be reassessed during the peri-partum period and study details will be reviewed again with the mother, and where possible, the father of the child. Women admitted to TJUH with *in utero* exposure to opioids who are not in the Family Center present will be screened and approached for consent during their inpatient stay.

Infants at risk for NAS will have abstinence assessed using the MOTHER scoring instrument (Jones 2005), which is based upon Finnegan Score and will hereafter be called the "NAS score" (Appendix 2). This is the standard instrument used at TJUH. A need for initiation of treatment will be defined as any consecutive 3 scores adding up to ≥ 24 or any single score ≥ 12. Randomization will take place following reaching of the threshold for initiation of treatment and a re-review of inclusion and exclusion criteria. Patients will be randomized to treatment groups of 1) oral morphine/sublingual placebo for buprenorphine or 2) oral placebo for morphine/sublingual buprenorphine. Oral morphine or placebo for morphine will be administered by mouth every 4 hours, while buprenorphine or placebo for buprenorphine will be administered every 8 hours. NAS scores will be obtained every 4 hours. Dose assessment will take place on a daily basis. If the three previous NAS scores are greater than 24, a dose advancement will take place. Morphine/placebo will be increased by 10% and buprenorphine/placebo will be increased by 25%. NNNS scoring with take place for all infants who provide consent at day 2 of life, or earlier if pharmacologic treatment is required before this time, on day 10 of life, and in the post therapy period (but no later than corrected post gestational age of 46 weeks). Corrected post gestational age is gestational age at birth + weeks of life.

b. Endpoints

i. Efficacy Endpoints

1. Length of Treatment (primary)

The primary endpoint is duration of treatment in days. This measure is most closely linked to efficacy of the allocated treatment. Length of treatment is defined as the number of calender days when treatment was initiated until the last dose of study drug using 12 midnight as the cut off between days.

2. Length of Stay (secondary)

Length of stay is defined as the number of calender days from date of birth to date of discharge from the hospital. Length of stay is less directly tied to treatment efficacy, as the lag time to treatment initiation varies, and occasionally social issues will delay a child's discharge after cessation of therapy for NAS. However,

this endpoint is of utility, as it has the potential to capture treatment-specific prolongation of hospital stay that is independent of length of treatment, for example, due to adverse events.

3. Need for Supplemental Phenobarbital Use (secondary)

The use of phenobarbital is often used as a rescue therapy when maximum opioid replacement therapy dose is reached without adequate resolution of symptoms, though it has also been used as an initial adjunct in combination therapy with an opioid (Coyle 2002) or as initial monotherapy (Jackson 2004). The current study design employs phenobarbital as rescue. Morphine and buprenorphine employ different up-titration rates and number of up-titrations until maximum dose is reached (6 for buprenorphine and 9 for morphine). Thus, the need for adjunctive phenobarbital is not necessarily a surrogate of "treatment failure" in infants with a more severe withdrawal symptom complex. Based upon pilot data, it is not clear where on the dose response curve the present maximum buprenorphine lies. It is possible, that as a partial agonist, buprenorphine may not be able to induce the dense signal generation at the mu opioid receptor obtained with morphine. However, there are no clear adverse events associated with short-term exposure to phenobarbital, and it is possible that a short course of phenobarbital may reduce total duration of treatment in children with more severe withdrawal symptoms.

4. Neurobehavioral Endpoint (exploratory)

The NAS score has been a favored research instrument due to the comprehensive capture of a large number of elements of neonatal withdrawal, (Jansson 2009) as well as widespread use in clinical care. There may be subtle neurobehavioral differences in the response to treatment which can be uncovered by use of the Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) system. (Lester 2004) NNNS assessment will be performed at day 2 of life and prior to initiation of pharmacologic therapy, 10 days of life, and in the post therapy period (but no later than corrected post gestational age of 46 weeks). Corrected post gestational age is gestational age at birth + weeks of life. Comparisons of interest will be, differences in NNNS scores between treatment arms, correlation of NNNS scores with the NAS, and an examination of day 2 NNNS scores in those infants requiring pharmacologic therapy for NAS and those infants who are discharged without need for therapy.

ii. Safety Endpoints

1. Adverse events

Adverse events will be recorded in the patient's research chart using a standardized form (Appendix 1). Events will be graded by blinded investigators. Adverse events will be analyzed in a per protocol fashion. Liver enzyme testing will be monitored in both treatment arms.

2. Severity and Causality Assessment

Adverse events will be graded by an investigator according to a severity score (mild, moderate, severe).

3. Serious adverse events

A serious adverse event is one that results in death, permanent disability, prolongation of hospitalization, or judged by an investigator to be a significant medical event. All serious adverse events will be reported to the Institutional Review Board, DSMB, and the FDA.

iii. Pharmacokinetic Endpoints

Sparse sampling will be used to generate an estimate of pharmacokinetic parameters and intersubject variability of sublingual buprenorphine and of oral morphine solution. Population PK techniques offer a powerful method of using the limited pharmacokinetic samples and observing the effects of covariates on drug disposition. This can relate ontogeny of body fat composition, drug metabolism, and excretion with pharmacokinetic and pharmacodynamic variables and can be used to rationally design dosing regimens.

iv. Pharmacogenetic Endpoints

An exploratory endpoint is the evaluation of the effects of pharmacogenetic variants on length of treatment in NAS and the propensity to develop NAS. The relationship between allele status, NAS scores, total amount of drug administered, and duration of therapy will be examined. The A118G polymorphism in the mu opioid receptor gene (OPRM1) (rs 1799971), among other things, alters the response to intravenous morphine, and perhaps vulnerability to heroin. (Drakenberg 2006) Polymorphisms in the ABCB1 gene encoding the multidrug resistance associated P-glycoprotein (rs1045642, rs2032582 and rs1128503) also appear to affect response to morphine in pain relief, and perhaps more importantly, methadone dose required for effective treatment of heroin addiction. There are reports linking SNPs in the dopamine receptor type 1, preproenkephalin and preprodynorphin genes to propensity to addiction which we may also consider for analysis, but support for their relevance to treatment is not as strong at this time.

c. Justification of the Study Design

A pilot feasibility study has demonstrated the safety and efficacy of buprenorphine delivered by the sublingual route. There are suggestions of increased efficacy of buprenorphine relative to oral morphine for the endpoints of length of treatment and length of hospitalization, though with a possibly higher incidence of required supplemental phenobarbital use. The pilot study was open label, which leaves open the possibility that the comparative advantage of buprenorphine was due to occult nursing or investigator bias. A randomized, double blinded design will remove this potential source of variability, especially as the weaning instrument has a number of subjective grading elements. The differing dose schedule (q8 vs. q4) and route of administration (sublingual vs. oral) make the use of a double dummy required to maintain the blind.

d. Justification of Dose

1. Morphine

The morphine treatment protocol used in this study is the standard treatment regimen used at the TJUH for the past 20 years. The initial dose of 0.4 mg/kg/day used is in the middle of the 0.12-0.6 range reported in a survey of 17 pediatric units in the United Kingdom. (Nandakumar 2006) The authors of this report opined that a higher initial dose may be associated with better control of symptoms, but freely admitted that evidence to support this intuition was lacking. The TJU dose is modestly below the 0.24 mg/kg/day recommended by the 1998 report of the American Academy of Pediatrics, though this protocol outlined drop unit doses which would make fine titration difficult.(American Academy of Pediatrics Committee on Drugs 1998) The Cochrane review of the topic does not specify a favored specific dose.(Osborn 2010a)

2. Buprenorphine

In the pilot study, the initial dose of buprenorphine and dosing regimen was determined using extrapolated adult data to generate a monoexponential pharmacokinetic model with a target steady state concentration of 2 ng/mL. In this pilot study 99.5% of pharmacokinetic samples had buprenorphine concentrations <0.6 ng/ml, with many samples below the limit of quantification of 0.1 ng/ml. What was striking was that there was excellent efficacy, even with buprenorphine concentrations well below the 0.7 ng/ml considered to be the level at which relief of adult abstinence symptoms begins. (Kuhlman 1998) Most patients required a relatively rapid up-titration of buprenorphine, possibly due to a longer half life and volume of distribution relative to morphine. Finally, there was evidence of suboptimal efficacy at the protocol-specified maximum dose of 39 mcg/kg/day on the basis of the need for supplemental phenobarbital in 3 of 12 infants treated with buprenorphine. Based upon these findings, a revised dosing scheme was introduced, which increased the initial dose to 15.9 mcg/kg/day, each step-up titration from 20 to 25%, and maximum dose to 60 mcg/kg. The length of treatment at this higher dose schedule remains intact relative to lower dose, while drug-related safety remains favorable.

e. Justification of Exclusion criteria

1. Breast Feeding

Methadone is passed on to neonates through breast milk, though the absolute amount is small (<0.2 mg/day) and does not appreciably change neonatal serum methadone concentrations. (Jansson 2008b). However, a pharmacodynamic effect is suggested, as breastfed infants have decreased severity of NAS or need for treatment with pharmacologic agents.(Jansson 2008b, Abdel-Latif 2006) While this effect may reflect the calming effect of the act of breastfeeding rather than drug effect, (Liu 2008) there is potential for breastfeeding to introduce bias in treatment effect or to cause a drug interaction between methadone and

buprenorphine. It is not expected that this exclusion will impact recruitment, as <5% of females maintained on methadone choose to breastfeed their infants at TJUH.

2. Benzodiazepine Exposure

A retrospective study at TJUH demonstrated that the length of NAS treatment for all non-benzodiazepine exposed infants between 2000-2006 was 31 days, while for all (including polydrug-exposed) term infants it was 38 days. (Seligman 2008) This value is comparable to that seen in the control group in our published study and follow up pilot clinical trial data in the buprenorphine trial (Kraft 2008), i.e. length of treatment of 34 days and length of stay of 39 days (n=24), though this cohort excluded infants with benzodiazepine exposure. What is striking is that benzodiazepine-exposed infants at TJUH have a 47-day length of stay, suggesting that these patients clearly represent a subpopulation which differs from those without this exposure.

3. Preterm Infants

Preterm infants have a well-described natural history of NAS and a need for treatment that differs from term infants. The current NAS scoring instruments have not been examined in this population. Length of stay is shorter in the preterm population.(Seligman 2008, Dysart 2007) The preterm population thus appears to be categorically different in terms to in utero opioid exposure. Finally, the safety of buprenorphine has not yet been fully defined in the preterm population.

12. STUDY POPULATION

a. Efficacy

Subject demographics, including sex, time to treatment, apgar scores, maternal methadone dose, tobacco exposure, and concomitant prepartum maternal medications will be collected. Length of treatment and length of stay will be collected in units of days. Group comparisons for continuous variables will be made using the Student's t-test or the Wilcoxon Rank Sum test where appropriate.

b. Safety

The safety and tolerability of sublingual buprenorphine and oral morphine will be evaluated by tabulating adverse events. Summary statistics will be used to describe relative rates in major organ systems.

c. Data unblinding plan

Unblinding of treatment allocation will take place for any infant who requires adjunctive treatment with phenobarbital. An investigator can unblind a study patient if he or she believes it is in the best interest of the infant. The analytical chemistry lab will be provided with an allocation schedule to facilitate analysis of the proper analyte. Investigators will be unblinded following receipt of analytical drug concentration data. No formal interim analysis for efficacy will be performed prior to the completion of the trial.

13. STUDY POPULATION

a. Inclusion Criteria

Patients eligible for participation include:

- ≥ 37 weeks gestation
- Exposure to opiates in utero
- Demonstration of signs and symptoms of neonatal abstinence syndrome requiring treatment

b. Exclusion Criteria

Patients ineligible for participation include:

- Major congenital malformations and/or intrauterine growth retardation (Mamelle 2001)
- Medical illness requiring intensification of medical therapy. This includes, but is not limited to suspected sepsis requiring antibiotic therapy.
- Hypoglycemia requiring treatment with intravenous dextrose.
- Bilirubin >20 mg/dL (The need for phototherapy is not exclusionary)
- Concomitant benzodiazepine or severe alcohol abuse, self-report of regular use of alcohol or of

benzodiazepines use in the past 30 days, and/or receipt of benzodiazepines by prescription (as determined by self-report or intake urine) by the mother 30 days prior to birth,

- Concomitant use of CYP 3A inhibitors (erythromycin, clarithromycin, ketoconazole, itraconazole, HIV protease inhibitors) or inducers (rifampin, carbamazepine, phenobarbital) prior to initiation of NAS treatment
- Seizure activity or other neurologic abnormality
- Breast feeding
- Inability of mother to give informed consent due to co-morbid psychiatric diagnosis

c. Randomization

A randomization key will be generated by a computerized method and maintained by the study statistician. Randomization will be managed by the unblinded pharmacy service. Patients will not be randomized until NAS score criteria for pharmacologic treatment are met. Patients will have stratified randomization based upon maternal use of buprenorphine or morphine use during pregnancy. Use will be defined as the plurality of days in the 30 days prior to birth during which either drug was used.

d. Patient Baseline and Allocation Numbers

All patients for whom consent is obtained will be given a four-digit baseline number that begins with sequence 1001. Patients randomized to treatment will receive a three-digit allocation number that begins with sequence 001.

14. STUDY POPULATION

1.1 Identity

All study drug will be prepared in a unit dose based upon weight in 1 mL pediatric dispensing vials (Healthcare Logistics Cat. # 7870 or equivalent). Preparation and stability for each product is listed in Appendix 3. Each syringe will be marked as “morphine/placebo for morphine” or “buprenorphine/placebo for buprenorphine”.

1.1.1 *Morphine Oral Solution*

Neonatal morphine solution will consist of morphine, water, and FD&C Green #3. The final concentration of morphine is 0.4 mg/mL total volume.

1.1.2 *Placebo for Morphine Oral Solution*

Placebo for neonatal morphine solution is water and FD&C Green #3.

1.1.3 *Buprenorphine*

Buprenorphine solution will consist of simple syrup, ethanol 30% final volume, and buprenorphine. The final concentration of buprenorphine is 0.075 mg/mL total volume.

1.1.4 *Placebo for Buprenorphine*

Placebo for buprenorphine solution will consist of water and simple syrup in a 1:1 ratio.

a. Calculation of Dose

i. Buprenorphine

The blinded pharmacist will use the patient <u>birth weight</u> for the calculation of all doses of buprenorphine. This includes protocol specified maximum of 60 mcg/kg/day for buprenorphine.

1.1.5 *Morphine*

The blinded pharmacist will use the patient's <u>ongoing daily weight</u> recorded in the medical record for the calculation of all doses of morphine. This includes protocol specified maximum of 1 mg/kg/day for morphine.

1.1.6 *Starting doses*

The starting daily dose for buprenorphine will be 15.9 mcg/kg/day (5.3 mcg/kg/q8 hours). The starting dose for morphine will be 0.4 mg/kg/day (0.7 mg/kg/q4 hours).

1.1.7 *Phenobarbital*

Actual weight at the time of reaching threshold maximum dose will be used for the calculation of phenobarbital loading and maintenance dose.

b. Administration

To allow for alterations in sleeping and feeding schedules, each dose of drug can be administered +/- 30 minutes around the nominal time point for that dose. Actual time that each dose was administered must be recorded in the medical record.

i. Order of Study Drug Administration

At the time points when both study drugs are administered, morphine/placebo for morphine will be

administered first. Approximately two minutes will separate administration of morphine/placebo for morphine and buprenorphine/placebo for buprenorphine.

ii. Morphine Oral Solution/Placebo for Morphine

The nurse administrator will administer drug into the oral cavity.

iii. Buprenorphine/Placebo for Buprenorphine

The study drug administrator will hold the child's head at approximately 45 degrees, gently move the tongue to the side, administer the drug under the tongue, and immediately place a pacifier in the mouth to reduce swallowing of drug. If the volume of the drug is >0.5 ml, half of the drug will be administered, followed by the remainder of the dose in approximately 2 minutes.

c. Drug Product Quality Control

i. Good Manufacturing Procedures

Preparation of stock solution for active drug and placebo will take place in the investigational drug pharmacy of TJUH.

ii. Stability

Buprenorphine/Placebo for Buprenorphine solution will be used within 30 days of stock drug preparation. The stability of the stock solution has been demonstrated by the liquid chromatography-electrospray ionization-tandem mass spectrometry. (Anagnostis 2011) Preservative-free morphine hydrochloride solution for neonatal administration is stable at 4 degrees celsius for at least 6 months. (Colombini 2008)

iii. Investigational New Drug (IND) Certification

This protocol is being conducted under existing IND # 68,403

15. STUDY PROCEDURES

a. Identification of potential study subjects

Potential subjects will be identified through the outpatient treatment clinics. Additional potential subjects will be identified by review of all infants at risk for NAS on the basis of maternally identified use of opioid therapy. All infants from whom consent has been obtained will have NAS graded according to the NAS score (Appendix #2) administered every 4 hours. Infants with the sum of three scores $\geq$ 24 or more or a single score of $\geq$ 12 will be eligible for randomization. The ultimate decision to initiate treatment will be

that of the treating pediatrician. A child will be randomized only after a definite decision to treat is made. All infants who have genetic consent obtained will have a blood sample for DNA analysis obtained, ideally at the time of a clinically indicated draw.

NNNS assessment will be performed at day 2 of life and prior to initiation of pharmacologic therapy, 10 days of life, and in the post therapy period (but no later than corrected post gestational age of 46 weeks). Corrected post gestational age is gestational age at birth + weeks of life. Comparisons of interest will be differences in NNNS scores between treatment arms, correlation of NNNS scores with the NAS, and an examination of day 2 NNNS scores in those infants requiring pharmacologic therapy for NAS and those infants who are discharged without need for therapy.

Infants not meeting the treatment threshold criteria will be observed at least 2 days postpartum in an inpatient setting prior to discharge. Infants who have the sum of three scores $\geq$18 but < 24 can be observed additional days as inpatients at the discretion of the attending physician.

Maternal opioid dose, defined as the current dose taken for at least 7 days, will be recorded. Other maternal demographic information to be collected will be type and dose of concomitant medications, tobacco use (none, <5 cigarettes/day, >5 cigarettes/day), maternal alcohol use, urine drug screen results and self report of other drugs of abuse. Neonatal demographics include birth weight, gestational age, apgar scores, bilirubin and urine drug screen results.

b. NAS Scoring procedures

NAS scoring for each subject will take place at 4 hour intervals (+/- 30 minutes to account for sleeping and feeding schedule). The exact time of scoring will be recorded in the medical record. A validated scorer will be used to train and observe nursing staff who will administer the NAS score.

c. Randomization

A randomization key will be kept by the unblinded pharmacist. The randomization procedure will consist of 1) a decision of the attending pediatrician to treat NAS with pharmacologic means, 2) contact of the study team with the investigational pharmacist, and 3) preparation of blinded study drug. An allocation number will only be used once.

d. Dose administration

Every eight hours each subject will receive sublingual buprenorphine/placebo for buprenorphine and oral

morphine/placebo for morphine. At the 4-hour time point between each of these intervals, each subject will receive oral morphine/placebo for morphine. There is a +/- 30 minute interval around each nominal time point to account for sleeping and feeding schedule. A dose should not be delayed more than 30 minutes past nominal dosing time due to sleep. If dosing occurs at a time different from the specified nominal time, the next dose will be scheduled to take place 4 hours following the actual dose administration. The exact time of drug administration will be recorded in the medical record. If unblinding takes place for any reason, co-administration of placebo treatment will stop.

e. Dose escalation

Dose escalation will take place if 1) the sum of 3 NAS scores is $\geq$ 24 or a single score is $\geq$ 12 OR 2) a rescue dose was administered [section 7.6]. To mimic actual clinical care, dose advancement will generally take place in daylight hours when the primary team caring for the patient is present. However, dose advancement may take place when the primary team is not present (such as would occur on evenings and nights). All dose decisions will be made based on the three most recent scores. No more than one dose escalation can take place each day, unless there is need for an additional rescue dose post-escalation.

f. Rescue dose

If, between scheduled doses, a child has a single score of $\geq$ 14, a rescue dose may be administered at the discretion of the treating physician. The rescue dose will be 50% of the previous dose. To maintain blinding, both active drug (morphine or buprenorphine) as well as placebo for morphine or buprenorphine will be administered at the same time. A rescue dose must be given at least 1 hour after and 1 hour before the next scheduled dose. If unblinding takes place for any reason, co-administration of placebo for rescue dose will stop.

g. Weaning

The initiation of weaning can take place as soon there are 48 hours of stability without dose advancement. Doses will be weaned when the sum of the previous three scores is < 18 and no single score is $\geq$ 8. Dose reductions in both the buprenorphine and morphine groups will occur at a rate of 10% per wean. If the sum of the previous three scores is $\geq$ 28, the standing dose will revert to the previous dose at which symptoms were controlled.

Weaning is expected to take place during daylight hours when the primary team is present, but dose adjustments can take place on evenings and nights. Only one wean of dose will take place each day. A rescue dose may be administered at the discretion of the treating physician during the weaning period if a single score $\geq$ 14. A rescue dose during the wean will be 50% of the previous dose. The administration of a

rescue dose in the weaning period will not trigger a dose escalation.

h. Dose cessation and observation

i. Buprenorphine cessation

Cessation of drug administration will occur in the buprenorphine arm when

- Dose is within 20% of the initial dose AND the sum of the previous three scores is < 10. If the sum three scores at this dose if ≥10 but < 18, the dose will be weaned to within 10% of the initial dose.

OR

- Dose is within 10% of the initial dose and the sum of the previous three scores is < 12, therapy.

ii. Morphine cessation

Cessation of drug administration will occur in the morphine arm when the dose is 0.15 mg/kg/day AND the sum of the previous three scores is < 10.

iii. Post Cessation Observation

Following cessation of dosing, children will be observed in an inpatient setting for at least 2 days, during which time scoring of NAS symptoms will continue. A rescue dose after cessation of therapy may be given at the discretion of the treating physician for any score of ≥ 12. The amount of drug administered will be the last dose the patient had received. An appropriate placebo for rescue dose will also be provided. If a post cessation rescue dose is given, patients must be observed at least 1 day following the last rescue dose.

i. Maximum dose and use of adjunctive phenobarbital

When the study pharmacist notes that a maximum dose of buprenorphine (60 mcg/kg/day) or morphine (1 mg/kg/day) has been achieved, he or she will notify the study team of a need for phenobarbital treatment. Phenobarbital will be initiated with a loading dose of 20 mg/kg followed by 5 mg/kg/day in two daily divided doses. Treatment with adjunctive phenobarbital will continue for at least two days. When the sum of the previous three scores is < 18 and no single score is ≥ 8, the attending physician may decrease the phenobarbital dose to 2.5 mg/kg/day in two daily doses and observe for at least 24 hours following dose reduction. When the sum of the previous three scores is < 18 and no single score is ≥ 8, the attending physician may discontinue phenobarbital. Primary therapy will not be weaned until phenobarbital is discontinued. Rescue doses of morphine or buprenorphine cannot be administered at the maximum dose. If symptoms of NAS are not controlled with phenobarbital 5 mg/kg/day, this can be titrated up by the treating physician to a serum concentration of 20-40 mg/dL.

j. Blinding

i. Unblinded Personnel

The staff of the Investigational Drug Service will maintain the randomization key, which will be generated by Pharmacy staff or by an unblinded statistician. All of the investigators, study coordinators, treating physicians, and nursing staff will remain blinded throughout the study. The Investigational Drug Service will provide the analytical collaborator a list of treatment allocation for each shipment of pharmacokinetic samples.

ii. Unblinding Due to Maximal Dose Triggers

Patients will have unblinding of treatment if 1) a patient allocated to buprenorphine requires adjunctive treatment with phenobarbital OR 2) a patient allocated to morphine has 7 up-titrations of dose. In both cases, these milestones will unblind treatment allocation to physicians and investigators. When this protocol-specified unblinding occurs, the placebo dummy treatment arm will cease and patients will be treated in an open-label fashion.

iii. Unblinding due to Emergent Cause

If an investigator or treating physician judges a need to unblind treatment, an investigator will contact the Investigational Drug Service to unblind treatment allocation. The rationale for the unblinding will be recorded in the patient's chart. When this emergent unblinding occurs, the placebo dummy treatment arm will cease and patients will be treated in an open-label fashion. The occurrence of a serious adverse event may, but not necessarily definitely, trigger unblinding.

The following medical issues that develop following randomization would not necessarily require withdrawal of the patient from double blinded treatment:

• Hyperbilirubinemia requiring phototherapy • Antibiotic therapy for suspected infection • Oral candidiasis • Candidal Skin Infection	• Paronychia requiring antimicrobial therapy • Gastroesophageal reflux requiring antisecretory therapy

k. Study Data

The following elements will be collected in the study data base:

Mode of birth, gestational age, gestational weight, apgar scores, NAS scores, daily weight, head circumference, concomitant medication, NNNS scores, and urine drug screen results.

Maternal elements to be collected include:

Methadone or buprenorphine dose, urine drug screen, tobacco use (none, <5 cigarettes/day, >5 cigarettes/day) and concomitant medication.

l. Blood samples for pharmacokinetics

Pharmacokinetic samples will be drawn on all patients randomized in the trial. Capillary blood samples will be drawn by heel stick with a goal volume of 0.4 ml blood into a lithium heparin tube. An outline of a sampling schedule is listed below. In light of the sparse sampling regimen, some allowance for variation from this schedule is anticipated to reflect feeding and sleeping schedules for the child. Wherever possible, pharmacokinetic samples will be paired with a standard of care blood draw. A description of blood processing is outlined in Appendix 4.

Table 5: Schedule of Pharmacokinetic Blood Draws

Week 1	Peak within 24 hours of initiation of therapy
	Peak and trough surrounding single dose x 2
	Single mid-interval dose
Weeks 2 onward	Peak and trough surrounding single dose
	Single mid-interval dose
Dose Cessation	Single sample between 12-24 hours after final dose
Periods of Co-administration of phenobarbital	Peak and trough surrounding single dose every three days

m. Urine samples for metabolite analysis

Two four-hour urine collections will take place while on treatment. Urine will be collected by means of a standard urine bag. A urine collection will take place during week one and week two to treatment. Patients randomized to morphine who have treatment allocation unblinded will not have urine collection. A description of urine processing is outlined in Appendix 4.

n. Blood samples for pharmacogenomics

A single 0.2-0.4 ml whole blood sample is collected in a lithium heparin tube. This sample will be collected prior to the initiation of therapy. If this is not possible, the sample can be collected at any time post randomization. Wherever possible, pharmacogenetic samples will be paired with a standard of care blood draw. A description of blood processing is outlined in Appendix 4.

o. Blood samples for liver function testing

All patients randomized for therapy will have blood drawn at predose, and at 7 (+/- 2) and 21 (+/- 2) days

after the first dose of study medication in both buprenorphine and morphine groups.

16. STATISTICAL/ANALYTICAL METHODS AND POWER ANALYSIS

a. Efficacy

The sample size of 40 neonates per treatment arm will provide 90% power to detect 28% decrease in length of treatment (LOT) on buprenorphine as compared to oral morphine (difference of 0.323 on the log scale) or 80% power to detect 24% decrease in LOT on buprenorphine as compared to oral morphine (difference of 0.279 on the log scale), assuming a common standard deviation of 0.44. Similarly, we will have 90% power to detect 23% decrease in LOS on buprenorphine as compared to oral morphine (difference of 0.264 on the log scale) and 80% power to detect 20% decrease in LOS on buprenorphine as compared to oral morphine (difference of 0.228 on the log scale), assuming a common standard deviation of 0.36.

Eighty opioid-exposed neonates will be randomized in 1:1 ratio to either buprenorphine or oral morphine. The primary endpoint for this trial is the LOT. Log transformed LOT will be compared between buprenorphine and oral morphine treated neonates using two-sample t-test with alpha=0.05. In secondary analysis, we will evaluate the effects of tobacco use, antidepressant use and methadone dose on LOT and LOS using the standard multivariate regression analysis. In the exploratory analysis, repeated over time NNNS scores will be analyzed in a linear mixed effects model incorporating random effects of subject and, respectively, correlation among the measurements from the same subject. If necessary, transformation of the NNNS scores will be used to satisfy the normality assumptions. The during-treatment (10 days post initiation of therapy) and post-treatment difference between treatment arms will be evaluated using the fitted mixed effects model. In a separate linear mixed effects model for NNNS measures we will incorporate the NAS score as a predictor of NNNS to evaluate the strength of association between NAS scores and NNNS scores. In addition, the difference in day 3 NNNS scores between infants requiring pharmacologic therapy for NAS and those infants who are discharged without need for therapy (as determined by the NAS scores) will be evaluated using either two-sample t-test or Wilcoxon test.

b. Pharmacometrics

The population PK/PD data will be analyzed using nonlinear mixed-effect modeling with the NONMEM software system (Version VI, Level 1.1, GlobalMax LLC, Hanover, MD, USA) with the PREDPP model library and NMTRAN subroutines. Sparse datasets for buprenorphine and morphine will also be analyzed using advanced modeling software (e.g. S-ADAPT/WinBUGs) to better support modeling and simulation efforts. Based on established population PK models, parent-metabolite relationship will be used to further

understand buprenorphine and morphine disposition characteristics and inter-individual and residual variability in the study population. Model selection criteria will be based on diagnostic plots (predicted versus observed concentrations, residuals versus predicted concentrations, weighted residual versus predicted concentrations), reasonable parameter estimates, precision of the parameter estimates, random residual variances, and objective function values. To discriminate between competing models, a decrease in the OFV > 10.83 will be considered significant ($p < 0.001$).

Drug-drug interaction and developmental changes in newborns with NAS will be evaluated using NONMEM and SIMCYP (Version 8.1, SIMCYP Inc, Sheffield, UK) with buprenorphine and morphine as probes. Comparison of effectiveness in two treatment arms will be assessed in terms of patient variables. Simulation will be performed based on the best model selected which provides accurate and precise estimates of inter-subject variability and the mean parameter values. The simulation would provide initial dose strategy for drug treatment and allow Baysian feedback analysis for dose individualization. No formal power analysis is performed. This analysis is primarily descriptive and will build upon existing buprenorphine and morphine neonatal models.

c. Pharmacogenetics

In addition to 80 patients enrolled in this trial, the data from ~60 subjects who did not require pharmacologic treatment for NAS will be available for analyses. Also the data from ~25 patients enrolled in the previous studies (15 requiring and 10 not requiring treatment) are available. Thus, we expect to analyze the rate of A118G allele in the total of 165 patients, 95 requiring and 70 not requiring treatment for NAS. The rate of A118G allele will be compared between the patients that do not require pharmacologic treatment for NAS (~70) combined with ~47 patients that did require the treatment but the length of treatment was below the median LOT in the corresponding treatment arm vs. ~48 patients that did require the treatment with LOT above or at the median LOT in the corresponding treatment arm. We expect that the A118G variant allele for the OPRM1 gene has a frequency ~12-15% in our population. (Drakenberg 2006) Our hypothesis is that the rate of A118G allele will be higher in the latter group of patients requiring longer LOT. This hypothesis will be tested using Fisher's exact test (two-sided, alpha=0.05). With our sample size, we have 80% power to detect as statistically significant the difference between 7.5% rate of A118G allele in 117 patients not treated or with LOT< median LOT and 26% rate of A118G allele in 48 patients with LOT> median LOT. These assumptions imply overall ~13% rate of A118G. In the secondary analyses, we will also compare the LOT between patients with and without A118G allele while controlling for the treatment difference. As secondary endpoints we will also consider the polymorphism status of dopamine receptor type 1, preproenkephalin and preprodynorphin genes. Finally, a multivariate logistic regression model to predict propensity to develop NAS using the genetic polymorphism status of known SNP's affecting response to

opioids as predictors will be built.

17. SAFETY PARAMETERS

a. Blood volume

No more than 12 ml of blood will be drawn over the course of the study. This represents a maximum, and not the typical amount drawn. This will include study-related blood draw as well as clinically indicated collection. Total blood volumes are estimated in Table 6.

Table 6: Blood Volume for Study Participants

Procedure	Research Related?	Total Number of Collections	Blood (mL) per Test	Total Blood (mL/test)
Newborn Hematology/chemistry	No	1	0.8	0.8
PKU screen	No	1	0.4	0.4
Pharmacokinetic sample	Yes	1	0.4	0.4
Liver Function Test	Yes	3	0.4	1.2
Buprenorphine or morphine assay	Yes	20*	0.4	8.0*
Maximum amount of blood drawn per female patient				10.8*
Circumcision blood loss (estimated)				0.2
Maximum amount of blood drawn per male patient				11.0*

* maximum

b. Stopping Rules

No further enrollment will take place following a SAE judged to be probably or definitely related to study treatment until review by the DSMB, FDA, and the NIDA Program Official. The treatment allocation for the infant with the SAE will be unblinded. There is no prespecified stopping rule for trial cessation. There is no predefined interim look for safety or efficacy. If the incidence of non-serious AE's is higher than expected, the PI can propose an ad hoc interim look to the DSMB and NIDA Program Official. The DSMB and NIDA Program Official will also receive biannual blinded summary statistics of adverse events. If both parties are in agreement, an interim look can be undertaken. The study statistician and investigational drug service staff will provide the unblinded adverse event data. All attempts will be made to maintain the blind of the principal investigator and clinical staff, unless DSMB makes a study-wide decision to unblind the study.

c. Unblinding Procedures

Either the Investigational Drug Service or study statistician(s) can provide treatment allocation if the

investigator believes it is in the interest of the study patient to have this information available for treating physicians. Unblinding can take place by verbal discussion, but will need written documentation to be placed in the subject's research chart.

d. Data Safety Monitoring Board

A DSMB will evaluate each serious adverse event judged by the investigator to be possibly, probably, or definitely related to study drug. The DSMB will provide binding recommendations to the investigators. There will be no planned interim analysis or prespecified stopping criteria for futility or efficacy.

e. Certificate of Confidentiality

A certificate of confidentiality will be obtained prior to enrollment of any protect to protect privacy of neonates and their mothers

f. Study-specific Risks

Respiratory Depression

A dose-dependent effect of all opioids is respiratory depression. In adults, buprenorphine (a partial agonist) causes less respiratory depression and fewer overdose-related deaths than full agonists such as morphine. While respiratory depression has been noted with accidental home ingestion, buprenorphine is anticipated to have less potential for hypoventilation than morphine in pediatric patients. Respiratory depression effects are mitigated by careful monitoring in an inpatient, high acuity setting. Excessive sedation and respiratory compromise with standard of care morphine is exceedingly rare in such settings. Following administration of >1600 doses of sublingual buprenorphine to 25 infants, no respiratory depression has been noted. In the unlikely event of buprenorphine-induced respiratory depression, naloxone has demonstrated reversibility in pediatric patients. Naloxone has the potential to precipitate acute withdrawal symptoms, so any use will be titrated to adequate respiration. Any treatment emergent use of naloxone will be considered an SAE, prompting immediate unblinding and halting of further enrollment.

Other Medical Risks

There are anticipated to be few mechanism-based risks specific to opioid treatment outside of theoretical risks of respiratory depression or excessive sedation. A symptom-driven dose titration serves to minimize risks of over-, or under-treatment with opioids. This approach in standard of care treatment of the neonatal abstinence syndrome is very effective in maintaining drug dose within a therapeutic window. All infants will be monitored in a high acuity of care setting on 24-hour telemetry monitoring of heart rate and respiratory function. Treatment of other emergent adverse events, whether judged to be drug related or not, will be

managed by the neonatology staff of the neonatal intensive care unit (NICU). The NICU has 24-hour senior level physician coverage and access to all subspecialty consultants. The case mix includes a wide spectrum of illness through critical care. The staff is able to manage, jaundice, vomiting, seizures, and infections.

If there is an SAE with unblinding, the decision to cease treatment (buprenorphine or morphine) will be informed by investigator assessment of causality to study drug. Depending upon the clinical situation, options could include 1) continuation of study treatment (buprenorphine or morphine) in an open label fashion with or without dose adjustment, 2) transition from buprenorphine to open label morphine, 3) transition from an opioid to phenobarbital monotherapy, or 4) cessation of all abstinence pharmacotherapy. In any case, treatment will follow clinician guidance and will not be protocol-driven. However, such infants will be followed in an intention to treat analysis and will be included in safety analysis.

Novel drug administration route

Buprenorphine will be administered by a sublingual route. An initial dose is 15.9 mcg/kg/day, with a maximum dose of 60 mcg/kg/day. Dose advancement is protocol driven based upon the NAS score. Due to limitations on blood volume in neonates, it is not possible to determine data-rich estimates of absorption rate constants or dose-to-dose variability.

It should be noted that there are no published data on the pharmacokinetics of oral morphine in neonates. Indeed, this is one of the primary goals of our proposed project. Intravenous morphine is associated with large intra-patient variability in serum concentration and pharmacodynamic response. (Bouwmeester 2003, Bouwmeester 2004, Barrett 1996) On theoretical grounds, buprenorphine-induced respiratory depression is less likely than the current standard of morphine. Morphine, as a full mu opioid receptor agonist, has a higher propensity for overdose-induced respiratory depression. The large first pass metabolism of morphine in adults with significant intra-patient variability is well recognized. (Lotsch 1999) Limited data for pediatric patients under the age of 11 suggest similar or greater variability. (Hunt 1999) The dynamic and variable nature of the ontogeny of pre- and post-systemic metabolism in neonates (Alcorn 2002a, Alcorn 2002b) likely contributes to greater variability in oral morphine disposition than in older pediatric patients for which data exist.

Taken together, these observations suggest that sublingual buprenorphine is safe based upon 1) extensive safety experience thus far, 2) all measured pharmacokinetic samples well within the safe therapeutic window, 3) pharmacokinetic modeling suggesting dose-to-dose variability that is comparable to adults, 4) monitoring of administration in a high acuity clinical setting, 5) established variability in oral morphine, which despite its potential for clinical adverse events as a full agonist, is well tolerated in the inpatient setting, and 6) pharmacologic properties of buprenorphine which make respiratory depression unlikely, even in the setting

of excessive dosing, and 7) administration of buprenorphine (with ~20 half-life in the study population) every 8 hours, which minimizes drug peak and trough concentrations.

APPENDIX 1: ADVERSE EVENT COLLECTION FORM

ADVERSE EXPERIENCE

Did patient have any adverse experiences? Yes ☐ No ☐ Initial/Date:_____

Is this AE serious? ☐ Yes ☐ No	Type of Adverse Experience: ☐ clinical; ☐ laboratory; ☐ other, specify:_____
AE Term:	
Worsening of pre-existing condition (Y/N)? _____	
Onset date:_____; Onset time:_____; Stop Date:_____; Stop Time_____; Duration (if <24 hrs):_____; Continuing (if >24 hrs):_____(Y/N)	

Maximum Intensity	☐	[1=mild, 2=moderate, 3=severe]
Test Drug Relationship:	☐	definitely not
	☐	probably not
	☐	possibly
	☐	Probably
	☐	definitely
Rx action taken:	☐	no action was taken with test drug
	☐	test drug was discontinued
	☐	test drug was increased
	☐	test drug was interrupted
	☐	test drug was reduced
Did AE result in:	☐	Death
	☐	Inpatient hospitalization or prolongation
	☐	Persistent or significant disability/incapacity
	☐	Immediately life-threatening
	☐	Due to overdose
	☐	Important medical event
	☐	None of the above

APPENDIX 2: NAS SCORING SYSTEM

Scored Elements	
Signs and Symptoms	*Score*
Crying: Excessive high pitched	2
Crying: Continuous high pitched	3
Sleeps < 1 hours after feeding	3
Sleeps < 2 hours after feeding	2
Sleeps < 3 hours after feeding	1
Hyperactive Moro Reflex	1
Markedly Hyperactive Moro Reflex	2
Mild Tremors: Disturbed	1
Moderate-Severe Tremors: Disturbed	2
Mild tremors: Undisturbed	1
Moderate-Severe Tremors: Undisturbed	2
Increased Muscle Tone	1-2
Excoriation (Indicate specific area):	1-2
Generalized Seizure (or convulsion)	8
Fever > 37.3 C (99.2 F)	1
Frequent Yawning (4 or more successive times)	1
Sweating	1
Nasal Stuffiness	1
Sneezing (4 or more successive times)	1
Tachypnea (Respiratory Rate >60/mm)	2
Poor feeding	2
Vomiting (or regurgitation)	2
Loose Stools	2
Failure to thrive (Current weight > 10% below birth weight 90% BWT=_____ *(record weight in score box 1 x day)*	2
Excessive Irritability	1-3
Total Score	
Unscored Elements	
Convulsions	Present/absent
Fever > 38.4 C (101.2 F)	Present/absent
Mottling	Present/absent
Excessive sucking	Present/absent
Watery Stools	Present/absent
Projectile vomiting	Present/absent
Retractions	Present/absent
Nasal flaring	Present/absent
Myoclonic jerks	Present/absent

APPENDIX 3: PREPARATION OF BUPRENORPHINE AND MORPHINE STOCK SOLUTIONS AND STABILITY

	Stability of Stock
Buprenorphine 0.075 mg/mL Composition of neonatal stock solution • One 0.3 mg ampule buprenorphine [Buprenex 0.3 mg/ 1 ml (Reckitt Benckiser) or generic buprenorphine for injection] • Ethanol to bring to final concentration of 30% (1.26 mL of 95% ethanol USP) • Simple syrup USP(Sucrose, Purified Water and 0.1% Sodium Benzoate) to bring to 4 mL total volume [Humco or equivalent] 0.3 mg buprenorphine per vial * 4 mL $^{-1}$ (final volume) = 0.075 mg/mL	3 days
Placebo for Buprenorphine Composition of neonatal stock solution 1:1 ratio of the following • Simple syrup USP (Sucrose, Purified Water and 0.1% Sodium Benzoate) [Humco or equivalent] • Sterile water for injection USP	1 month
Morphine 0.4 mg/ml Composition of neonatal stock solution • 1 part Morphine oral solution 4mg/ml [Roxane or equivalent] • 9 parts sterile water for injection USP	1 month
Placebo for Morphine Composition of neonatal stock solution • Sterile water for injection USP • FD&C Green #3	1 month

APPENDIX 4: PROCESSING OF PHARMACOKINETIC AND PHARMACOGENETIC SAMPLES

Buprenorphine and its metabolites are stable in frozen plasma at -20 C for at least 6 months. (Huang 2006) In urine, there is stability for 16 hours at 22 C, 72 hr at 4 C and through 3 freeze/thaw cycles. (Kacinko 2008)

Pharmacokinetic Serum Samples

Samples will be obtained by capillary heel stick into lithium heparin pediatric tubes (BD Microtainer, Ref # 365971 or equivalent). A goal of 400 microliters should be collected. Blood is spun at 3,000 RPM on a refrigerated tabletop centrifuge for 10 minutes, and plasma transferred to storage tubes and frozen at -20 C. Blood from an indwelling catheter can be used if one is present for medical care unrelated to the treatment of NAS.

Pharmacokinetic Urine Samples

Volume of urine will be recorded, as well as time of start and stop of urine collection. Uncentrifuged urine from the collection bag will be transferred to a polypropylene tube and frozen at – 20 C. Volume in excess of 10 ml will be discarded.

Blood for DNA

Blood for DNA analysis will be collected by capillary heel stick into an uncoated capillary pediatric tube. Blood can also be collected in tubes containing anticoagulants.

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2 AMENDMENT HISTORY

A Randomized, Active-Control, Double-Blind, Double-Dummy Clinical Trial Comparing Sublingual Buprenorphine and Morphine Solution for The Treatment Of Neonatal Opioid Abstinence Syndrome

2.1 Amendment 1

Existing Protocol Version: 1

Revised Protocol Version: 2

Amendment Date: October 13, 2011

Number of subjects Enrolled at time of amendment: 0

Major Changes

Major Change

Stratification of randomization to in utero exposure of buprenorphine or methadone.

Rationale

The MOTHER study (Jones HE, et al.: N Engl J Med; 2010; 9;363(24):2320-31) demonstrated improved neonatal abstinence outcomes for infants exposed to buprenorphine compared to methadone. Stratification will ensure that equal numbers of infants with each exposure will be equally represented in each treatment arm. Currently, a minority of infants at risk for NAS at Thomas Jefferson University Hospital (TJUH) have in utero buprenorphine exposure. Buprenorphine use among mothers is expected to increase through the duration of the study period.

Potential change in risk

This change will not entail increased risk to study subjects.

Minor Changes

1. Study Synopsis and Appendix 2. Change of concentration of simple syrup in buprenorphine placebo from a 1:1 ratio to the ratio used in the active drug solution. *The rationale is to standardize the amount of sucrose in each dose in light of the analgesic effects of sucrose solutions in neonates.*
2. Section 1.2.1.3 the "Bogenschutz 2008" abstract reference was updated to reflect the publication of full results in a 2010 manuscript.
3. Appendix 1 (adverse event source document) was removed. All source documents will be maintained in a location separate from the protocol. All appendix numbers have been updated.

4. Section 4, "STUDY POPULATION" has been changed to "STUDY DATA". *This will remove repetitive elements outlined in other sections of the protocol and standardize location of protocol sections.*
5. Section 5.3. The investigational drug service pharmacist will generate and maintain the randomization code rather than the study biostatistician. *This section is now consistent with existing section 7.10.1.*
6. Section 6.1. Clarification that drug will be delivered to the floors in bulk rather than unit dosed. *This will eliminate the prior practice of having multiple syringes in the drawer, which could lead to misdosing.*
7. Section 6.2. Clarification that for both arms, weight at time of allocation will be used to fix initial dose and that there will not be dose adjustments based upon weight after this time. *This is the mode of dose calculation used in the pilot study and current standard of care therapy.*
8. Section 7.15. Data parameters for the 4 hour urine collection are specified.
9. Section 7.16. Adding language the DNA can be collected by either cheek swab or heel stick. *The current plans are to continue heel stick blood collection, but this has been added to allow the future substitution of cheek swab if the quality of DNA can be assured.*
10. Section 7.17. Specific liver function tests to be measured are listed.
11. Section 7.18. Additional clarification of procedures for the Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) are provided.
12. Section 8.4. Final unblinding procedures are provided.
13. Table 6. "Pharmacokinetic" was replaced with "Pharmacogenetic".
14. Reference. Update Anagnostis EA article to "In Press".

Typographical Corrections

1. Study Synopsis, correction of buprenorphine concentration from "0.4" to "0.075".
2. Section 1.1, change "or" to "of".
3. Section 1.2.1.3, clarification of unclear language.
4. Section 3.2.1.2, change "calender" to "calendar".
5. Multiple capitalization changes of section headings were made to standardize format.

2.2 Amendment 2

Existing Protocol Version: 2

Revised Protocol Version: 3

Amendment Date: April 19, 2012

Number of subjects Enrolled at time of amendment: 4

Major Changes

1. Addition of an exploratory endpoint examining effects of treatment on feeding (Section 2.3)

Optimizing growth and development is a primary reason to use pharmacologic treatment for NAS. Understanding the amount and types of feedings may help optimize dose and treatment choices. Collection of this information has been facilitated by use of the hospital electronic medical record.

2. Morphine dose
    a. Increase morphine dose advancement rate from 10 to 20%. (Sections 3.1, 6.2.2)

This change reflects a change in practice at Thomas Jefferson University Hospital for more aggressive up titration since the pilot phase 1 study. Matching standard of care will help increase the external validity of study results. This dose advancement schedule has been used extensively without evidence of respiratory depression. The very small increase in risk with increased dose is balanced with likely decreased length of hospitalization and associated risks of nosocomial complications and impairment of maternal bonding.

    b. Increase the maximum morphine dose to 1.25 mg/kg day. (Section 3.4.1.1)

The rationale for this change is to more closely match recent practice patterns and facilitate blinding of treatment allocation. Coupled with the increase in the uptitration of morphine, this will allow the attainment of maximum dose to match that of buprenorphine at 6 uptitrations. This change will prevent the unblinding at maximum dose seen with the previous morphine regimen. The 1.25 mg/kg/day dose is well within the range employed at TJUH and other institutions.

    c. Change cessation weight to be based upon actual rather than birth weight. (Section 6.2.1)

This clarifies protocol language which was not clear about cessation dose. Standard practice at TJUH is to fix a titration schedule based upon birth weight, but to establish cessation dose based upon actual weight. This corrects for infant weight and shortens treatment duration, especially for infants who are treated for an extended time period.

3. Blinding.
    a. Removal of unblinding for phenobarbital treatment. (Sections 4.1, 7.10.2)

Revision of the dosing schema will allow continuation of blinding when adjunctive therapy with phenobarbital is needed. This is expected to markedly decrease the potential for bias and increase the internal validity of the study.

4. Change in dose titration criteria.
    a. Rescue dose. (Section 7.6)

The trigger for rescue dose is lowered to 12 from 14. The dose of rescue medicine will be the full amount of the previous dose. Both of these practices mimic current standard treatment at TJUH.

    b. Weaning. (Section 7.7)

The treating physician is given discretion to revert to a previous dose. This will allow scores beyond the previous three to be considered. This is especially important for older

infants who may have fussy periods at certain times of the day. The potential for bias is markedly reduced through use of proper blinding.

c. Cessation dose for buprenorphine standardized. (Section 7.8.1)

The prior open label study employed two discreet stopping rules for cessation of buprenorphine. To reduce the potential for dosing and protocol error in a more complicated blinded protocol, the cessation dose is standardized. This change will also help with eventual generalization of use.

d. Cessation criteria. (Section 7.8)

Cessation criteria for NAS scores are increased to allow cessation to occur at higher scores. This is important especially for older infants who have prolonged stay and can have elevated scores as a part of normal behavior.

5. Phenobarbital adjunctive dose. (Section 7.9)

The weaning of phenobarbital will be changed to more closely match current TJUH clinical practice of weaning down opioid down from maximum dose prior to cessation of phenobarbital. Practice patterns have become less heterogeneous since the conduct of the initial pilot phase 1 protocol.

Minor Changes

1. *Update version number and date*
2. *Change FD&C Green to Blue to match optimized blinding solution*
3. *Updating Figure 2 (Study Algorithm) and Table 2 (Dose Schema) to reflect protocol changes*
4. *Updating the screening and allocation number convention. (Section 5.4)*
5. *Removal of repeated listing of collected data variables. (Section 7.1)*
6. *Clarify duration of buprenorphine stability in plastic syringes. (Section 6.4.2)*
7. *Increase flexibility of age at which initial NNNS is collected from 2 to 2-3 days.*
8. *Change formatting to highlight dose escalation. (Section 7.5)*
9. *Addition of demographics listed previously in protocol or which are study specific measures. (Section 7.11)*
10. *Correction of minor typographical errors (Various Sections)*

2.3 Amendment 3

Existing Protocol Version: 3

Revised Protocol Version: 4 (FINAL)

Amendment Date: October 17, 2013

Number of subjects Enrolled at time of amendment: 19

MAJOR CHANGES

1) The overall rationale for the changes is to expand inclusion criteria to include women who are breast feeding. This reflects local and national trends to

strongly encourage breastfeeding for women prescribed methadone or buprenorphine. Jefferson has become a "Breastfeeding Hospital", which has markedly increased the number of mothers who at least attempt to breast feed. The inclusion of such infants will increase the generalizability of results, and eventually provide guidance for clinicians treating breastfed infants. Only mothers with no other contraindications to breastfeeding (i.e., no current illicit drug use) will breast feed infants. NIH and FDA have both agreed to this proposed change.

2) Maternal medical record numbers will be added to facilitate the linking of infant to maternal data in the electronic medical record such that data can be directly exported into the database, which will significantly increase study data accuracy and validity.

3) Some of the PK samples will be reserved to assay for ethanol (which is an excipient in the buprenorphine solution). Blood volume collection will not change. Ethanol has been assayed in buprenorphine-exposed infants before in our investigations, but we will use a much more sensitive assay which will also detect endogenous alcohols produced as part of normal metabolism in non-ethanol exposed (i.e. morphine group) infants.

4) Respiratory pattern exploratory endpoint has been added

PROTOCOL CHANGES

Topic	**Section**	**Page** (edits showing version)	**Change**
Title page		1	Addition of Short Title B-BORN Protocol Date Version Removed Di Wu, PhD
Footers			Updated Protocol version number to 4.0
Synopsis		3	Addition of respiratory endpoint. Removal of exclusion criteria of breast feeding
Table of Contents		4-6	Updated sections and page numbers
Endpoint	2.3	16	Addition of respiratory exploratory endpoint.
Respiratory Endpoint	3.2.6	19-20	Description of respiratory endpoint
Justification of Exclusion Criteria	3.5.1.1	20	Breast feeding exclusion removed
Justification of Inclusion of Breast Feeding	3.6	21	Addition of rationale for addition of breast feeding
Exclusion Criteria	5.2	23	Removal of exclusion criteria of breast feeding
Patient Baseline and Allocation Numbers	5.4	24	Addition of stratification and allocation numbers for breast feeding status
Stratification of Randomization	7.3	27	Addition of stratification and allocation numbers for breast feeding status
Phenobarbital Adjunctive	7.9	29	Procedures for weaning of phenobarbital have been clarified
Study Data	7.11	31	Added data elements to be collected

			Infant • medical record number • adverse events • respiratory patterns Maternal • medical record number • date of birth Changed "<5 cigarettes/day" to "≤5 cigarettes/day" Typographical change to lower case of "Opioid"
Blood Samples	7.14	32	Specify that some PK samples will be assayed for ethanol.
Blood Volume	9.1	35	Update analytes to be examined in PK samples
References		42	Add references

3 FINAL PROTOCOL VERSION 4.0

Title	A Randomized, Active-Control, Double-Blind, Double-Dummy Clinical Trial Comparing Sublingual Buprenorphine And Morphine Solution For The Treatment Of Neonatal Opioid Abstinence Syndrome
Short Title	**B-BORN study** **(B**linded **B**uprenorphine **OR N**eonatal morphine solution)
Clinical Study Site	Thomas Jefferson University Hospital Philadelphia, PA
Principal Investigator	Walter K. Kraft, MD Department of Pharmacology and Experimental Therapeutics 1170 Main Building 132 S. 10th St Philadelphia, PA 19107
Co-Investigators	Susan Adeniyi-Jones, MD Jay Greenspan, MD Karol Kaltenbach, PhD Heather L. Aldridge, CRNP, NNP-BC
Statisticians	Thomas Jefferson University Inna Chervoneva, PhD Ed Pequignot, MS
Analytic Chemistry	University of Utah David Moody, PhD
Pharmacometrics	Children's Hospital of Philadelphia Jeffrey Barrett, PhD
Pharmacogenetics	Mt. Sinai School of Medicine Michelle E. Ehrlich, MD
Protocol Date	October 4,2013
IND	68,403
Institutional Review Board	Thomas Jefferson University
Version	4.0

PROTOCOL SYNOPSIS

Primary Objective	To compare length of treatment using sublingual buprenorphine or oral morphine solution in the pharmacologic treatment of the neonatal abstinence syndrome (NAS).	
Secondary Objectives	To compare length of stay using sublingual buprenorphine or morphine solution. To compare requirement for use of supplemental phenobarbital using sublingual buprenorphine or morphine solution. To compare the safety of using sublingual buprenorphine or morphine solution. To estimate the pharmacokinetic parameters of buprenorphine in the treatment of NAS. To estimate the pharmacokinetic parameters of morphine in the treatment of NAS.	
Exploratory Objectives	To examine the influence of genetic variants in the ABCB1 and mu opioid receptor genes on the need for treatment in NAS. To examine the influence of genetic variants in the ABCB1 and mu opioid receptor genes on the duration of treatment in NAS. To explore buprenorphine drug disposition by urinary analysis. To compare Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) values in infants treated with buprenorphine vs. those treated with morphine for NAS. To compare the feeding patterns, weight gain, and incidence of feeding dysfunction in infants treated with buprenorphine and morphine for NAS To compare the respiratory patterns of infants receiving sublingual buprenorphine or morphine solution for the pharmacologic treatment of NAS.	
Design	Randomized, Blinded, Double Blind, Double Dummy Clinical Trial	
Study Treatments	**Buprenorphine Arm** <u>Buprenorphine</u> Sublingual, q8 hours 0.075 mg ml solution *(Buprenex, Reckitt Benckiser, Richmond, VA) , 30% ethanol USP, in simple syrup USP* **AND** <u>Placebo for Morphine</u> Oral, q4 hours *FD&C Blue #3* *water USP*	**Morphine Arm** <u>Morphine Solution</u> Oral, q4 hours 0.4 mg ml solution *Morphine in sterile water* **AND** <u>Placebo for Buprenorphine</u> Sublingual, q8 hours *Water USP* *Simple syrup USP*
Number of Subjects	80 infants, (40 in each treatment arm with stratified randomization according to maternal use of methadone or buprenorphine)	
Population: Inclusion Criteria	Patients eligible for participation include: 4. $\geq$ 37 weeks gestation	

	5. Exposure to opiates in utero 6. Demonstration of signs and symptoms of neonatal abstinence syndrome requiring treatment
Population: Exclusion Criteria	Patients ineligible for participation include: 18. Major congenital malformations and/or intrauterine growth retardation, defined as birth weight <2200 gm (Mamelle 2001) 19. Medical illness requiring intensification of medical therapy. This includes, but is not limited to suspected sepsis requiring antibiotic therapy. 20. Hypoglycemia requiring treatment with intravenous dextrose 21. Bilirubin >20 mg/dL (The need for phototherapy is not exclusionary) 22. Concomitant benzodiazepine or severe alcohol abuse , self-report of regular use of alcohol or of benzodiazepines use in the past 30 days, and/or receipt of benzodiazepines by prescription (as determined by self-report or urine drug screen) by the mother 30 days prior to birth 23. Concomitant use of CYP 3A inhibitors (erythromycin, clarithromycin, ketoconazole, itraconazole, HIV protease inhibitors) or inducers (rifampin, carbamazepine, phenobarbital) prior to initiation of NAS treatment 24. Seizure activity or other neurologic abnormality 25. Inability of mother to give informed consent due to co-morbid psychiatric diagnosis

Table 1: List of Abbreviations

ADR	Adverse Drug Reaction
AE	Adverse Event
DSMB	Data Safety Monitoring Board
CMV	Cytomegalovirus
FDA	Food and Drug Administration
LOT	Length of Treatment
LOS	Length of Stay
NAS	Neonatal Abstinence Syndrome
NICU	Neonatal Intensive Care Unit

NNNS	Neonatal Intensive Care Unit Network Neurobehavioral Scale
SAE	Serious Adverse Event
TJUH	Thomas Jefferson University Hospital

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Figure 1: Study Schema

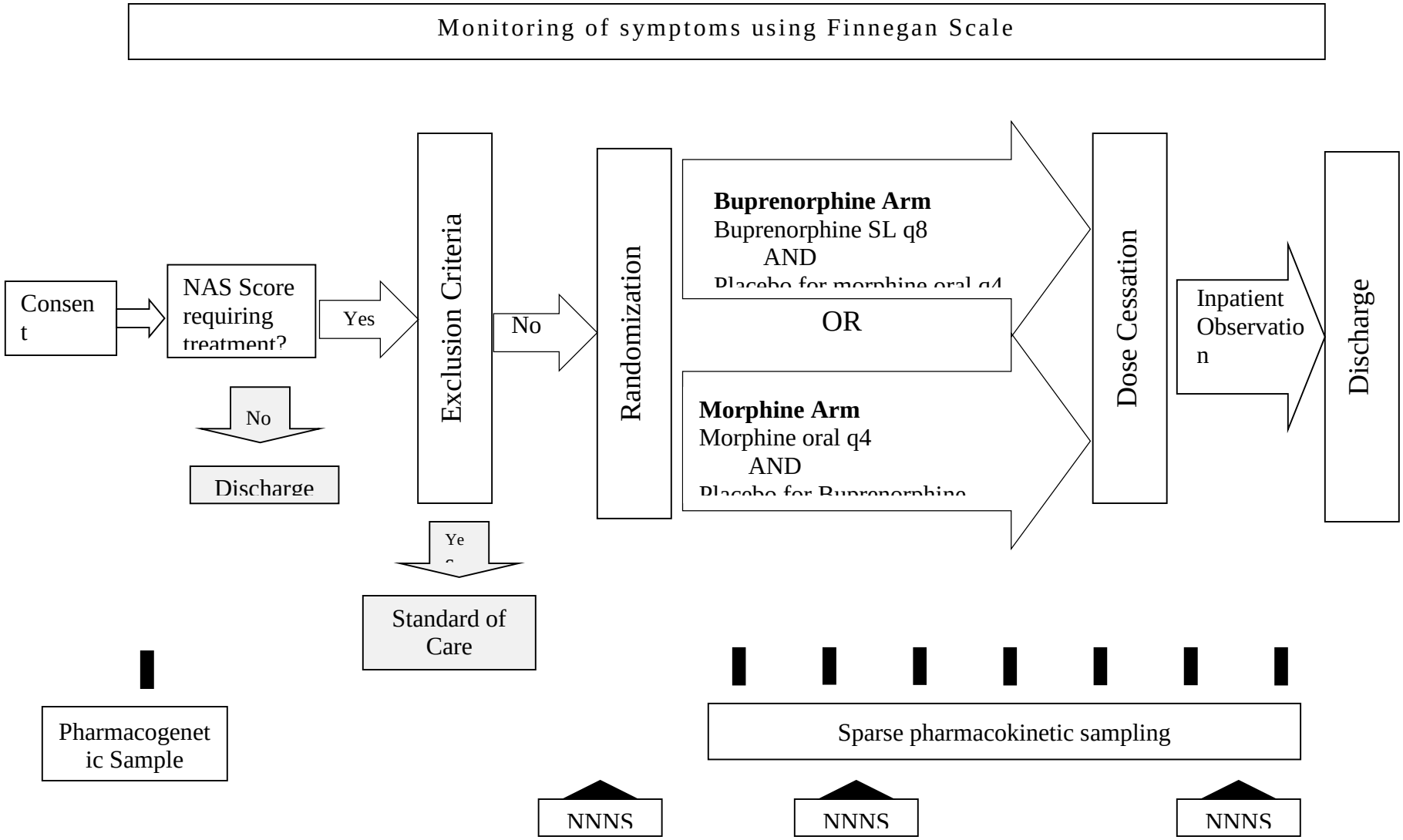


Figure 2: Study Algorithm

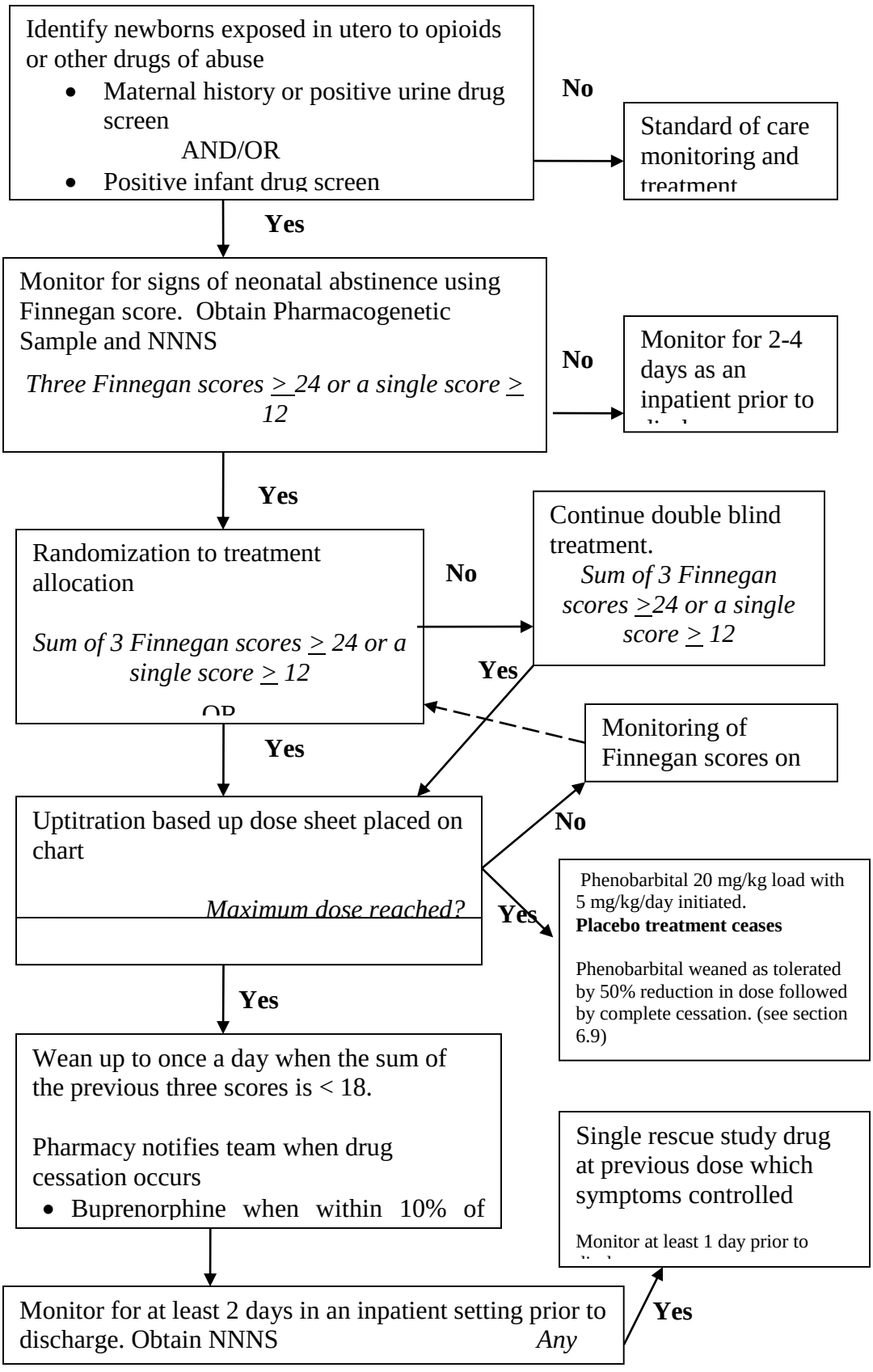


Table 2: Dose Schema for Buprenorphine and Morphine

	Buprenorphine	**Morphine**
Initial daily dose	15.9 mcg/kg/day	0.4 mg/kg/day
Initial unit dose	5.3 mcg/kg q8 hours	0.07 mg/kg q 4 hours
Maximum daily dose	60 mcg/kg/day	1.25 mg/kg/day
Maximum unit dose	20 mcg/kg q8 hours	0.17 mg/kg q 4 hours
Up-titration rate	25%	20%
Maximum # of up-titrations	6	6
Weaning rate	10%	10%
Cessation Dose	Within 10% of starting dose	0.025 mg/kg q 4 hours
Inpatient observation following cessation of last scheduled dose	At least 2 days	At least 2 days
Inpatient observation following last rescue dose	At least 2 days	At least 2 days

1 INTRODUCTION

Background

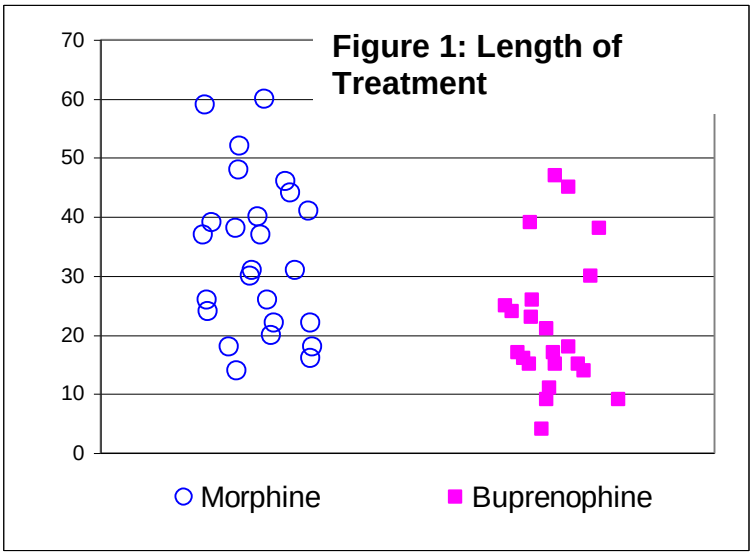
Neonatal withdrawal symptoms have been noted following prenatal exposure to a number of drugs. Examples include opioids, benzodiazepines, mood stabilizing medications, (ACOG Committee on Practice Bulletins--Obstetrics 2008) selective serotonin reuptake inhibitors, (Koren 2005) and nicotine. (Law 2003) For all drug classes except opioids, these symptoms are usually self limited and do not require specific pharmacologic therapy. Neonates exposed to opioids can develop the Neonatal Abstinence Syndrome (NAS), a complex of signs and symptoms in the postnatal period associated with the sudden withdrawal of maternally transferred opioids. Cardinal manifestations include increased muscle tone, autonomic instability, irritability, poor sucking reflex, gastrointestinal symptoms, and impaired weight gain. In epidemiologic studies, maternal opioid abuse is common, with toxicological evidence of use in approximately 1% of births. (Vega 1993) This includes methadone, and increasingly buprenorphine, both of which are used to treat women with physical dependency for opioids. The degree of abstinence symptoms vary in each child. Those with milder symptoms respond well to supportive treatments of swaddling, small calorically dense formula (if not breastfeeding) and minimization of distractive external stimuli. NAS symptoms severe enough to require pharmacologic treatment has been reported to occur in 55-94% of infants born to opioid-dependent mothers. (American Academy of Pediatrics Committee on Drugs 1998) It should be noted that the societal burden of NAS has been difficult to assess based upon limited self report of drug use and underreporting of the condition using ICD classifications. (Burns 2007).

There continues to exist significant heterogeneity in the diagnosis and treatment of neonates at risk for opiate withdrawal. (Crocetti 2007, Sarkar 2006) This is reflected in the variability in length of hospital stay and treatment for such patients. (Jackson 2004, Coyle 2002, Langenfeld 2005, Colombini 2008) Cochrane reviews, (Osborn 2010a, Osborn 2010b) the American Academy of Pediatrics, (American Academy of Pediatrics Committee on Drugs 1998) and expert review identify opioid replacement as the ideal treatment for the withdrawal symptoms associated with *in utero* exposure to opiates. (Johnson 2003)

Buprenorphine is a partial mu opioid receptor antagonist used in the treatment of adult opioid dependency. Cochrane reviews have demonstrated efficacy (retention in treatment programs and suppression of heroin use) of buprenorphine that is comparable to methadone treatment. (Mattick 2008, Gowing 2006) Use of buprenorphine has gained favor due to properties of improved safety, particularly with regard to respiratory depression. Buprenorphine can control abstinence in adults with every other day dosing, due in part to the long residence time at the opioid receptor. (Greenwald 2007) A recent exciting finding is demonstration of improved outcomes in infants with in utero exposure of buprenorphine compared to methadone. (Jones 2010)

Buprenorphine has a number of characteristics that make it an attractive agent in the treatment of NAS and a candidate for the unmet need of improved NAS treatment. As an agonist/antagonist, buprenorphine has a ceiling effect for respiratory depression. There is a lack of the cardiovascular liability associated with methadone as well as an established safety profile in adults. The long half-life and duration of action prevents the rapid change in receptor occupancy that can precipitate withdrawal symptoms. Finally, there is limited abuse liability, which makes consideration of outpatient treatment for NAS a possibility for carefully screened caregivers. Based on these characteristics, the current team of investigators planned and executed the first and only human clinical trial employing buprenorphine and comparing it to oral morphine in the treatment of NAS. The results of the first cohort of 26 randomized infants was reported in a peer reviewed journal. (Kraft 2008) Based upon the experience gained, a second cohort of 24 patients randomized with a revised dose protocol was undertaken. (Kraft 2011) While the goals of the study were demonstration of the feasibility and safety of buprenorphine use in NAS, an analysis of all randomized patients reveals substantial efficacy advantage of buprenorphine over standard of care morphine. Indeed, patients treated with oral morphine had a mean length of treatment of 34 days, whereas those treated with buprenorphine had a mean length of treatment of 23 days. Following log transformation to satisfy normality assumptions, the length of treatment was on average 34% shorter (95% CI: 13%, 50%; p=0.005) in the buprenorphine arm than in those administered oral morphine, and the length of stay was on average 26% shorter (95% CI: 5%, 43%; p=0.020).

Figure 3: Efficacy Outcomes of Buprenorphine compared to Oral Morphine



When a buprenorphine-treated infant with complications and prolonged stay due to an unrelated cytomegalus virus (CMV) is excluded, length of treatment was on average 37% shorter (95% CI: 16%, 52%) on buprenorphine (20 days) than on morphine, and the length of stay was on average 31% shorter (95% CI: 13%, 45%) on buprenorphine. In both analyses, this difference was statistically significant (p=0.002 for both). However, as this was a first human use phase 1 trial in neonates, results must be verified in a double-blind trial.

Rationale for Study Design and Dose

Standard of Care arm

A consensus does not exist on the use of weight vs. symptom driven dosing regimens of oral morphine. (Jansson 2008a) In addition, there exists considerable heterogeneity in specific morphine-based regimens. (Nandakumar 2006) A symptom-based morphine regimen has been the standard of care treatment at Thomas Jefferson University Hospital (TJUH) for at least 30 years, and was the comparator group used in the phase 1 trial of buprenorphine.

The morphine regimen used as the active control is the standard of care at TJUH. The initial dose is 0.07 mg/kg every 4 hours (0.4 mg/kg/day). Morphine is the most commonly used replacement opioid therapy for NAS. While intravenous morphine pharmacokinetics have been described in neonates, there are no published reports of morphine disposition when administered by the oral route in neonates being treated for NAS or for any other condition. The ontogeny of presystemic morphine metabolism has not been described.

Buprenorphine arm

Buprenorphine administered via the sublingual route is efficacious in the treatment of NAS as demonstrated in a pilot study performed at TJUH. (Kraft 2008, Kraft 2011) As the first investigation of sublingual buprenorphine in neonates, the study goals were to explore the safety, feasibility, and pharmacokinetic properties of sublingual buprenorphine. The initial experience was not a formal exploration of efficacy relative to standard of care, but instead a phase 1 trial in a patient population. In the initial cohort, 13 of 26 patients were treated with buprenorphine. Though not statistically significant ($p=0.077$), buprenorphine was associated with a 22 day length of treatment, compared to 32 for standard of care neonatal opium solution. Observations of need for rapid up-titration and increased need of phenobarbital adjunctive therapy, paired with pharmacokinetic data led to a revised dose schedule for buprenorphine. At the same time, TJUH transitioned from neonatal opium solution to an oral morphine solution, which was equipotent for morphine equivalents. A similar efficacy outcome has been noted in this second cohort of 24 patients. A summary of the doses used in patients thus far is presented in Table 3.

Table 3: Summary of Buprenorphine Dose Cohorts

Dose Cohort	Duration	Total subjects enrolled	Buprenorphine		
			Initial dose mcg/kg/day	Maximum dose mcg/kg/day	up-titration rate
1	4/05-1/08	26 *(13 buprenorphine, 13 neonatal opium solution)*	13.2	39	20%
2	3/08- 11/09	24 *(12 buprenorphine,12 morphine)*	15.9	60	25%
Total enrolled		**50** ***(25 buprenorphine, 25 morphine)***			

Of scoring systems available to gauge severity of symptoms and titrate drug dose, the Finnegan score (Finnegan 1992) is most commonly used. (Sarkar 2006) All scoring systems, however, rely on semi-quantitative provider assessments. A double-blinded approach is required to remove any potential for grading bias based upon treatment allocation. The differences in route and frequency of administration between morphine and buprenorphine will require the use of a double dummy design to maintain blinding.

Some clinics treat selected children with NAS on an outpatient basis. This approach decreases inpatient duration of stay, but increases total duration of treatment with an opioid. (Oei 2001) Buprenorphine has less of a potential for diversion than does morphine and less propensity for respiratory depression. For these reasons, buprenorphine may be ideally suited for widening the use of outpatient treatment for NAS.

Maternal use of antidepressants during pregnancy can be associated with neonatal symptoms of the central nervous and respiratory systems. (Koren 2005, Ferreira 2007) The maternal dose to neonatal severity relationship is not established. These symptoms have a temporal onset similar to the abstinence symptoms of methadone abstinence. This exposure is a potential confounder for comparative efficacy examination. However, prospective stratification by self-report of adherence to prescribed psychotropics is not feasible and effective randomization should control for these potential confounders. In addition, as SSRI withdrawal is limited to the first few days of life, it is not expected to significantly impact the primary endpoint of length of stay, a hypothesis supported by lack of effect of SSRI exposure on length of stay at TJUH between 2000-06. (Seligman 2008)

Comparative Safety

Treatment of NAS in a highly monitored inpatient setting is very safe. The most concerning side effect of morphine in the treatment of abstinence is the potential for respiratory depression on the basis of an inadvertent overdose. In adults, buprenorphine is associated with a ceiling effect on respiratory depression, with deaths in adults primarily

occurring with the co-administration of benzodiazepines. (Megarbane 2006) Preliminary data from TJUH constitutes the majority of what is known about buprenorphine in neonates, and the side effect profile has been favorable. There have been no observed episodes of excessive sedation, respiratory depression, or aspiration after >1600 doses of sublingual buprenorphine administered to 25 infants. There is a theoretic possibility of precipitation of withdrawal with administration of a partial opioid agonist such as buprenorphine. There has not been an observed worsening of symptoms, possibly because receptor occupancy by methadone is likely already low at the time NAS symptoms necessitate treatment.

Table 4: All Adverse Events Observed in the Clinical Investigation

Subject	**Treatment**	**Adverse Event**	**Serious AE**	**Causality**
007	buprenorphine	seizure	yes	probably not related
027	buprenorphine	paronychia of finger	no	unrelated
039	morphine	oral thrush	no	unrelated
047	buprenorphine	paronychia of finger	no	unrelated
047	buprenorphine	reflux/poor feeding	yes	probably not related
047	buprenorphine	elevated transaminases	no	probably not related
047	buprenorphine	CMV infection	no	unrelated
047	buprenorphine	aminoaciduria	no	unrelated
049	morphine	conjunctivitis	no	unrelated
050	morphine	oral thrush	no	unrelated
051	buprenorphine	clavicle birth fracture	no	unrelated
052	morphine	reflux	no	unrelated

Two serious AEs took place in the study. The second patient randomized to buprenorphine developed tonic clonic seizures 78 hours after the initial dose. The infant had 4 up titrations prior to this event, but no alteration in dose in the 16 hours immediately preceding the event and had demonstrated improved symptomatic control over this time period with NAS scores between 6 and 8. Buprenorphine was halted and treatment initiated with phenobarbital and oral morphine. Post event evaluation revealed normal serum hematology, chemistry, C-reactive protein, and lumbar puncture indices, and negative cultures. An interictal EEG was negative and MRI of the brain revealed a small amount of dependent subdural hemorrhage within the posterior fossa likely related to the birthing process and deemed unlikely to be symptomatic, with no parenchymal abnormalities. This child's total length of stay was 28 days. At one-year follow up, the child was developmentally normal. A causal link of under-treatment of withdrawal or a dose dependent effect of buprenorphine was not immediately apparent to the investigators. Independent review by the Data Safety Monitoring Board and the Thomas Jefferson University IRB was performed and it was recommended that the trial be

resumed using the established protocol.

AN 047, the 22nd patient randomized to buprenorphine, had a number of adverse events, possibly as a result of a cytomegalovirus (CMV) infection acquired late in the inter-uterine period. The infant had hypoglycemia prior to initiation of treatment with buprenorphine, which was considered to be transient. AE's included prolonged reflux/poor feeding, elevated transaminases, aminoaciduria, and paronychia of a finger. Lack of resolution on buprenorphine and phenobarbital, and indeed absence of worsening when these drugs were removed, led to further medical evaluation. CMV was noted in urine, and a lack of cerebral calcifications or microcephaly at birth suggested a late *in utero* infection. Length of buprenorphine treatment was 45 days and length of stay was 98 days. On the last day of buprenorphine weaning, alanine transaminase was noted to be 30x the upper limit of normal (ULN), while aspartate transaminase (AST) was noted to be 20x ULN. These values remained elevated 6 weeks following the cessation of buprenorphine. Bilirubin was never elevated. Following completion of therapy in this infant, further enrollment was halted and a Data Safety Monitoring Board (DSMB) meeting was called to review the case. The DSMB found causality of the syndrome complex to be unlikely to be related to the administration of buprenorphine and recommended the resumption of the clinical trial with some minor modifications. A revised protocol including these changes was approved by the Thomas Jefferson University Institutional Review Board. These modifications included:

- *addition of liver function tests measured at predose, and 7 (+/- 2) and 21 (+/- 2) days after the first dose of study medication in both buprenorphine and morphine groups*
- *inclusion of hypoglycemia as an exclusion criterion*

Buprenorphine has been associated with elevated liver enzymes, primarily in patients with underlying hepatic dysfunction from viral hepatitis. It should be noted, however, that cohort studies have been reassuring with regard to hepatic safety, (Bogenschutz 2010) and no hepatic toxicity was noted in a 2008 case series of 84 pediatric overdoses of buprenorphine. (Hayes 2008) Buprenorphine has been safely administered to patients with acute hepatitis C and elevated transaminases. In clinical trial experience at TJUH, transaminase levels in the seven other tested infants administered buprenorphine were normal.

Pharmacogenetics as a potential diagnostic aid

Individualization of therapy is a primary goal of all of those who prescribe medication. Differential responses to drug therapy based upon heritable factors has been well described in multiple disease states, and there is a great deal of interest in genetic factors leading to a propensity to addiction and/or pharmacodynamic response to various opioids. (Haile 2008) It is important to note that the factors in those two classifications may not be the same. It is highly unlikely that the variability in either infants or adults can be reduced to a monogenic etiology, but several single nucleotide polymorphisms (SNPs) in particular candidate genes have been identified that appear to determine response

to opioids for pain or replacement abstinence therapy in adults, and/or for predilection to addiction. (Drakenberg 2006) NAS is a condition with wide variation in the need for pharmacologic therapy, severity of disease, as well as in the duration of therapy. The study of pharmacogenetic effects in newborn patients presents a particular advantage since mood, personality, socioeconomic and concomitant medical illness covariates are expected to be markedly reduced relative to adults. Toward this end, DNA samples will be collected in those infants at risk and/or treated for NAS. In this fashion, linkages between the propensity to require pharmacologic therapy for NAS, as well as response to existing therapies, can be investigated.

STUDY OBJECTIVES

Primary Objective

- To compare length of treatment using sublingual buprenorphine or oral morphine solution in the pharmacologic treatment of the NAS.

Secondary Objectives

- To compare length of stay using sublingual buprenorphine or morphine solution.
- To compare requirement for use of rescue phenobarbital using sublingual buprenorphine or morphine solution.
- To compare the safety of using sublingual buprenorphine or morphine solution.
- To estimate the pharmacokinetic parameters of buprenorphine in the treatment of NAS.
- To estimate the pharmacokinetic parameters of morphine in the treatment of NAS.

Exploratory Objectives

- To examine the influence of pharmacogenetic variants in the ABCB1 and mu opioid receptor genes on the need for treatment in NAS.
- To examine the influence of pharmacogenetic variants in the ABCB1 and mu opioid receptor genes on the duration of treatment in NAS.
- To explore buprenorphine drug disposition by urinary analysis.
- To compare Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) values in infants treated with buprenorphine vs. those treated with morphine for NAS.

- To compare the feeding patterns, weight gain, and incidence of feeding dysfunction in infants treated with buprenorphine and morphine for NAS.

- To compare the respiratory patterns of infants receiving sublingual buprenorphine or morphine solution for the pharmacologic treatment of NAS

OVERALL DESIGN AND PLAN OF THE STUDY

Overview

This is a single-site, randomized, double-blind, double-dummy, parallel-group clinical trial. Potential patients will be identified in the pre-natal period by staff of the Thomas Jefferson University Family center. Mothers who provide consent will be contacted upon admission to TJUH. Inclusion and exclusion criteria will be reassessed during the peri-partum period and study details will be reviewed again with the mother, and where possible, the father of the child. Women admitted to TJUH with *in utero* exposure to opioids who are not in the Family Center present will be screened and approached for consent during their inpatient stay.

Infants at risk for NAS will have abstinence assessed using the MOTHER scoring instrument (Jones 2005), which is based upon Finnegan Score and will hereafter be called the "NAS score" (Appendix 1). This is the standard instrument used at TJUH. A need for initiation of treatment will be defined as any consecutive 3 scores adding up to ≥ 24 or any single score ≥12, and the clinical decision of the attending physician that the infant requires pharmacologic therapy. Randomization will take place following reaching of the threshold for initiation of treatment and a re-review of inclusion and exclusion criteria. Patients will be randomized to treatment groups of 1) oral morphine/sublingual placebo for buprenorphine or 2) oral placebo for morphine/sublingual buprenorphine. Randomization will be stratified according to in utero exposure to methadone or buprenorphine (Section 7.3). Oral morphine or placebo for morphine will be administered by mouth every 4 hours, while buprenorphine or placebo for buprenorphine will be administered every 8 hours. NAS scores will be obtained every 4 hours. Dose assessment will take place on a daily basis. If the three previous NAS scores are greater than 24, a dose advancement will take place (at the discretion of the neonatologist). Morphine/placebo will be increased by 20% and buprenorphine/placebo will be increased by 25%. NNNS scoring with take place for all infants who provide consent at day 2-3 of life, or earlier if pharmacologic treatment is required before this time, on day 10 of life, and in the post therapy period (but no later than corrected post gestational age of 46 weeks). Corrected post gestational age is gestational age at birth + weeks of life.

Endpoints

Efficacy Endpoints

Length of Treatment (primary)

The primary endpoint is duration of treatment in days. This measure is most closely linked to efficacy of the allocated treatment. Length of treatment is defined as the number of calendar days when treatment was initiated until the last dose of study drug using 12 midnight as the cut off between days.

Length of Stay (secondary)

Length of stay is defined as the number of calendar days from date of birth to date of discharge from the hospital. Length of stay is less directly tied to treatment efficacy, as the lag time to treatment initiation varies, and occasionally social issues will delay a child's discharge after cessation of therapy for NAS. However, this endpoint is of utility, as it has the potential to capture treatment-specific prolongation of hospital stay that is independent of length of treatment, for example, due to adverse events.

Need for Supplemental Phenobarbital Use (secondary)

The use of phenobarbital is often used as a rescue therapy when maximum opioid replacement therapy dose is reached without adequate resolution of symptoms, though it has also been used as an initial adjunct in combination therapy with an opioid (Coyle 2002) or as initial monotherapy (Jackson 2004). The current study design employs phenobarbital as rescue. Morphine and buprenorphine employ different up-titration rates and number of up-titrations until maximum dose is reached (6 for buprenorphine and 9 for morphine). Thus, the need for adjunctive phenobarbital is not necessarily a surrogate of "treatment failure" in infants with a more severe withdrawal symptom complex. Based upon pilot data, it is not clear where on the dose response curve the present maximum buprenorphine lies. It is possible, that as a partial agonist, buprenorphine may not be able to induce the dense signal generation at the mu opioid receptor obtained with morphine. However, there are no clear adverse events associated with short-term exposure to phenobarbital, and it is possible that a short course of phenobarbital may reduce total duration of treatment in children with more severe withdrawal symptoms.

Neurobehavioral Endpoint (exploratory)

The NAS score has been a favored research instrument due to the comprehensive capture of a large number of elements of neonatal withdrawal, (Jansson 2009) as well as widespread use in clinical care. There may be subtle neurobehavioral differences in the response to treatment which can be uncovered by use of the Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) system. (Lester 2004) NNNS assessment will be performed at day 2 to 3 of life and prior to initiation of pharmacologic therapy, 10 days of life, and in the post therapy period (but no later than corrected post gestational age of 46 weeks). Corrected post gestational age is gestational age at birth + weeks of life. Comparisons of interest will be differences in NNNS scores between treatment arms, correlation of NNNS scores with the NAS, and an examination of day 2 NNNS scores in those infants requiring pharmacologic

therapy for NAS and those infants who are discharged without need for therapy.

Safety Endpoints

Adverse events

Adverse events will be recorded in the patient's research chart using a standardized form. Events will be graded by blinded investigators. Adverse events will be analyzed in a per protocol fashion. Liver enzyme testing will be monitored in both treatment arms.

Severity and Causality Assessment

Adverse events will be graded by an investigator according to a severity score (mild, moderate, severe).

Serious adverse events

A serious adverse event is one that results in death, permanent disability, prolongation of hospitalization, or judged by an investigator to be a significant medical event. All serious adverse events will be reported to the Institutional Review Board, DSMB, and the FDA.

Pharmacokinetic Endpoints

Sparse sampling will be used to generate an estimate of pharmacokinetic parameters and intersubject variability of sublingual buprenorphine and of oral morphine solution. Population PK techniques offer a powerful method of using the limited pharmacokinetic samples and observing the effects of covariates on drug disposition. This can relate ontogeny of body fat composition, drug metabolism, and excretion with pharmacokinetic and pharmacodynamic variables and can be used to rationally design dosing regimens.

Pharmacogenetic Endpoints

An exploratory endpoint is the evaluation of the effects of pharmacogenetic variants on length of treatment in NAS and the propensity to develop NAS. The relationship between allele status, NAS scores, total amount of drug administered, and duration of therapy will be examined. The A118G polymorphism in the mu opioid receptor gene (OPRM1) (rs 1799971), among other things, alters the response to intravenous morphine, and perhaps vulnerability to heroin. (Drakenberg 2006) Polymorphisms in the ABCB1 gene encoding the multidrug resistance associated P-glycoprotein (rs1045642, rs2032582 and rs1128503) also appear to affect response to morphine in pain relief, and perhaps more importantly, methadone dose required for effective treatment of heroin addiction. There are reports linking SNPs in the dopamine receptor type 1, preproenkephalin and preprodynorphin genes to propensity to addiction which we may also consider for analysis, but support for their relevance to treatment is not as strong at this time.

Nutritional Endpoints

Similar to the CNS, there are high concentrations of opioid receptors in the gastrointestinal tract. Gastrointestinal dysfunction is common in infants with NAS. The symptoms include, poor feeding due to uncoordinated or excessive

suck, hyperphagia with excessive suck, vomiting, watery stools, constipation, abdominal discomfort/gassiness, dehydration and skin breakdown or diaper rash. Impaired nutritional intake and weight gain occur. Consequently, impaired nutritional intake and poor weight gain may occur. Meeting the increased caloric demands imposed by NAS is often challenging and may involve changing from standard to soy-based formula and ultimately, or elemental formula to diminish GI symptoms and facilitate growth. Impaired nutritional intake and weight gain occur. Comparisons of GI symptoms, formula/caloric intake and weight gain will be made between treatment allocation, NAS severity, and medication dose.

Respiratory Patterns

Doses of Neonatal morphine solution as high as 1.8 mg/kg/day have been used in the treatment of NAS following in utero exposure to opioids alone or opioids and benzodiazepines without overt clinically evident disturbance in cardiorespiratory pattern. Using trend analysis, Chasnoff et al (Chasnoff 1989) noted increased apnea density and periodic breathing in cocaine-exposed infants compared to methadone-exposed infants. Continuous cardiorespiratory monitoring is standard of care for all infants at Thomas Jefferson University Hospital with event surveillance and recording of the cardiorespiratory patterns for up to 24 hours for review and print out. The respiratory patterns of benzodiazepine-exposed and non-exposed infants with NAS being treated with buprenorphine or neonatal opium solution will be compared.

Justification of the Study Design

A pilot feasibility study has demonstrated the safety and efficacy of buprenorphine delivered by the sublingual route. There are suggestions of increased efficacy of buprenorphine relative to oral morphine for the endpoints of length of treatment and length of hospitalization, though with a possibly higher incidence of required supplemental phenobarbital use. The pilot study was open label, which leaves open the possibility that the comparative advantage of buprenorphine was due to occult nursing or investigator bias. A randomized, double blinded design will remove this potential source of variability, especially as the weaning instrument has a number of subjective grading elements. The differing dose schedule (q8 vs. q4) and route of administration (sublingual vs. oral) make the use of a double dummy required to maintain the blind.

Justification of Dose

Morphine

The initial morphine treatment dose used in this study is the standard treatment regimen used at the TJUH for the past 20 years. The initial dose of 0.4 mg/kg/day used is in the middle of the 0.12-0.6 range reported in a survey of 17 pediatric units in the United Kingdom. (Nandakumar 2006) The authors of this report opined that a higher initial dose may be associated with better control of symptoms, but freely admitted that evidence to support this intuition was lacking. The TJU initial dose is modestly above the 0.24 mg/kg/day recommended by the 1998 report of the American Academy of Pediatrics, though this protocol outlined drop unit doses which would make fine titration difficult.

(American Academy of Pediatrics Committee on Drugs 1998) The Cochrane review of the topic does not specify a favored specific dose. (Osborn 2010a)

Maximum dose of morphine will be 1.25 mg/kg/day. There is no generally accepted maximum dose of morphine used for NAS. A survey of neonatal units in the UK revealed that typical doses were up to 1.3 mg/kg/day, and that one third determine maximum dose according to symptom control. (O'Grady 2009) Doses higher than 1.5 mg/kg/day have been used at TJUH. An additional benefit of 1.25 mg/kg/day is that when paired with a 20% increase in dose, an equal uptitration rate as buprenorphine is maintained. This will allow the maintenance of blinded treatment even when phenobarbital is added as an adjunct treatment.

Buprenorphine

In the pilot study, the initial dose of buprenorphine and dosing regimen was determined using extrapolated adult data to generate a monoexponential pharmacokinetic model with a target steady state concentration of 2 ng/mL. In this pilot study 99.5% of pharmacokinetic samples had buprenorphine concentrations <0.6 ng/ml, with many samples below the limit of quantification of 0.1 ng/ml. What was striking was that there was excellent efficacy, even with buprenorphine concentrations well below the 0.7 ng/ml considered to be the level at which relief of adult abstinence symptoms begins. (Kuhlman 1998) Most patients required a relatively rapid up-titration of buprenorphine, possibly due to a longer half life and volume of distribution relative to morphine. Finally, there was evidence of suboptimal efficacy at the protocol-specified maximum dose of 39 mcg/kg/day on the basis of the need for supplemental phenobarbital in 3 of 12 infants treated with buprenorphine. Based upon these findings, a revised dosing scheme was introduced, which increased the initial dose to 15.9 mcg/kg/day, each step-up titration from 20 to 25%, and maximum dose to 60 mcg/kg. The length of treatment at this higher dose schedule remains intact relative to lower dose, while drug-related safety remains favorable.

Justification of Exclusion Criteria

Benzodiazepine Exposure

A retrospective study at TJUH demonstrated that the length of NAS treatment for all non-benzodiazepine exposed infants between 2000-2006 was 31 days, while for all (including polydrug-exposed) term infants it was 38 days. (Seligman 2008) This value is comparable to that seen in the control group in our published study and follow up pilot clinical trial data in the buprenorphine trial (Kraft 2008), i.e. length of treatment of 34 days and length of stay of 39 days (n=24), though this cohort excluded infants with benzodiazepine exposure. What is striking is that benzodiazepine-exposed infants at TJUH have a 47-day length of stay, suggesting that these patients clearly represent a subpopulation which differs from those without this exposure.

Preterm Infants

Preterm infants have a well-described natural history of NAS and a need for treatment that differs from term infants. The current NAS scoring instruments have not been examined in this population. Length of stay is shorter in the

preterm population.(Seligman 2008, Dysart 2007) The preterm population thus appears to be categorically different in terms of in utero opioid exposure. Finally, the safety of buprenorphine has not yet been fully defined in the preterm population.

Justification of Inclusion of Breast Feeding

Generalizability of Study Results

In 2011 the United States Surgeon General released A Call to Action to Support Breastfeeding, which calls for expansion of breastfeeding for American infants. This is a position supported by the Department of Health and Human Services in Healthy People 2020, as well as major medical societies. (Mass 2011) The number of females maintained on methadone at TJUH who choose to breastfeed their infants has been <5%. Recent local and national initiatives to increase breast feeding in mothers maintained on methadone have increased this percentage to >15% at Thomas Jefferson University Hospital. It is, however, expected that this number will continue to rise both locally and nationwide due to directed campaigns. The inclusion of breastfeeding infants will increase the future generalizability of study results.

Transfer of Methadone and Buprenorphine in Breast Milk

Methadone is passed on to neonates through breast milk, though the absolute amount is small (<0.2 mg/day) and does not appreciably change neonatal serum methadone concentrations. (Jansson 2008b). However, a pharmacodynamic effect is suggested, as breastfed infants have decreased severity of NAS or need for treatment with pharmacologic agents. (Jansson 2008b, Abdel-Latif 2006) Based upon the small doses of drug transferred to the infant, it is not clear if this effect reflects the calming effect of the act of breastfeeding or drug effect. (Liu 2008)

For mothers maintained on usual abstinence doses, the amount of breast milk transferred buprenorphine is 0.1-1.2 mcg/kg/day, which represents ~0.02% of the maternal dose. (Grimm 2005, Marquet 1997, Ilett 2011, Lindemalm 2009) The bioavailability buprenorphine transferred in breast milk is not characterized, but appears low based upon measurement in neonatal blood and urine, (Lindemalm 2009) and by minimal effects in suppression of NAS symptomatology. (Johnson 2003, Lejeune 2001, Loustauneau 2002) There are not reported safety concerns associated with breast feeding, and so despite the product insert which advises against breastfeeding, current national guidelines advocate breastfeeding for mothers prescribed buprenorphine as long as there are no contraindications. (SAMHSA 2004)

Potential for Interaction Between Breast-Derived Buprenorphine and Methadone

The absolute amount of methadone and buprenorphine transferred and absorbed by breast milk are low. Buprenorphine can act as a partial antagonist, so there is a theoretical basis for impaired efficacy of morphine in infants

receiving buprenorphine derived in breast milk. This effect is seen typically at higher doses of buprenorphine. During the conduct of the open label pilot investigations of buprenorphine in NAS, one infant was transitioned from buprenorphine to morphine due to an adverse event of seizure. Another infant received a weight appropriate dose of morphine in lieu of buprenorphine due to a nursing error. In both cases, there was no evidence of precipitation of withdrawal or change in NAS scores, suggesting a lack of significant attenuation of morphine efficacy. Treatment allocation will be randomized and thus the distribution of breastfeeding thus would be expected to be generally equally distributed between dose cohorts. The careful monitoring and symptom driven dose adjustments should provide a good measure of safety should a subtle interaction be present.

STUDY DATA

Data Unblinding Plan

An investigator can unblind a study patient if he or she believes it is in the best interest of the infant. The analytical chemistry lab will be provided with an allocation schedule to facilitate analysis of the proper analyte. Investigators will be unblinded following receipt of analytical drug concentration data. No formal interim analysis for efficacy will be performed prior to the completion of the trial.

STUDY POPULATION

Inclusion Criteria

Patients eligible for participation include:

- ≥ 37 weeks gestation
- Exposure to opiates in utero
- Demonstration of signs and symptoms of neonatal abstinence syndrome requiring treatment

Exclusion Criteria

Patients ineligible for participation include:

- Major congenital malformations and/or intrauterine growth retardation, defined as birth weight <2200 gm (Mamelle 2001)
- Medical illness requiring intensification of medical therapy. This includes, but is not limited to suspected sepsis requiring antibiotic therapy.
- Hypoglycemia requiring treatment with intravenous dextrose
- Bilirubin >20 mg/dL (The need for phototherapy is not exclusionary)

- Concomitant benzodiazepine or severe alcohol abuse, self-report of regular use of alcohol or of benzodiazepines use in the past 30 days, and/or receipt of benzodiazepines by prescription (as determined by self-report or urine drug screen) by the mother 30 days prior to birth
- Concomitant use of CYP 3A inhibitors (erythromycin, clarithromycin, ketoconazole, itraconazole, HIV protease inhibitors) or inducers (rifampin, carbamazepine, phenobarbital) prior to initiation of NAS treatment
- Seizure activity or other neurologic abnormality
- Inability of mother to give informed consent due to co-morbid psychiatric diagnosis

Randomization

A randomization key will be generated by a computerized method and maintained by the investigational drug service pharmacist. Randomization will be managed by the unblinded pharmacy service. Patients will not be randomized until NAS score criteria for pharmacologic treatment are met. Patients will have stratified randomization based upon maternal use of buprenorphine or morphine use during pregnancy. Use will be defined as the plurality of days in the 30 days prior to birth during which either drug was used.

Patient Baseline and Allocation Numbers

All patients for whom consent is obtained will be given a four-digit baseline screening number that begins with sequence 1001. Patients randomized to treatment will receive a three-digit allocation number that begins with sequence 101 (in utero methadone exposure not breast feeding), 201 (in utero buprenorphine exposure not breast feeding), 301 (in utero methadone exposure breast feeding), and 401 (in utero buprenorphine exposure breast feeding). . (See section 7.3)

STUDY POPULATION

3.1 Identity

All study drug will be prepared in bulk solution, distributed to the patient ward, and unit dosed by nursing in1 mL pediatric dispensing vials (Healthcare Logistics Cat. # 7870 or equivalent). Preparation and stability for each product is listed in Appendix 2. Each syringe will be marked as "morphine/placebo for morphine" or "buprenorphine/placebo for buprenorphine".

3.1.1 *Morphine Oral Solution*

Neonatal morphine solution will consist of morphine, water, and FD&C Green #3. The final concentration of morphine is 0.4 mg/mL total volume.

3.1.2 *Placebo for Morphine Oral Solution*

Placebo for neonatal morphine solution is water and FD&C Blue #3.

3.1.3 *Buprenorphine*

Buprenorphine solution will consist of simple syrup, ethanol 30% final volume, and buprenorphine. The final concentration of buprenorphine is 0.075 mg/mL total volume.

3.1.4 *Placebo for Buprenorphine*

Placebo for buprenorphine solution will consist of water and simple syrup.

Calculation of Dose

Buprenorphine

The blinded pharmacist or study team will use the patient weight at the time of randomization for the calculation of all doses of buprenorphine/placebo. These doses will be communicated with the primary team by way of a dose sheet. The primary team will order the buprenorphine/placebo using the electronic medical record. This includes protocol specified maximum of 60 mcg/kg/day for buprenorphine.

3.1.5 *Morphine*

The blinded pharmacist or study team will use the patient's weight at the time of randomization recorded in the medical record for the calculation of all doses of morphine/placebo. These doses will be communicated with the primary team by way of a dose sheet. The primary team will order the morphine/placebo using the electronic medical record. This includes protocol specified maximum of 1.25 mg/kg/day for morphine. There will not be adjustment of dose for weight after allocation for treatment, but final dose will be based upon actual (most recent daily) and not initial weight.

3.1.6 *Starting doses*

The starting daily dose for buprenorphine will be 15.9 mcg/kg/day (5.3 mcg/kg/q8 hours). The starting dose for morphine will be 0.4 mg/kg/day (0.07 mg/kg/q4 hours).

3.1.7 *Phenobarbital*

Actual weight at the time of reaching threshold maximum dose will be used for the calculation of phenobarbital loading and maintenance dose.

Administration

To allow for alterations in sleeping and feeding schedules, each dose of drug can be administered +/- 30 minutes around the nominal time point for that dose. Actual time that each dose was administered must be recorded in the medical record.

Order of Study Drug Administration

At the time points when both study drugs are administered, morphine/placebo for morphine will be administered first. Approximately two minutes will separate administration of morphine/placebo for morphine and buprenorphine/placebo for buprenorphine.

Morphine Oral Solution/Placebo for Morphine

The nurse administrator will administer drug into the oral cavity.

Buprenorphine/Placebo for Buprenorphine

The study drug administrator will hold the child's head at approximately 45 degrees, gently move the tongue to the side, administer the drug under the tongue, and immediately place a pacifier in the mouth to reduce swallowing of drug. If the volume of the drug is >0.5 ml, half of the drug will be administered, followed by the remainder of the dose in approximately 2 minutes.

Drug Product Quality Control

Good Manufacturing Procedures

Preparation of stock solution for active drug and placebo will take place in the investigational drug pharmacy of TJUH.

Stability

Buprenorphine/Placebo for Buprenorphine solution will be used within 30 days of stock drug preparation. Buprenorphine has stability in plastic syringes for 7 days at room temperature. The stability of the stock solution has been demonstrated by the liquid chromatography-electrospray ionization-tandem mass spectrometry. (Anagnostis 2011) Preservative-free morphine hydrochloride solution for neonatal administration is stable at 4 degrees celsius for at least 6 months. (Colombini 2008) All study drug will be labeled with a 7 day stability to maintain blinding of treatment allocation.

Investigational New Drug (IND) Certification

This protocol is being conducted under existing IND # 68,403

STUDY PROCEDURES

Identification of Potential Study Subjects

Potential subjects will be identified through the outpatient treatment clinics. Additional potential subjects will be identified by review of all infants at risk for NAS on the basis of maternally identified use of opioid therapy. All infants from whom consent has been obtained will have NAS graded according to the NAS score (Appendix 1)

administered every 4 hours. Infants with the sum of three scores ≥ 24 or more or a single score of ≥ 12 will be eligible for randomization. The ultimate decision to initiate treatment will be that of the treating pediatrician. A child will be randomized only after a definite decision to treat is made. All infants who have genetic consent obtained will have a blood sample for DNA analysis obtained, ideally at the time of a clinically indicated draw.

NNNS assessment will be performed at day 2 to 3 of life and prior to initiation of pharmacologic therapy, at day 10 of life, and in the post therapy period (but no later than corrected post gestational age of 46 weeks). Corrected post gestational age is gestational age at birth + weeks of life. Comparisons of interest will be differences in NNNS scores between treatment arms, correlation of NNNS scores with the NAS, and an examination of day 2 to 3 NNNS scores in those infants requiring pharmacologic therapy for NAS and those infants who are discharged without need for therapy.

Infants not meeting the treatment threshold criteria will be observed at least 3 days postpartum in an inpatient setting prior to discharge. Infants who have the sum of three scores ≥18 but < 24 can be observed additional days as inpatients at the discretion of the attending physician.

NAS Scoring Procedures

NAS scoring for each subject will take place at 4 hour intervals (+/- 30 minutes to account for sleeping and feeding schedule). The exact time of scoring will be recorded in the medical record. A validated scorer will be used to train and observe nursing staff who will administer the NAS score.

Randomization and Stratification According to Maternal Opioid Replacement Therapy and Breast Feeding

A randomization key will be kept by the unblinded pharmacist. The randomization procedure will consist of 1) a decision of the attending pediatrician to treat NAS with pharmacologic means, 2) contact of the study team with the investigational pharmacist, and 3) preparation of blinded study drug. An allocation number will only be used once. Randomization will be stratified according to maternal use of buprenorphine or methadone (or any other opioid) and breast feeding status. Mothers maintained on buprenorphine will be considered to be in the buprenorphine group, so long as there is reasonable evidence that buprenorphine was used regularly. Allocation number according to stratification is outlined in Table 5.

Table 5: Allocation number according to maternal use of opioid

Maternal use of	Methadone (or other non-buprenorphine opioid)	Buprenorphine

Infant Allocation Number	100-180 (non breast feeding) 300-380 (breast feeding)	200-280 (non breast feeding) 400-480 (breast feeding)

Dose Administration

Every eight hours each subject will receive sublingual buprenorphine/placebo for buprenorphine and oral morphine/placebo for morphine. At the 4-hour time point between each of these intervals, each subject will receive oral morphine/placebo for morphine. There is a +/- 30 minute interval around each nominal time point to account for sleeping and feeding schedule. A dose should not be delayed more than 30 minutes past nominal dosing time due to sleep. If dosing occurs at a time different from the specified nominal time, the next dose will be scheduled to take place 4 hours following the actual dose administration. The exact time of drug administration will be recorded in the medical record. If unblinding takes place for any reason, co-administration of placebo treatment will stop.

Dose Escalation

Dose escalation will take place if

- the sum of 3 NAS scores is $\geq$ 24 or a single score is $\geq$ 12

  OR

- a rescue dose was administered [section 7.6].

To mimic actual clinical care, dose advancement will generally take place in daylight hours when the primary team caring for the patient is present. However, dose advancement may take place when the primary team is not present (such as would occur on evenings and nights). All dose decisions will be made based on the three most recent scores. No more than one dose escalation can take place each day, unless there is need for an additional rescue dose post-escalation.

Rescue Dose

If, between scheduled doses, a child has a single score of $\geq$ 12, a rescue dose may be administered at the discretion of the treating physician. The rescue dose will be same as the previous dose. To maintain blinding, both active drug (morphine or buprenorphine) as well as placebo for morphine or buprenorphine will be administered at the same time. A rescue dose must be given at least 1 hour after and 1 hour before the next scheduled dose. If unblinding takes place for any reason, co-administration of placebo for rescue dose will stop.

Weaning

The initiation of weaning can take place as soon there are 48 hours of stability without dose advancement.

- Doses will be weaned when the sum of the previous three scores is < 18 and no single score is $\geq$ 8.

Dose reductions in both the buprenorphine and morphine groups will occur at a rate of 10% per wean.

- If the sum of the previous three scores is $\geq$ 28 and at the discretion of the treating physician, the standing dose will revert to the previous dose at which symptoms were controlled.

Weaning is expected to take place during daylight hours when the primary team is present, but dose adjustments can take place on evenings and nights. Only one wean of dose will take place each day. A rescue dose may be administered at the discretion of the treating physician during the weaning period if a single score $\geq$ 12. A rescue dose during the wean will be the same as the previous dose. The administration of a rescue dose in the weaning period will not trigger a dose escalation.

Dose Cessation and Observation

Buprenorphine cessation

- Cessation of drug administration will occur in the buprenorphine arm when the dose is within 10% of the initial dose and the sum of the previous three scores is < 18, therapy.

The pharmacy will notify the primary team when the buprenorphine dose meets cessation criteria.

Morphine cessation

- Cessation of drug administration will occur in the morphine arm when the dose is 0.15 mg/kg/day AND the sum of the previous three scores is < 18.

The pharmacy will notify the primary team when the morphine dose meets cessation criteria

Post Cessation Observation

Following cessation of dosing, children will be observed in an inpatient setting for at least 2 days, during which time scoring of NAS symptoms will continue. A rescue dose after cessation of therapy may be given at the discretion of the treating physician for any score of $\geq$ 12. The amount of drug administered will be the last dose the patient had received. An appropriate placebo for rescue dose will also be provided. If a post cessation rescue dose is given, patients must be observed at least 2 days following the last rescue dose.

Maximum Dose and Use of Adjunctive Phenobarbital

When the maximum dose of buprenorphine (60 mcg/kg/day) or morphine (1 mg/kg/day) has been achieved, phenobarbital will be initiated with a loading dose of 20 mg/kg followed by 5 mg/kg/day. Rescue doses of morphine or buprenorphine cannot be administered at the maximum dose of morphine/buprenorphine. If symptoms of NAS are not controlled with phenobarbital 5 mg/kg/day, this can be titrated up by the treating physician to a serum concentration of 20-40 mg/dL. If symptoms are not controlled at 5 mg/kg/day, the attending physician may adjust the

dose clinically as needed, with or without the use of phenobarbital therapeutic drug monitoring.

Treatment with adjunctive phenobarbital will continue for at least two days. When buprenorphine or morphine have been weaned to at least 50% of the maximal dose and the sum of the previous three scores is < 18 and no single score is ≥ 8, the attending physician will decrease the phenobarbital dose to 2.5 mg/kg/day. The morphine/buprenorphine doses will not be changed when phenobarbital is weaned. The half life of phenobarbital in neonates decreases from 115 hr after 1 week to 67 hr after 4 weeks. (Pitlick 1978) As such, phenobarbital will be continued for three dose titrations. When the sum of the previous three scores is < 18 and no single score is ≥ 8, the attending physician may discontinue phenobarbital. The morphine/buprenorphine doses will not be weaned on the step when phenobarbital is discontinued.

Blinding

Unblinded Personnel

The staff of the Investigational Drug Service will maintain the randomization key, which will be generated by pharmacy staff or by an unblinded statistician. All of the investigators, study coordinators, treating physicians, and nursing staff will remain blinded throughout the study. The Investigational Drug Service will provide the analytical collaborator a list of treatment allocation for each shipment of pharmacokinetic samples.

Unblinding due to Emergent Cause

If an investigator or treating physician judges a need to unblind treatment, an investigator will contact the Investigational Drug Service to unblind treatment allocation. The rationale for the unblinding will be recorded in the patient's chart. When this emergent unblinding occurs, the placebo dummy treatment arm will cease and patients will be treated in an open-label fashion. The occurrence of a serious adverse event may, but not necessarily definitely, trigger unblinding.

The following medical issues that develop following randomization would not necessarily require withdrawal of the patient from double blinded treatment:

• Hyperbilirubinemia requiring phototherapy • Antibiotic therapy for suspected infection • Oral candidiasis • Candidal skin infection	• Paronychia requiring antimicrobial therapy • Gastroesophageal reflux requiring antisecretory therapy

Study Data

The following elements will be collected in the study data base:

- mode of birth
- gestational age
- birth weight
- gender
- Apgar scores
- NAS scores
- daily intake (cc/kg/day) and formula type
- stool number and characteristics
- daily weight
- head circumference
- concomitant medication
- NNNS scores
- urine drug screen results
- liver function test results
- dates and times of primary treatment and phenobarbital (if applicable)
- medical record number
- adverse events
- respiratory patterns

Maternal elements to be collected include:

- opioid (methadone, buprenorphine, or other) dose
- urine drug screen
- tobacco use (none, $\leq$5 cigarettes/day, >5 cigarettes/day)
- concomitant medication during gestation
- medical record number
- date of birth

Efficacy

Subject demographics, (including sex and gestational age, birth weight, time to treatment, Apgar scores, maternal methadone dose, tobacco exposure, and concomitant prepartum maternal medications will be collected. Length of treatment and length of stay will be collected in units of days. Group comparisons for continuous variables will be made using the Student's t-test or the Wilcoxon Rank Sum test where appropriate.

Safety

The safety and tolerability of sublingual buprenorphine and oral morphine will be evaluated by tabulating adverse events. Summary statistics will be used to describe relative rates in major organ systems.

Blood Samples for Pharmacokinetics

Pharmacokinetic samples will be drawn on all patients randomized in the trial. Capillary blood samples will be drawn by heel stick with a goal volume of 0.4 ml blood into a lithium heparin tube. An outline of a sampling schedule is listed below. In light of the sparse sampling regimen, some allowance for variation from this schedule is anticipated to reflect feeding and sleeping schedules for the child. Wherever possible, pharmacokinetic samples will be paired with a standard of care blood draw. A description of blood processing is outlined in Appendix 3.

One or two samples collected for pharmacokinetic analysis will be retained to examine ethanol pharmacokinetics. This will help establish the total ethanol exposure following buprenorphine administration, and differentiate from ethyl and non-ethyl alcohol generated by normal metabolic processes in non-ethanol exposed infants.

Table 6: Schedule of Pharmacokinetic Blood Draws

Week 1	Peak within 24 hours of initiation of therapy
	Peak and trough surrounding single dose x 2
	Single mid-interval dose
Weeks 2 onward	Peak and trough surrounding single dose
	Single mid-interval dose
Dose Cessation	Single sample between 12-24 hours after final dose
Periods of Co-administration of phenobarbital	Peak and trough surrounding single dose every three days

Urine Samples for Metabolite Analysis

Two four-hour urine collections will take place while on treatment. Urine will be collected by means of a standard urine bag. A urine collection will take place during week one and week two to treatment. Patients randomized to morphine who have treatment allocation unblinded will not have urine collection. A description of urine processing is outlined in Appendix 3.

Due to the difficulty of reliably collecting full 4 hour urines from infants, and the role of this assessment as an exploratory endpoint, failure to collect a full 4 hour urine will not be considered a protocol deviation or violation.

Blood Samples for Pharmacogenomics

A single 0.2-0.4 ml whole blood sample is collected in a lithium heparin tube. This sample will be collected prior to the initiation of therapy. If this is not possible, the sample can be collected at any time post randomization. Wherever possible, pharmacogenetic samples will be paired with a standard of care blood draw. A description of blood processing is outlined in Appendix 3. Alternatively, DNA can be obtained through the use of a cheek swab.

Blood samples for Liver Function Testing

All patients randomized for therapy will have blood drawn at predose, and at 7 (+/- 2) and 21 (+/- 2) days after the first dose of study medication in both buprenorphine and morphine groups. Liver function testing will include alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (direct and indirect), and alkaline phosphatase.

Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS)

NNNS scoring with take place for all infants who provide consent at day 2-3 of life, or earlier if pharmacologic treatment is required before this time, on day 10 of life (+/- 2 days), and in the post therapy period (but no later than corrected post gestational age of 46 weeks). Corrected post gestational age is gestational age at birth + weeks of life.

Administration of study drug will not be delayed due to inability to have trained staff immediately available to perform the NNNS. Due to the urgency of need for therapy and the role of this assessment as an exploratory endpoint, having an NNNS fall outside of specified windows will not be considered a protocol deviation or violation.

STATISTICAL/ANALYTICAL METHODS AND POWER ANALYSIS

Efficacy

The sample size of 40 neonates per treatment arm will provide 90% power to detect 28% decrease in length of treatment (LOT) on buprenorphine as compared to oral morphine (difference of 0.323 on the log scale) or 80% power to detect 24% decrease in LOT on buprenorphine as compared to oral morphine (difference of 0.279 on the log scale), assuming a common standard deviation of 0.44. Similarly, we will have 90% power to detect 23% decrease in LOS on buprenorphine as compared to oral morphine (difference of 0.264 on the log scale) and 80% power to detect 20% decrease in LOS on buprenorphine as compared to oral morphine (difference of 0.228 on the log scale), assuming a common standard deviation of 0.36.

Eighty opioid-exposed neonates will be randomized in 1:1 ratio to either buprenorphine or oral morphine. The primary endpoint for this trial is the LOT. Log transformed LOT will be compared between buprenorphine and oral morphine treated neonates using two-sample t-test with alpha=0.05. In secondary analysis, we will evaluate the effects of tobacco use, antidepressant use and methadone dose on LOT and LOS using the standard multivariate regression analysis. In the exploratory analysis, repeated over time NNNS scores will be analyzed in a linear mixed effects model incorporating random effects of subject and, respectively, correlation among the measurements from the same subject. If necessary, transformation of the NNNS scores will be used to satisfy the normality assumptions. The during-treatment (10 days post initiation of therapy) and post-treatment difference between treatment arms will be evaluated using the fitted mixed effects model. In a separate linear mixed effects model for NNNS measures we will incorporate the NAS score as a predictor of NNNS to evaluate the strength of association between NAS scores and NNNS scores. In addition, the difference in day 3 NNNS scores between infants requiring pharmacologic therapy for NAS and those infants who are discharged without need for therapy (as determined by the NAS scores) will be evaluated using either two-sample t-test or Wilcoxon test.

Pharmacometrics

The population PK/PD data will be analyzed using nonlinear mixed-effect modeling with the NONMEM software system (Version VI, Level 1.1, GlobalMax LLC, Hanover, MD, USA) with the PREDPP model library and NMTRAN subroutines. Sparse datasets for buprenorphine and morphine will also be analyzed using advanced modeling software (e.g. S-ADAPT/WinBUGs) to better support modeling and simulation efforts. Based on established population PK models, parent-metabolite relationship will be used to further understand buprenorphine and morphine disposition characteristics and inter-individual and residual variability in the study population. Model selection criteria will be based on diagnostic plots (predicted versus observed concentrations, residuals versus predicted concentrations, weighted residual versus predicted concentrations), reasonable parameter estimates, precision of the parameter estimates, random residual variances, and objective function values. To discriminate between competing models, a decrease in the OFV > 10.83 will be considered significant ($p < 0.001$).

Drug-drug interaction and developmental changes in newborns with NAS will be evaluated using NONMEM and SIMCYP (Version 8.1, SIMCYP Inc, Sheffield, UK) with buprenorphine and morphine as probes. Comparison of effectiveness in two treatment arms will be assessed in terms of patient variables. Simulation will be performed based on the best model selected which provides accurate and precise estimates of inter-subject variability and the mean parameter values. The simulation would provide initial dose strategy for drug treatment and allow Baysian feedback analysis for dose individualization. No formal power analysis is performed. This analysis is primarily descriptive and will build upon existing buprenorphine and morphine neonatal models.

Pharmacogenetics

In addition to 80 patients enrolled in this trial, the data from ~60 subjects who did not require pharmacologic treatment for NAS will be available for analyses. Also the data from ~25 patients enrolled in the previous studies (15 requiring and 10 not requiring treatment) are available. Thus, we expect to analyze the rate of A118G allele in the total of 165 patients, 95 requiring and 70 not requiring treatment for NAS. The rate of A118G allele will be compared between the patients that do not require pharmacologic treatment for NAS (~70) combined with ~47 patients that did require the treatment but the length of treatment was below the median LOT in the corresponding treatment arm vs. ~48 patients that did require the treatment with LOT above or at the median LOT in the corresponding treatment arm. We expect that the A118G variant allele for the OPRM1 gene has a frequency ~12-15% in our population. (Drakenberg 2006) Our hypothesis is that the rate of A118G allele will be higher in the latter group of patients requiring longer LOT. This hypothesis will be tested using Fisher's exact test (two-sided, alpha=0.05). With our sample size, we have 80% power to detect as statistically significant the difference between 7.5% rate of A118G allele in 117 patients not treated or with LOT< median LOT and 26% rate of A118G allele in 48 patients with LOT> median LOT. These assumptions imply overall ~13% rate of A118G. In the secondary analyses, we will also compare the LOT between patients with and without A118G allele while controlling for the treatment difference. As secondary endpoints we will also consider the polymorphism status of dopamine receptor type 1, preproenkephalin and preprodynorphin genes.

Finally, a multivariate logistic regression model to predict propensity to develop NAS using the genetic polymorphism status of known SNP's affecting response to opioids as predictors will be built.

Nutrition and Growth

Nutritional and growth data for infants treated with buprenorphine and morphine will be compared and analyzed to determine the and compare incidence, frequency and duration of breast feeding, standard and non-standard formula, mean daily intake, peak intake, mean daily weight gain, time to regain birth weight, incidence and duration of diaper rash needing treatment. Continuous and categorical variables will be analyzed using Fisher's Exact Test and unpaired t test, respectively.

SAFETY PARAMETERS

Blood volume

No more than 12 ml of blood will be drawn over the course of the study. This represents a maximum, and not the typical amount drawn. This will include study-related blood draw as well as clinically indicated collection. Total blood volumes are estimated in Table 7.

Table 7: Blood Volume for Study Participants

Procedure	Research Related?	Total Number of Collections	Blood (mL) per Test	Total Blood (mL/test)
Newborn Hematology/chemistry	No	1	0.8	0.8
PKU screen	No	1	0.4	0.4
Pharmacogenetic sample	Yes	1	0.4	0.4
Liver Function Test	Yes	3	0.4	1.2
Buprenorphine, morphine or ethanol assay	Yes	20*	0.4	8.0*
Maximum amount of blood drawn per female patient				10.8*
Circumcision blood loss (estimated)				0.2
Maximum amount of blood drawn per male patient				11.0*

* maximum

Stopping Rules

No further enrollment will take place following a SAE judged to be probably or definitely related to study treatment until review by the DSMB, FDA, and the NIDA Program Official. The treatment allocation for the infant with the SAE will be unblinded. There is no prespecified stopping rule for trial cessation. There is no predefined interim look for safety or efficacy. If the incidence of non-serious AE's is higher than expected, the PI can propose an ad hoc

interim look to the DSMB and NIDA Program Official. The DSMB and NIDA Program Official will also receive biannual blinded summary statistics of adverse events. If both parties are in agreement, an interim look can be undertaken. The study statistician and investigational drug service staff will provide the unblinded adverse event data. All attempts will be made to maintain the blind of the principal investigator and clinical staff, unless DSMB makes a study-wide decision to unblind the study.

Final Unblinding Procedure

Either the Investigational Drug Service or study statistician(s) can provide treatment allocation if the investigator believes it is in the interest of the study patient to have this information available for treating physicians. Unblinding can take place by verbal discussion, but will need written documentation to be placed in the subject's research chart.

Data Safety Monitoring Board

A DSMB will evaluate each serious adverse event judged by the investigator to be possibly, probably, or definitely related to study drug. The DSMB will provide binding recommendations to the investigators. There will be no planned interim analysis or prespecified stopping criteria for futility or efficacy.

Certificate of Confidentiality

A certificate of confidentiality will be obtained prior to enrollment of any protect to protect privacy of neonates and their mothers

Study-specific Risks

Respiratory Depression

A dose-dependent effect of all opioids is respiratory depression. In adults, buprenorphine (a partial agonist) causes less respiratory depression and fewer overdose-related deaths than full agonists such as morphine. While respiratory depression has been noted with accidental home ingestion, buprenorphine is anticipated to have less potential for hypoventilation than morphine in pediatric patients. Respiratory depression effects are mitigated by careful monitoring in an inpatient, high acuity setting. Excessive sedation and respiratory compromise with standard of care morphine is exceedingly rare in such settings. Following administration of >1600 doses of sublingual buprenorphine to 25 infants, no respiratory depression has been noted. In the unlikely event of buprenorphine-induced respiratory depression, naloxone has demonstrated reversibility in pediatric patients. Naloxone has the potential to precipitate acute withdrawal symptoms, so any use will be titrated to adequate respiration. Any treatment emergent use of naloxone will be considered an SAE, prompting immediate unblinding and halting of further enrollment.

Other Medical Risks

There are anticipated to be few mechanism-based risks specific to opioid treatment outside of theoretical risks of respiratory depression or excessive sedation. A symptom-driven dose titration serves to minimize risks of over-, or

under-treatment with opioids. This approach in standard of care treatment of the neonatal abstinence syndrome is very effective in maintaining drug dose within a therapeutic window. All infants will be monitored in a high acuity of care setting on 24-hour telemetry monitoring of heart rate and respiratory function. Treatment of other emergent adverse events, whether judged to be drug related or not, will be managed by the neonatology staff of the neonatal intensive care unit (NICU). The NICU has 24-hour senior level physician coverage and access to all subspecialty consultants. The case mix includes a wide spectrum of illness through critical care. The staff is able to manage, jaundice, vomiting, seizures, and infections.

If there is an SAE with unblinding, the decision to cease treatment (buprenorphine or morphine) will be informed by investigator assessment of causality to study drug. Depending upon the clinical situation, options could include 1) continuation of study treatment (buprenorphine or morphine) in an open label fashion with or without dose adjustment, 2) transition from buprenorphine to open label morphine, 3) transition from an opioid to phenobarbital monotherapy, or 4) cessation of all abstinence pharmacotherapy. In any case, treatment will follow clinician guidance and will not be protocol-driven. However, such infants will be followed in an intention to treat analysis and will be included in safety analysis.

Novel drug administration route

Buprenorphine will be administered by a sublingual route. An initial dose is 15.9 mcg/kg/day, with a maximum dose of 60 mcg/kg/day. Dose advancement is protocol driven based upon the NAS score. Due to limitations on blood volume in neonates, it is not possible to determine data-rich estimates of absorption rate constants or dose-to-dose variability.

It should be noted that there are no published data on the pharmacokinetics of oral morphine in neonates. Indeed, this is one of the primary goals of our proposed project. Intravenous morphine is associated with large intra-patient variability in serum concentration and pharmacodynamic response. (Bouwmeester 2003, Bouwmeester 2004, Barrett 1996) On theoretical grounds, buprenorphine-induced respiratory depression is less likely than the current standard of morphine. Morphine, as a full mu opioid receptor agonist, has a higher propensity for overdose-induced respiratory depression. The large first pass metabolism of morphine in adults with significant intra-patient variability is well recognized. (Lotsch 1999) Limited data for pediatric patients under the age of 11 suggest similar or greater variability. (Hunt 1999) The dynamic and variable nature of the ontogeny of pre- and post-systemic metabolism in neonates (Alcorn 2002a, Alcorn 2002b) likely contributes to greater variability in oral morphine disposition than in older pediatric patients for which data exist.

Taken together, these observations suggest that sublingual buprenorphine is safe based upon 1) extensive safety experience thus far, 2) all measured pharmacokinetic samples well within the safe therapeutic window, 3) pharmacokinetic modeling suggesting dose-to-dose variability that is comparable to adults, 4) monitoring of

administration in a high acuity clinical setting, 5) established variability in oral morphine, which despite its potential for clinical adverse events as a full agonist, is well tolerated in the inpatient setting, and 6) pharmacologic properties of buprenorphine which make respiratory depression unlikely, even in the setting of excessive dosing, and 7) administration of buprenorphine (with ~20 half-life in the study population) every 8 hours, which minimizes drug peak and trough concentrations.

APPENDIX 1: NAS SCORING SYSTEM

Scored Elements	
Signs and Symptoms	*Score*
Crying: Excessive high pitched	2
Crying: Continuous high pitched	3
Sleeps < 1 hours after feeding	3
Sleeps < 2 hours after feeding	2
Sleeps < 3 hours after feeding	1
Hyperactive Moro Reflex	1
Markedly Hyperactive Moro Reflex	2
Mild Tremors: Disturbed	1
Moderate-Severe Tremors: Disturbed	2
Mild tremors: Undisturbed	1
Moderate-Severe Tremors: Undisturbed	2
Increased Muscle Tone	1-2
Excoriation (Indicate specific area):	1-2
Generalized Seizure (or convulsion)	8
Fever > 37.3 C (99.2 F)	1
Frequent Yawning (4 or more successive times)	1
Sweating	1
Nasal Stuffiness	1
Sneezing (4 or more successive times)	1
Tachypnea (Respiratory Rate >60/mm)	2
Poor feeding	2
Vomiting (or regurgitation)	2
Loose Stools	2
Failure to thrive (Current weight > 10% below birth weight 90% BWT=_____ *(record weight in score box 1 x day)*	2
Excessive Irritability	1-3
Total Score	
Unscored Elements	
Convulsions	Present/absent
Fever > 38.4 C (101.2 F)	Present/absent
Mottling	Present/absent
Excessive sucking	Present/absent
Watery Stools	Present/absent
Projectile vomiting	Present/absent
Retractions	Present/absent
Nasal flaring	Present/absent
Myoclonic jerks	Present/absent

APPENDIX 2: PREPARATION OF BUPRENORPHINE AND MORPHINE STOCK SOLUTIONS AND STABILITY

	Stability of Stock
Buprenorphine 0.075 mg/mL Composition of neonatal stock solution • One 0.3 mg ampule buprenorphine [Buprenex 0.3 mg/ 1 ml (Reckitt Benckiser) or generic buprenorphine for injection] • Ethanol to bring to final concentration of 30% (1.26 mL of 95% ethanol USP) • Simple syrup USP(Sucrose, Purified Water and 0.1% Sodium Benzoate) to bring to 4 mL total volume [Humco or equivalent] 0.3 mg buprenorphine per vial * 4 mL $^{-1}$ (final volume) = 0.075 mg/mL	3 days
Placebo for Buprenorphine Composition of neonatal stock solution • Per 4 ml solution1.75 ml simple syrup USP (Sucrose, Purified Water and 0.1% Sodium Benzoate) [Humco or equivalent] • 2.25 ml Sterile water for injection USP	1 month
Morphine 0.4 mg/ml Composition of neonatal stock solution • 1 part Morphine oral solution 4mg/ml [Roxane or equivalent] • 9 parts sterile water for injection USP	1 month
Placebo for Morphine Composition of neonatal stock solution • Sterile water for injection USP • FD&C Blue #3	1 month

APPENDIX 3: PROCESSING OF PHARMACOKINETIC AND PHARMACOGENETIC SAMPLES

Buprenorphine and its metabolites are stable in frozen plasma at -20 C for at least 6 months. (Huang 2006) In urine, there is stability for 16 hours at 22 C, 72 hr at 4 C and through 3 freeze/thaw cycles. (Kacinko 2008)

Pharmacokinetic Serum Samples

Samples will be obtained by capillary heel stick into lithium heparin pediatric tubes (BD Microtainer, Ref # 365971 or equivalent). A goal of 400 microliters should be collected. Blood is spun at 3,000 RPM on a refrigerated tabletop centrifuge for 10 minutes, and plasma transferred to storage tubes and frozen at -20 C. Blood from an indwelling catheter can be used if one is present for medical care unrelated to the treatment of NAS.

Pharmacokinetic Urine Samples

Volume of urine will be recorded, as well as time of start and stop of urine collection. Uncentrifuged urine from the collection bag will be transferred to a polypropylene tube and frozen at – 20 C. Volume in excess of 10 ml will be discarded.

Blood for DNA

Blood for DNA analysis will be collected by capillary heel stick into an uncoated capillary pediatric tube. Blood can also be collected in tubes containing anticoagulants.

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4 DETAILED DATA SAFETY MONITORING PLAN

"A Randomized, Active-Control, Double-Blind, Double-Dummy Clinical Trial Comparing Sublingual Buprenorphine And Morphine Solution For The Treatment Of Neonatal Opioid Abstinence Syndrome" (1R01DA029076-01A1)

Principal Investigator and Medical Monitor: Walter Kraft, MD

Study Site: Thomas Jefferson University

Summary of the Protocol:

Brief description of the protocol (Study design)

This single-site study tests the hypothesis that in the treatment of the neonatal abstinence syndrome (NAS) sublingual buprenorphine will have shorter duration of therapy and length of hospitalization compared to oral morphine. Consent will be obtained from the parent(s) of infants at risk for development of NAS in this single site study. Approximately 140 infants will be consented to enroll 80 infants randomized to either sublingual buprenorphine or oral morphine. The need for pharmacologic treatment will be determined by a standard NAS scoring instrument. Treatment allocation will be blinded by use of a double-dummy design. Heel stick blood collection will be used for population pharmacokinetic model refinement and pharmacogenetic evaluation. Neurobehavioral assessments will take place before and after treatment using the NNNS instrument.

Primary and secondary outcome measures

The primary outcome will be length of treatment. Secondary outcome measures are 1) length of hospitalization, 2) need for adjunct phenobarbital, 3) safety and tolerability of each treatment, and 4) pharmacokinetic parameters of buprenorphine and morphine. Exploratory endpoints are 1) neurobehavioral effects of buprenorphine and morphine, and 2) pharmacogenetic determinants of response to treatment for NAS.

Inclusion/exclusion criteria

Inclusion Criteria

1. ≥ 37 weeks gestation
2. Exposure to opiates in utero
3. Demonstration of signs and symptoms of neonatal abstinence syndrome requiring treatment

Exclusion Criteria

1. Major congenital malformations and/or intrauterine growth retardation
2. Medical illness requiring intensification of medical therapy. This includes, but is not limited to suspected sepsis requiring antibiotic therapy.
3. Hypoglycemia requiring treatment with intravenous dextrose
4. Bilirubin >20 mg/dL (The need for phototherapy is not exclusionary)
5. Concomitant benzodiazepine or severe alcohol abuse, self-report of regular use of alcohol or of benzodiazepines use in the past 30 days, and/or receipt of benzodiazepines by prescription (as determined by self-report or intake urine) by the mother 30 days prior to birth
6. Concomitant use of CYP 3A inhibitors (erythromycin, clarithromycin, ketoconazole, itraconazole, HIV protease inhibitors) or inducers (rifampin, carbamazepine, phenobarbital) prior to initiation of NAS treatment

7. Seizure activity or other neurologic abnormality
8. Breast feeding
9. Inability of mother to give informed consent due to co-morbid psychiatric diagnosis.

Power calculation and sample size
The primary endpoint for this trial is the length of treatment (LOT). Log transformed LOT will be compared between buprenorphine and morphine treated neonates using two-sample t-test with alpha=0.05. The sample size of 40 neonates per treatment arm will provide 90% power to detect 28% decrease in LOT on buprenorphine as compared to morphine (difference of 0.323 on the log scale) or 80% power to detect 24% decrease in LOT on buprenorphine as compared to NOS (difference of 0.279 on the log scale), assuming a common standard deviation of 0.44. Similarly, there is 90% power to detect 23% decrease in LOS on buprenorphine as compared to morphine (difference of 0.264 on the log scale) and 80% power to detect 20% decrease in LOS on buprenorphine as compared to morphine (difference of 0.228 on the log scale), assuming a common standard deviation of 0.36 (an estimate obtained from the preliminary data analysis). The differences assumed in theses power calculations are slightly conservative as compared to the mean differences between buprenorphine and morphine observed in preliminary studies to offset the uncertainty of preliminary estimates based on a moderate sample size.

Approximately 65% of infants will require pharmacologic treatment of NAS. The rate of A118G allele will be compared between the patients that do not require pharmacologic treatment for NAS (~70) combined with ~47 patients that did require the treatment but the length of treatment was below the median LOT in the corresponding treatment arm vs. ~48 patients that did require the treatment with LOT above or at the median LOT in the corresponding treatment arm. It is expected that the A118G variant allele for the OPRM1 gene has a frequency 12-15% in the study population. The hypothesis is that the rate of A118G allele will be higher in the latter group of patients requiring longer LOT. This hypothesis will be tested using Fisher's exact test (two-sided, alpha=0.05). The study will have 80% power to detect as statistically significant the difference between 7.5% rate of A118G allele in 117 patients not treated or with LOT< median LOT and 26% rate of A118G allele in 48 patients with LOT> median LOT. These assumptions imply overall ~13% rate of A118G.

Trial Management
List of participating enrolling clinics or data collection centers
The proposed trial is a single site investigation at Thomas Jefferson University Hospital.

Projected timetable

	Year 1	Year 2	Year 3	Year 4	Year 5
IRB approval of protocol	X				
Obtain Certificate of Confidentiality	X				
Create Case Report Forms and Database	X				
Operationalize Protocol	X				
Enroll Subjects	X	X	X	X	X
Pharmacogenetic Analysis		X	X	X	
Pharmacokinetic Analysis		X	X	X	X
Data Analysis: Efficacy and Safety					X
Data Analysis: Pharmacometrics					X
Data Analysis: Pharmacogenetics					X
DSMB Meeting	X	X	X	X	X

Target population distribution (e.g, women, minorities, etc)
There is not pre-specified sex, racial, or socioeconomic target population. Based upon the epidemiology of opioid abuse in Philadelphia, we anticipate the large majority of infants enrolled will be white.

Data Management and Analysis
Data acquisition, transmission, and entry methods
Patient data will be collected on paper source documents separate from the clinical chart. A data manager will enter source data into a password protected Access (Microsoft Corp.) electronic case report form (eCRF) file located on a central server with redundant backup.

Data analysis plan
No formal interim analysis for efficacy or futility will be performed prior to the completion of the trial. The primary endpoint for this trial is the length of treatment (LOT). Log transformed LOT will be compared between buprenorphine and morphine treated neonates using two-sample t-test with alpha=0.05.

Databases will be constructed to allow eventual transfer of final results according to ClinicalTrials.gov Basic Results Data Element Definitions detailed in http://prsinfo.clinicaltrials.gov/results_definitions.html. Pharmacokinetic data will be transferred to the data manager, who will format the data, generate a verified file, and send a copy to the pharmacometrician collaborators, along with deidentified patient data. Pharmacogenetic data will be similarly formatted. Following verification of all study data and resolution of all queries, the database will be locked and transferred to the biostatistician for analysis.

Quality Assurance
Procedures in place to ensure the validity and integrity of the data
The allocation schedule and drug accountability forms will be maintained by the unblinded Investigational Drug Service of Thomas Jefferson University Hospital.

A research nurse will be trained to a "gold standard", i.e. 90% concordance, using an inter-rater reliability program developed by Dr. Karol Kaltenbach, to assess abstinence signs and symptoms with the NAS Scoring Tool. Dr. Kaltenbach will be responsible for training and maintaining inter-rater reliability for nursing staff who will administer all NAS assessments for study participants. This procedure has been utilized in the MOTHER trial (ClinTrials.gov ID NCT00271219) in which the treatment of NAS was also a primary outcome measure. NNNS will employ a lead and back up assessor, each with full training and certification.

The study coordinator will be primarily responsible for collecting data into case report forms, or transferring electronic medical records (NAS scores) onto a server with redundant backup. A back up staff member will perform this task when the coordinator is out or on vacation. The data manager will transfer paper source documents into the electronic case report forms. The data manager will be responsible for custody and version control for electronic files. A delegation of authority form will be maintained in the study regulatory file for all key personnel and investigators.

Procedures to guarantee the accuracy and completeness of the data, during data collection, entry, transmission, and analysis.
Program staff will source document verify (medical record and/or research source):

- 100% of patient informed consent forms, SAEs, AEs

- 100% of patient drug dose and time administration, PK sample times, and length of hospitalization
- 100% of all data from the first 5 patients
- 100% of all data from a random selection of 20% of patients.

Source document verification will be the primary responsibility of the study coordinator. If there is discrepancy between source documents and eCRF, a query will be generated. Documentation of the query and its resolution will be maintained.

Regulatory Issues

Reporting of SAEs to the IRB, NIDA, and, FDA.

SAEs will be reported to TJU IRB according to Policy and Procedure "GA 120", which specifies that "*on-site SAEs that are deemed to be possibly or definitely related to the study article should be reported within 48 hours of knowledge of the event, except that death should be reported within 24 hours (one working day). Unrelated SAEs should be reported within 5 working days.*" The principal investigator will notify the NIDA Program Official of SAEs according to this timeline.

All SAEs and non-serious AEs will be reported to the Thomas Jefferson University IRB. On-site AEs and SAEs will be reported using the electronic AE reporting system (eSAEy) accessed via the university intranet at http://www3.kimmelcancercenter.org/clinicaltrials/ae/ .

A summary of all AEs will be submitted quarterly to DSMB. Additionally, the PI of the study will report all non-serious AEs in the IND Annual Report to the FDA. SAEs will be reported to the FDA in accordance with Regulation 21 CFR 312.32 – IND Safety Reports.

Reporting of IRB actions to NIDA

All IRB actions that would halt or significantly impair recruitment will be reported to the NIDA Program Official within 72 hours. Results of IRB audit that contains serious protocol violation or consent deviations will be reported to the NIDA Program Official within one week of receipt of the formal written notification from the IRB. The annual report from the investigator to the NIDA Program Official will provide an update of routine updates from the IRB such as ongoing review and the results of audits without significant findings.

Report of changes or amendments to the protocol

The IRB will receive proposals for amendments to the protocol according to IRB standard operating procedures. Any proposed protocol changes will be discussed with the NIDA Program Official for programmatic approval before submission to the IRB.

Trial stopping rules

No further enrollment will take place following a SAE judged to be probably or definitely related to study treatment until review by the DSMB, FDA, and the NIDA Program Official. The treatment allocation for the infant with the SAE will be unblinded. There is no prespecified stopping rule for trial cessation. There is no predefined interim look for safety or efficacy. If the incidence of non-serious AE's is higher than expected, the PI can propose an ad hoc interim look to the DSMB and NIDA Program Official. The DSMB and NIDA Program Official will also receive biannual blinded summary statistics of adverse events. If both parties are in agreement, an interim look can be undertaken. The study statistician and investigational drug service staff will provide the unblinded adverse event data. All attempts will be made to maintain the blind of the principal investigator and clinical staff, unless DSMB makes a study-wide decision to unblind the study.

Disclosure of any conflict of interest in the DSM

All investigators who are faculty of Thomas Jefferson University will have a conflict of interest form filed annually with university legal counsel. Non-Thomas Jefferson University investigators will be asked to provide conflict of interest information at the beginning and end of the trial period. These documents will be held in the study regulatory file. All published data will also include disclosure of any potential conflicts of interest for all authors.

Trial Safety

Potential risks for participants

Serious Adverse Events

While there is a large clinical database of buprenorphine use in adults, data for the NAS population is limited to the 25 patients treated in Phase I of this clinical trial. The outcomes have been favorable thus far. Two infants randomized to buprenorphine had a SAE. The first was a seizure, and the second was cytomegalovirus (CMV) infection with associated transaminitis, poor enteral feeding, and aminoaciduria. For both infants, the DSMB and TJU IRB reviewed the events, judged them to be unlikely to caused by study drug, and advocated continuation of the clinical trial. However, contribution in part due to buprenorphine cannot be excluded. In both cases, the investigative team acted to place the study on hold until formal review by the DSMB, IRB, and FDA. The pilot study was restarted only after approval of each oversight organization.

Respiratory Depression

A dose-dependent effect of all opioids is respiratory depression. In adults, buprenorphine (a partial agonist) causes less respiratory depression and fewer overdose-related deaths than full agonists such as morphine. While respiratory depression has been noted with accidental home ingestion, buprenorphine is anticipated to have less potential for hypoventilation than morphine in pediatric patients. Respiratory depression effects are mitigated by careful monitoring in an inpatient, high acuity setting. Excessive sedation and respiratory compromise with standard of care morphine is exceedingly rare in such settings. Following administration of >1600 doses of sublingual buprenorphine to 25 infants, no respiratory depression has been noted. In the unlikely event of buprenorphine-induced respiratory depression, naloxone has demonstrated reversibility in pediatric patients. Naloxone has the potential to precipitate acute withdrawal symptoms, so any use will be titrated to adequate respiration. Any treatment emergent use of naloxone will be considered an SAE, prompting immediate unblinding and halting of further enrollment.

Other Medical Risks

There are anticipated to be few mechanism-based risks specific to opioid treatment outside of theoretical risks of respiratory depression or excessive sedation. A symptom-driven dose titration serves to minimize risks of over-, or under-treatment with opioids. This approach in standard of care treatment of the neonatal abstinence syndrome is very effective in maintaining drug dose within a therapeutic window. All infants will be monitored in a high acuity of care setting on 24-hour telemetry monitoring of heart rate and respiratory function. Treatment of other emergent adverse events, whether judged to be drug related or not, will be managed by the neonatology staff of the neonatal intensive care unit (NICU). The NICU has 24-hour senior level physician coverage and access to all subspecialty consultants. The case mix includes a wide spectrum of illness through critical care. The staff is able to manage, jaundice, vomiting, seizures, and infections.

If there is an SAE with unblinding, the decision to cease treatment (buprenorphine or morphine) will be informed by investigator assessment of causality to study drug. Depending upon the clinical situation, options could include 1) continuation of study treatment (buprenorphine or morphine) in an open label fashion with or without dose adjustment, 2) transition from buprenorphine to open label morphine, 3) transition from an opioid to phenobarbital monotherapy,

or 4) cessation of all abstinence pharmacotherapy. In any case, treatment will follow clinician guidance and will not be protocol-driven. However, such infants will be followed in an intention to treat analysis and will be included in safety analysis.

Novel drug administration route

Buprenorphine will be administered by a sublingual route. An initial dose is 15.9 mcg/kg/day, with a maximum dose of 60 mcg/kg/day. Dose advancement is protocol driven based upon the NAS score. Due to limitations on blood volume in neonates, it is not possible to determine data-rich estimates of absorption rate constants or dose-to-dose variability. Sublingual of administration has a limited potential for intoxication due to the following evidence:

1) Extensive safety experience
2) All measured pharmacokinetic samples reveal drug concentration well within the safe therapeutic window
3) Modeling-based data suggesting comparable dose-to-dose variability as in adults
4) Monitoring of administration in a high acuity clinical setting
5) Probable large variability in oral morphine, which despite danger as a full agonist, is well tolerated in an inpatient dosing setting
6) Partial agonist properties of buprenorphine that make respiratory depression unlikely
7) Administration of buprenorphine (with ~20 hour half-life in the study population) every 8 hours, which minimizes drug peak and trough concentrations.

Morphine is orally administered in an initial dose of 0.4 mg/kg/day to a maximum of 1.0 mg/kg/day. Dose advancement is protocol driven based upon the NAS score.

Specimen collection

The number of heel sticks and volume of blood collected from each infant will depend upon the length of treatment. The expected average volume of blood to be collected over the course of the study is 11 ml (including clinical samples), with a maximum of 20 blood collections. Urine will be collected over two 4-hour periods by way of a collection bag, to investigate glucuronidated metabolites. Such samples would not be conducted in the standard treatment of infants with NAS. Risks of these interventions include infection and discomfort. In the Phase I study, no infants suffered AEs from heel stick blood collection.

Potential benefits for participants

If the comparative advantage of buprenorphine over morphine noted in the Phase I trial is maintained, infants randomized to this treatment will benefit from decreased length of hospitalization and length of treatment.

Collection and reporting of AEs and SAEs

All study participants will be monitored by study staff or investigators on a daily basis for any treatment emergent AEs. All AEs will be reported on paper case report forms and transferred to the Thomas Jefferson University electronic AE reporting system (eSAEy) accessed at http://www3.kimmelcancercenter.org/clinicaltrials/ae/ .

Each AE will be classified by an investigator as SERIOUS (including life-threatening) or NON-SERIOUS. This classification of the gravity of the event determines the reporting procedures to be followed.

An AE that meets one or more of the following criteria/outcomes is classified as SERIOUS:

- Death
- Life-threatening (i.e., immediate risk of death)

- Prolongation of hospitalization
- Persistent or significant disability/incapacity
- An event not included in the list above, but one that may jeopardize the subject or require intervention to prevent one of the outcomes listed above, will be reported as a serious adverse event (SAE).

A NON-SERIOUS event is one that does not meet the criteria described for a serious or life-threatening event.

A project investigator will assign an attribute to each AE that occurs, in accordance with the current version of Common Toxicity Criteria as follows:

5	Definite –	The AE is *clearly* related to the treatment.
4	Probable –	The AE is *likely* related to the treatment.
3	Possible –	The AE *may be* related to the treatment.
2	Unlikely –	The AE is *doubtfully* related to the treatment.
1	Unrelated –	The AE is *clearly not* related to the treatment.

Management of SAEs or other study risks
Potential risks to infants enrolled in the trial are minimized by the use of careful inpatient monitoring in a high-acuity clinical ward. Study staff will evaluate infants daily for the development of AEs. Should an AE develop, qualified physicians, nurses and support staff are immediately available. The medical care of infants will be assured by a collaborator who is a board certified neonatologist. Following each SAE judged to be *possibly, probably* or *definitely* related to study medication, no further enrollment will take place until the DSMB review of the event is completed.

Trial Efficacy
Plans for interim analysis of efficacy data
There is no planned interim analysis for safety or efficacy. If the incidence of non-serious AE's is higher than expected, the PI can propose an ad hoc interim look to the DSMB and NIDA Program Official. The DSMB and NIDA Program Official will also receive biannual blinded summary statistics of adverse events. If both parties are in agreement, an interim look can be undertaken. The study statistician and investigational drug service staff will provide the unblinded adverse event data. All attempts will be made to maintain the blind of the principal investigator and clinical staff, unless DSMB makes a study-wide decision to unblind the study.

DSM Plan Administration
Responsibility for data and safety monitoring
The principal investigator Dr. Walter Kraft is the medical monitor for the study and will be responsible for the overall conduct of the trial, including all aspects of safety monitoring and reporting. Dr. Susan Adenyi-Jones is the neonatologist investigator who will be responsible for coordinating the medical care for infants with treatment-emergent adverse events. The study coordinator will be responsible for documentation of safety measures.

Frequency of DSM
A DSM report will be produced by the principal investigator every 6 months. This report will be forwarded to all members of the DSMB. The report will contain a brief description of the trial, baseline sociodemographic characteristics, retention, and disposition of study participants, and a listing of AEs. If applicable, quality assurance and audit findings, regulatory Issues, and SAE narratives will be included. Overall length of treatment, length of stay, and unblinding rate will be

included, but there will be no unblinding of completed research subjects or formal review of efficacy.

Data Safety Monitoring Board (DSMB) Plan

Members and affiliation

A Data Safety Monitoring Board (DSMB) overseeing the trial includes four independent, external experts who have agreed to serve. Dr. Wade Berrettini, Professor of Psychiatry at the University of Pennsylvania and an expert in the pharmacology of substance abuse, has agreed to serve as chair of the DSMB. He has held this role in the Phase I trial of buprenorphine in NAS performed at Thomas Jefferson University. Dr. Laura McNicholas is also a faculty at the University of Pennsylvania and was a member of the Phase I DSMB. She is a national expert in buprenorphine pharmacology and clinical trials. Two additional members have agreed to serve on the DSMB. Dr. John van den Anker is a neonatologist and the Chair of Pediatric Clinical Pharmacology at Children's National Medical Center. Dr. Arzu Onu-Thomas is a biostatistician at St. Jude's Research Hospital and an expert in pediatric clinical trial design. The varied expertise and background of the members will ensure a qualified and independent evaluation of patient safety. A formal charter detailing DSMB conduct will be used to guide decisions.

Frequency of meetings and monitoring activities

The DSMB will meet annually. Additionally, the DSMB will be provided a copy of the 6-month interim DSM report between annual meetings. At the annual meeting the principal investigator will provide a comprehensive assessment of enrollment rates and current AEs. The assessment will indicate the significance of the AE, and whether these toxicities have affected the conduct of the trial. The DSMB chair will lead a discussion on general conduct of the trial, a review of outcome results and factors external to the study (such as scientific or therapeutic developments). The members will then make recommendations and vote on the status of the study. There is no planned interim look for safety. Between annual meetings, the DSMB Chair may call an ad hoc meeting based upon findings in the DSM report. The mechanism by which a meeting can be called will be described in a DSMB charter.

In the event of an SAE or emergent safety concern, the principal investigator or his designee will report the event to the IRB, the NIDA Program Official, FDA and DSMB. The DSMB will meet within 5 days (via conference call) following the notification of an SAE. No new infants will be enrolled until the DSMB has reviewed each SAE graded as possibly, probably, or definitely study drug related. Following each SAE, the principal investigator will make a determination of the need to unblind treatment for each patient. Even if withdrawn from the study, infants will be monitored until resolution of the SAE.

Conflict of interest

All members of the DSMB will be asked to disclose any potential conflicts of interest before the initiation of the study. Before each annual meeting, members will be asked to update their conflict of interest. The conflict of interest forms will be kept in the study regulatory binder maintained by the principal investigator.

Protection of confidentiality

The DSMB will be provided with anonymized subject data. Risk to the parents from disclosure of illicit drug use and prescription drug abuse will be minimized by use of a Certificate of Confidentiality. The investigators have obtained this protection for the parents of subjects who have participated in their NAS investigations and will do so in the proposed investigation. Prospective subjects will be advised of issues related to confidentiality in accordance with HIPAA

guidelines. All patient or maternal data containing protected health information (PHI) will be stored in secured areas of the principal investigator's office, in the Thomas Jefferson University Hospital medical records, or in the secured office of the Investigational Drug Service. Any publications resulting from these studies will be produced in a fashion such that no features that might identify individual participants are included.

Communication plan to IRB, NIDA, and FDA (if applicable)
The principal investigator will be responsible for monitoring the safety and efficacy of this trial, executing the DSM plan, and complying with the reporting requirements. The PI will provide a summary of the DSM report to the NIDA Program Official on an annual basis as part of the progress report. The FDA will be updated on a yearly basis with the submission of the IND annual report. The NIDA Program Official and FDA will be notified of any SAEs judged to be *possibly, probably*, or *definitely* related to study drug reported within 48 hours, except death, which will be reported within 24 hours (one working day). SAEs judged to be *probably not* or *definitely not* related to study drug will be reported via the Medwatch system within 5 days.