Title	Modeled Dose Exposure of Sublingual Buprenorphine in the Neonatal Opioid Abstinence Syndrome
Short Title	**B-PHORE** (**B**uprenorphine **Ph**armacometric **O**pen Label **R**esearch study of Drug **E**xposure)
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Protocol Date	April 27, 2018
IND	68,403
Institutional Review Board	Thomas Jefferson University
Version	1.1

PROTOCOL SYNOPSIS

Primary Objective	To define buprenorphine pharmacokinetic exposure in infants treated with buprenorphine for neonatal abstinence syndrome (NAS) using a model-based optimized dose.
Exploratory Objectives	To examine the safety of a model-based optimized dose of buprenorphine in neonates. To estimate efficacy of length of treatment of a model-based optimized dose of buprenorphine for infants treated for NAS. To evaluate possible developmental trajectory of urinary glucuronidated metabolites of buprenorphine.
Design	Open label, single arm clinical trial
Study Treatment	Buprenorphine Sublingual 0.075 mg ml solution *(Buprenex, Indivior, Richmond, VA or generic equivalent) , 30% ethanol USP, in simple syrup USP*
Number of Subjects	10 infants
Population: Inclusion Criteria	Patients eligible for participation include: 1. ≥ 36 weeks gestation 2. Exposure to opioids in utero 3. Demonstration of signs and symptoms of neonatal abstinence syndrome requiring pharmacologic treatment
Population: Exclusion Criteria	Patients ineligible for participation include: 1. Major congenital malformations and/or intrauterine growth retardation, defined as birth weight <2000 gm 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. Inability of mother to give informed consent due to co-morbid psychiatric diagnosis

PROTOCOL VERSIONS

Version	Date	Description
1.0	Feb 19, 2018	Sent to FDA for IND
1.1	Apr 27, 2018	• Added reference for Moore et. al. buprenorphine PK/PD publication (PMID 29516490) • Adding collection of hours of duration of treatment to current days, if feasible (section 3.2.4.1) • Change phenobarbital weight used in dose calculation to birth weight (section 5.2.2) • Removal of “investigational” from pharmacy site of drug preparation. Drug can be made by standard hospital pharmacy (section 5.3.1) • Added “dose interval” to “dose” for reversion to control situation at which drug can be resumed following score $\geq$ 28 (section 6.6) • Various minor typographical, formatting, and stylistic changes

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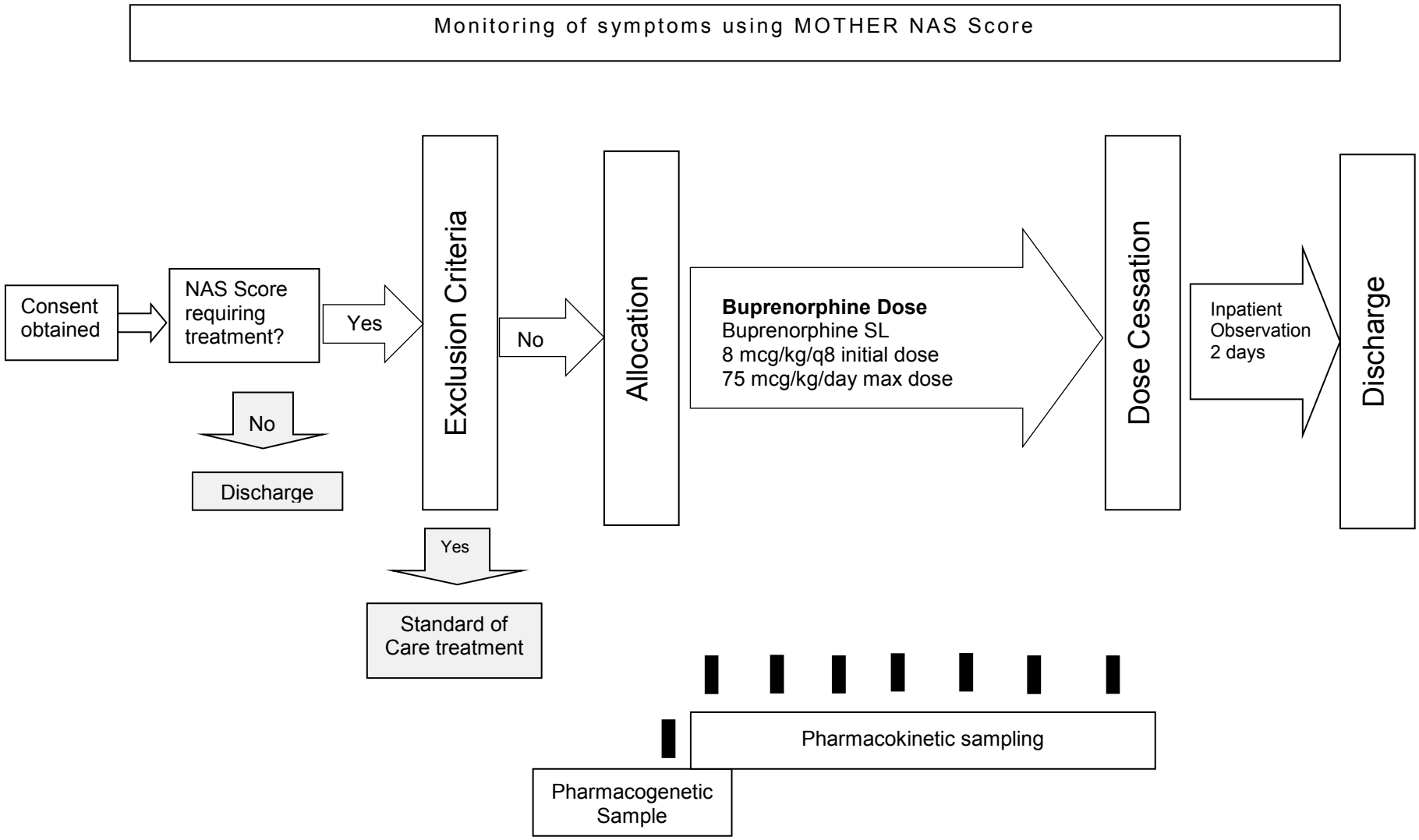


Figure 2: Study Algorithm

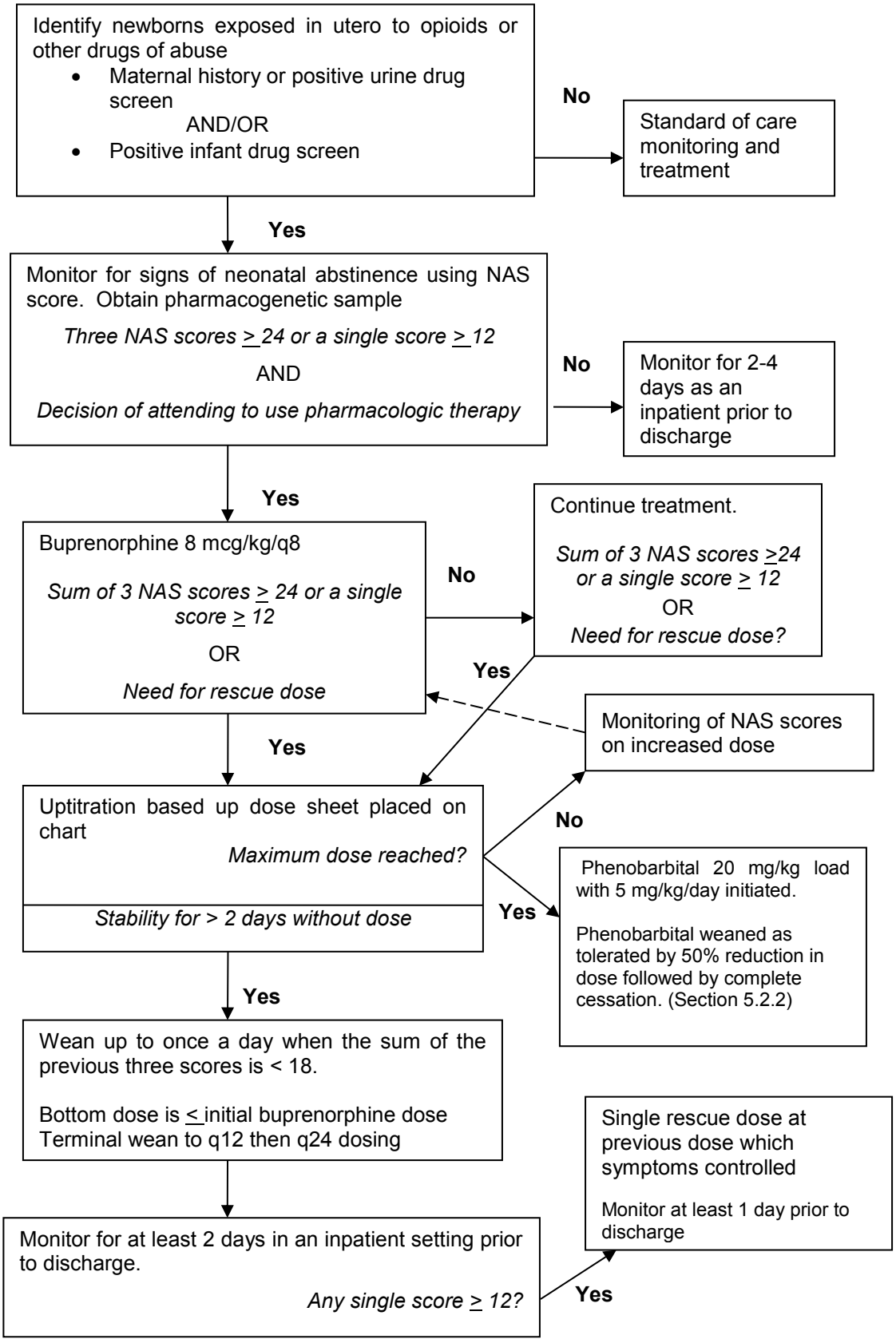
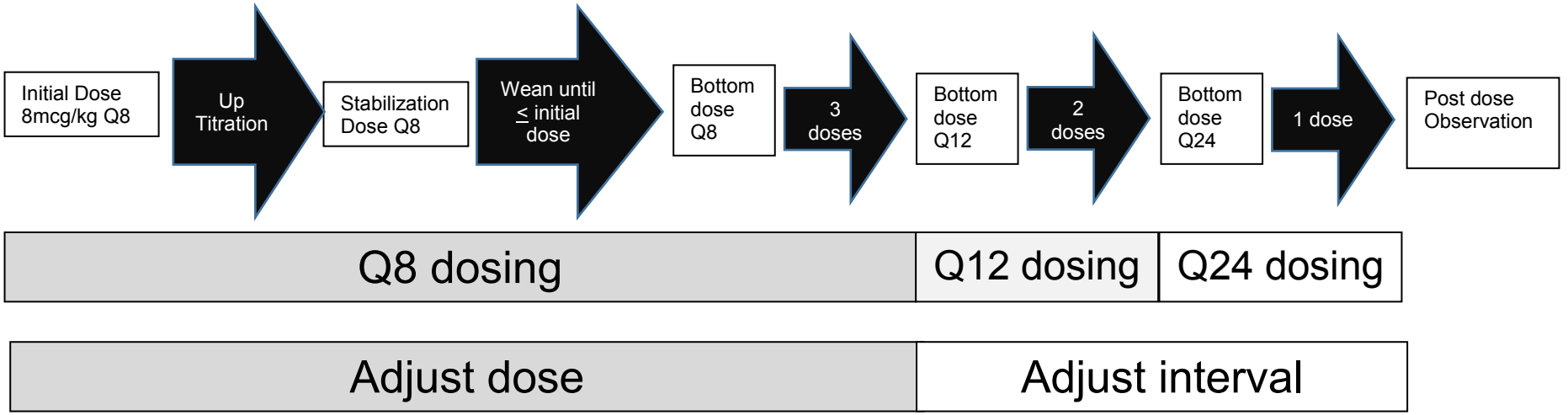


Table 1: Dose Schema for Buprenorphine

	Buprenorphine
Initial daily dose	24 mcg/kg/day
Initial unit dose	8 mcg/kg q8 hours
Maximum daily dose	75 mcg/kg/day
Maximum unit dose	25 mcg/kg q8 hours
Up-titration rate	33%
Maximum # of up-titrations	4
Weaning rate	15%
Cessation (bottom) dose	≤ Initial dose
Dosing interval until bottom dose (hrs)	8
Dose interval extension #1 at bottom dose (hrs)	12
Dose interval extension #2 at bottom dose (hrs)	24
Inpatient observation following cessation of last scheduled dose	At least 2 days
Inpatient observation following last rescue dose	At least 1 day

Figure 3: Study Algorithm



1 INTRODUCTION

1.1 Background

Neonatal abstinence syndrome (NAS) is a set of signs of withdrawal in an infant with in utero exposure to opioids.[1] Cardinal manifestations include increased muscle tone, autonomic instability, irritability, poor sucking reflex, gastrointestinal symptoms, and impaired weight gain. All infants are treated with non-pharmacologic methods such as swaddling, rooming in with mother, and minimization of stimuli.[2] Despite these measures, ~ 50% of infants require pharmacologic treatment to ensure proper growth and development. While the optimal pharmacologic treatment for NAS has not been identified, expert review identifies an opioid as the primary therapy.[3] In the US 80% of infants are treated with morphine and 20% with methadone.[4] Sublingual buprenorphine has been demonstrated to be safe and effective in an open label clinical trial conducted by Thomas Jefferson University investigators [NCT00521248].[5,6] The BBORN (**B**linded **B**uprenorphine **OR** **N**eonatal morphine solution) double blinded clinical trial [NCT01452789] comparing buprenorphine to morphine for NAS confirmed the efficacy in a double blind fashion.[7] The external validity of this finding was supported by retrospective examination of buprenorphine used in a treatment paradigm, with a reduction in length of treatment of ~30%.[8,9]

Dose selection for both the phase 1 trial and the efficacy trial (BBORN) were empirically derived. A population pharmacokinetic model for buprenorphine in NAS has been published.[10] In addition a pre-specified endpoint for the BBORN trial was a pharmacokinetic analysis of buprenorphine. A pharmacokinetic/pharmacodynamic model from the BBORN study has been created.[11] The time to control of symptoms was directly tied to buprenorphine exposure, which itself appeared to be driven primarily by clearance. Among the strengths of pharmacometric models is the ability to simulate in silico many potential dose regimens. In this manner, a dose regimen can be chosen that achieves exposures associated with efficacy. This approach also allows for incorporation of covariates of drug exposure or response to treatment. This is much safer and efficient than the traditional approach of choosing an empiric dose that would need to be tested in clinical trial.[12] An ideal dose would quickly reach this exposure while maintaining a good safety margin. There was no evidence of decline in respiratory rate in infants treated with higher doses of buprenorphine compared to lower doses, or those treated with buprenorphine compared to those treated with morphine. This may allow a higher initial dose to more quickly reach therapeutic buprenorphine concentrations. This ultimately could lead to shorter lengths of treatment and stay, though this goal is outside of the scope of the current proposed project.

In summary, buprenorphine at the dose and schedule used in clinical trials has been demonstrated to be safe and effective. The goal of the proposed study is to simulate a dose of sublingual buprenorphine for

NAS using pharmacometric modeling techniques. This dose will be tested in infants requiring treatment for NAS. Pharmacokinetic samples would be collected and used to confirm and refine the pharmacokinetic model. The proposed study would allow broad examination and refinement of the exposure/response relationship. This optimized dose could later be used in an efficacy trial, and can serve other modeling purposes such as an eventual changes to the weaning dose to twice or even once a day for outpatient treatment.

1.2 Proposed Study Design

This is an open label, single arm, single site, dose finding clinical trial.

1.3 Rationale for Study Design and Dose

1.3.1 *Single arm*

Buprenorphine became the current standard of care for NAS pharmacologic treatment at Thomas Jefferson University Hospital. The goal of this study is to examine buprenorphine pharmacokinetics following an altered dose regimen. The study will not explore comparative safety or efficacy of buprenorphine or morphine. Therefore, a buprenorphine or morphine standard of care group would provide limited additional information.

1.3.2 *Buprenorphine Safety*

Pharmacologic treatment of NAS in a highly monitored inpatient setting is safe.[13] This has proved true for buprenorphine. A listing of all adverse events occurring in three clinical trials of buprenorphine at Thomas Jefferson University Hospital is listed in Table 2. This represents total adverse events, and some infants had more than one adverse event. All were “definitely not” or “probably not” related to study drug. There were four serious adverse events, none of which was drug related. This safety profile has been mirrored in published studies of 212 infants treated in Cincinnati, Ohio.[8,9]

Modeling from BBORN data demonstrated no respiratory relationship between either buprenorphine or norbuprenorphine exposure. Buprenorphine in adults has a modestly correlated dose response relationship, with a much better exposure response as defined by amelioration of symptoms[14] and CNS mu opioid binding.[15,16] There have been no observed episodes of excessive sedation, respiratory depression, or aspiration after >3,500 doses of sublingual buprenorphine administered to >60 infants in the phase 1 and BBORN trials. No respiratory depression was described by Hall in the clinical use of buprenorphine for NAS in Cincinnati (personal communication, S Wexelblatt), nor in the current therapeutic use at Thomas

Jefferson University Hospital. These findings mirror years of clinical experience with morphine administered in a monitored setting, as well as wide experience with methadone at other sites. Hepatic safety is similarly reassuring based on an adolescent cohort study.[17] There was no hepatic toxicity noted in a 2008 case series of 84 pediatric overdoses of buprenorphine.[18] In clinical trial experience at Thomas Jefferson University Hospital, there was an episode of elevated transaminase level in an infant with unrelated cytomegalovirus. There were no other elevations of liver enzymes in any other subjects. These lines of evidence strongly support the wide therapeutic index of the partial agonist buprenorphine at exposures anticipated using a new dosing regimen.

Table 2: Adverse Events Observed in Buprenorphine Clinical Trials at Thomas Jefferson University

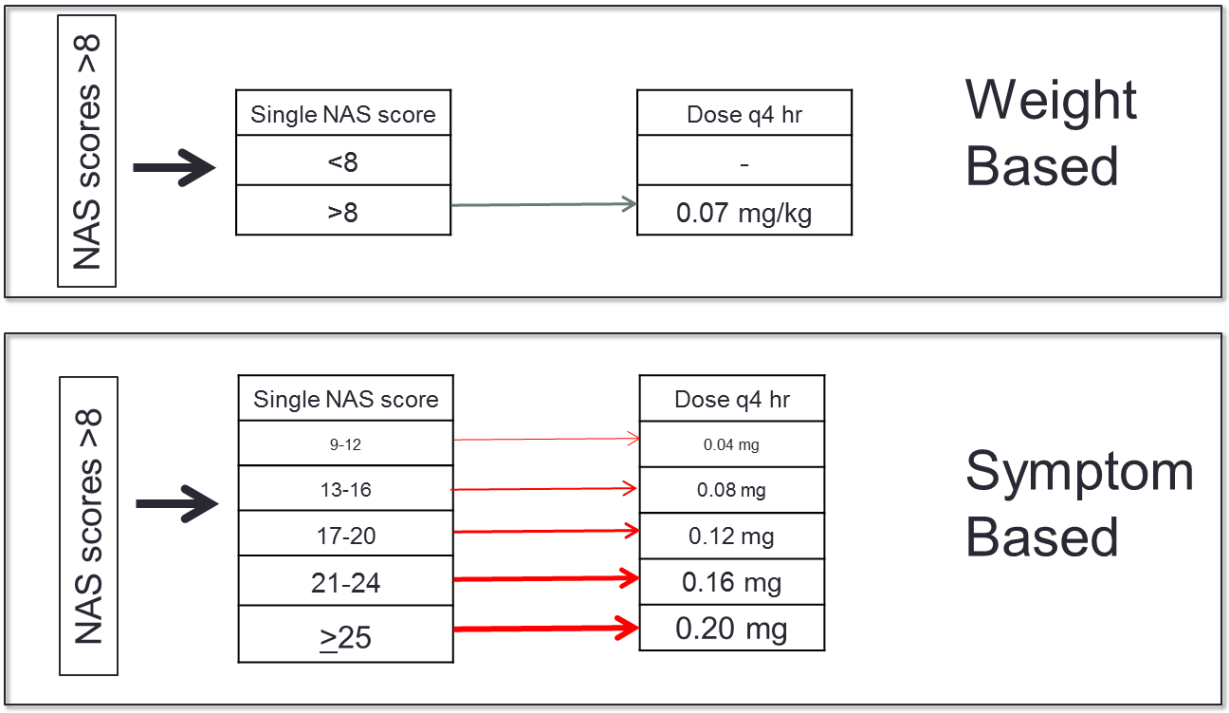
	Buprenorphine (n=64)	Morphine (n=60)
Anemia	1	0
Skin conditions	7	3
Gastrointestinal conditions	4	6
Respiratory conditions	1	2
Tachycardia	1	0
Umbilical granuloma	1	0
Urinary conditions	1	1
Ocular conditions	0	1
Clavicle birth fracture	1	0
Hepatic conditions	1	0
Inguinal hernia repair*	0	1
Supraglottoplasty*	1	0
Cytomegalovirus infection*	1	0
Seizure*	1	0
Total number of adverse events	21 (33%)	14 (23%)

*unrelated serious adverse events

1.3.3 *Weight based vs Symptom based dosing*

This trial will use a weight-based approach, which is regimen in all published buprenorphine clinical trials in NAS. This method employs a single weight-based dose for all infants regardless of the initial NAS score. A small number other centers using morphine employ a symptom-based approach. This symptom-based regimen uses a weight-independent dose that varies with the severity of disease. (Figure 3) The weight-based approach accounts for variability due to infant mass, at the expense of being less tailored to individual disease severity. The weight-based approach is most commonly used with almost all other medications used in the neonatology unit. Symptom based dosing captures variability in disease severity but will result in more varied initial dose. While the weight based method is much more commonly used, there is no consensus as to which is the preferred method, nor are there any comparative trials.[19,20]

Figure 4: Representative Weight and Symptom Based Approaches to Morphine Dosing



A concern about a weight-independent dose calculation is variability in buprenorphine exposure based upon infant size. For smaller infants with severe symptoms, there is a theoretical concern of an excess dose leading to respiratory depression. However, this concern is less likely due to partial agonism of buprenorphine and a ceiling effect on respiratory depression in adults.[21] Despite this safety profile that is likely to be favorable, the weight based approach has a number of compelling advantages. Foremost is that the original modeling exercise is based upon the BBORN trial, in which doses and titration schedules were weight based. Adding the additional variable of a new titration regimen will complicate simulation of doses. Secondly, the culture and practice of clinicians at Thomas Jefferson University Hospital is to use a weight-based approach. Maintaining this approach for both study and non-study infants will help to standardize care within the context of the clinical trial. There is greater clinician acceptance of the weight-based approach external to Thomas Jefferson University. Lastly, the use of a weight-based approach in this pharmacokinetic-driven protocol will not substantially impact the ability to test the symptom based approach in subsequent phase 2 and 3 studies. The primary goal of the current investigation is primarily investigating exposures with alternated dose regimens, with a secondary goal of attempting to define exposure response.

1.3.4 *Justification of Buprenorphine Dose*

This protocol seeks to explore additional dose regimens which will be used to refine a pharmacometric

model of buprenorphine in NAS. This model refinement will aid in increasing precision with which future simulations can be performed. The goal of these simulations will be to inform subsequent clinical trials to optimize drug dosing. The parameters to be examined are 1) initial dose, 2) uptitration rate, 3) maximum dose, 4) weaning rate, 5) cessation dose, and 6) dosing interval. The study regimen employs a terminal wean in which the final adjustments are an elongation of the dosing interval rather than a reduction in the dose. To reduce confusion to parents and clinicians, what has been termed until now the "cessation dose" will be generally referred to as the "bottom dose".

Table 3: Final Dosing Plan. Proposed dose schema to be used in this trial (BPHORE) is compared to that used in the BBORN trial

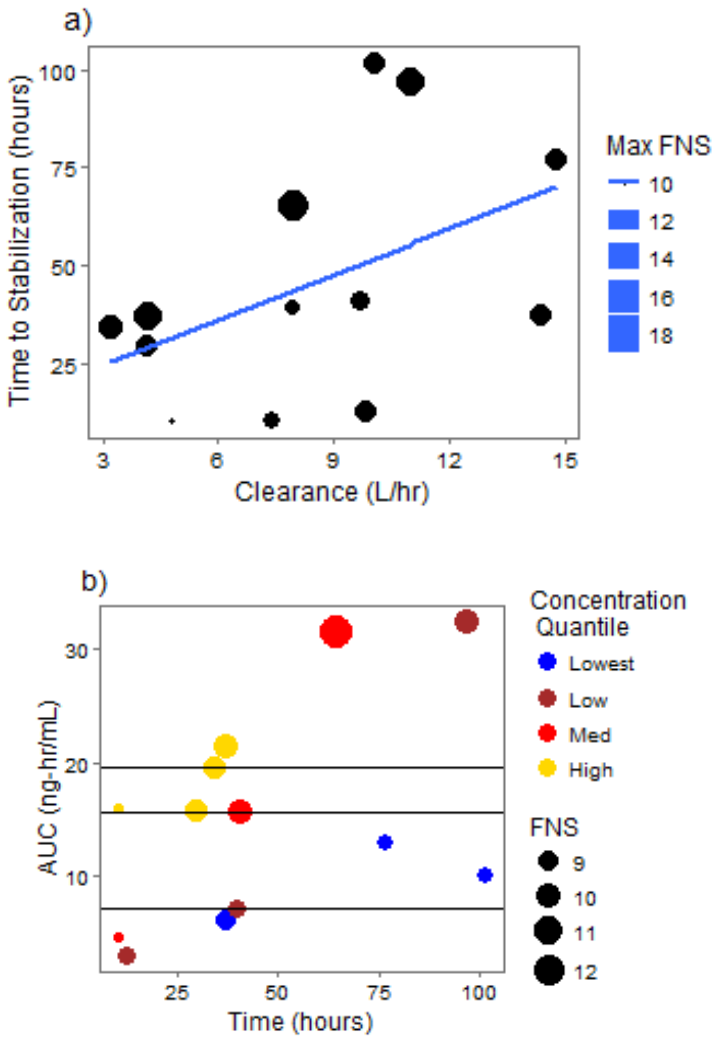
Trial	BPHORE	BBORN
Initial dose (mcg/kg)	8	5.3
Uptitration rate	33%	25%
Maximum number of up-titrations	4	6
Maximum dose (mcg/kg)	25	20
Weaning rate	15%	10%
Cessation ("bottom") dose	≤ 100% of initial dose	≤ 110% of initial dose
Dosing interval until bottom dose (hrs)	8	8
Dose interval extension #1 at bottom dose (hrs)	12	N/A
Dose interval extension #2 at bottom dose (hrs)	24	N/A

1.3.4.1 Pharmacokinetic/Pharmacodynamic (PK/PD) core model

The BBORN trial used an initial dose of 5.3 mcg/kg/q8, with a maximum dose of 20 mcg/kg/q8. This approach was associated with a mean length of treatment of 15 days, compared to 28 days with the standard of care morphine regimen. Based upon the pharmacometric model developed by Ng from the phase 1 data at Thomas Jefferson University Hospital,[10] Moore and Ng developed a revised model from the BBORN trial.[11] This model was based upon 265 samples of buprenorphine and norbuprenorphine, and 4,373 NAS scores collected from 28 infants. The model was shown to reasonably predict the BBORN data with a mean squared error of 0.062 and root mean squared error of 0.251. An adapted PK model was fit to the data and extended to describe the metabolite concentration-time profile. An exposure-response relationship was found between buprenorphine and NAS symptom progression, but not for respiratory rate. Time to NAS stabilization was found to decrease with increasing buprenorphine AUC. Neonates with a lower clearance had a shorter time to stabilization as shown in Figure 4a. Time to stabilization of NAS scores was best correlated with buprenorphine exposure. Neonates with similar severity of NAS generally stabilized around the same time. Furthermore, for any given group of NAS severity, neonates exposed to higher concentrations of buprenorphine tended to stabilize faster. While buprenorphine and

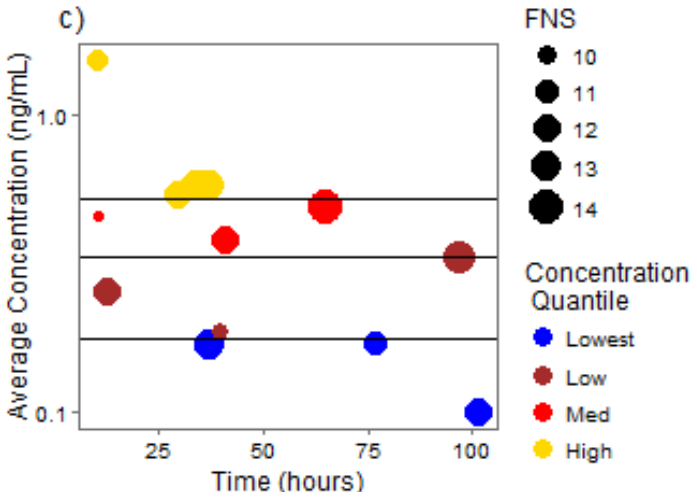
norbuprenorphine concentrations were highly correlated, buprenorphine was found to be a more significant driver of PD effects. Though the primary metabolite norbuprenorphine has a greater respiratory effect than the parent in animal models,[22] it is a substrate of p-glycoprotein and with very little CNS penetration.[23,24] For this reason dosing simulations will be based primarily on buprenorphine rather than metabolite.

Figure 5: Relationship of Buprenorphine Concentration to Control of NAS Symptoms

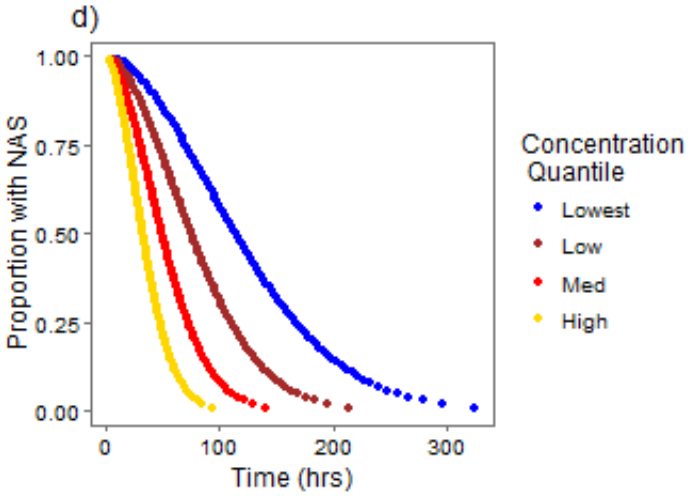


A) Effect of Changing Clearance on Time to NAS Stabilization (TNS). The size of each dot represents each neonate's maximum Finnegan Score (FNS).

B) Relationship between TNS and AUC. Each neonate included is represented by a point, which represents the TNS and AUC of buprenorphine until that time. The graph is divided into quartiles of AUC by the 4 black lines and is divided into quartiles of average concentration by the colors. The max FNS score is represented by the size of each dot.



C) Relationship between TNS and average concentration. Each neonate included is represented by a point, which represents the TNS and average concentration of buprenorphine until that time. The graph is divided into quartiles of average concentration by the colors and the black lines. The max FNS score is represented by the size of each dot.



D) NAS Survival Analysis. This graph represents the predicted stabilization of NAS over time for a theoretical neonate at one of the 4 quartiles of buprenorphine concentration and a max FNS of 11.

Based upon the exposure response relationship, several summary observations can be made from the PK/PD modeling of buprenorphine in NAS.

- Efficacy of time to stabilization is driven primarily by buprenorphine exposure, implying that most of the variability in dose response has a pharmacokinetic basis.
- Exposure is driven primarily by changes in clearance, with no clearly identified covariates that can predict pharmacokinetic variability.
- There was no identifiable change in respiratory rate associated with buprenorphine exposure.
- In light of limited sampling close to dose administration, absorption kinetics are not well defined.

A pharmacometric simulation created using the model was employed with a goal of exploring variations in dosage regimens. The final PK/PD model was utilized to explore exposure differences by performing dose ranging simulations, from 0.1 to 15 mcg/kg doses. An adaptive simulation design was developed to account for the dynamic changes in withdrawal due to buprenorphine exposure based on each of the simulated doses. Stochastic simulations used fixed parameter values for PK parameters and inter-individual

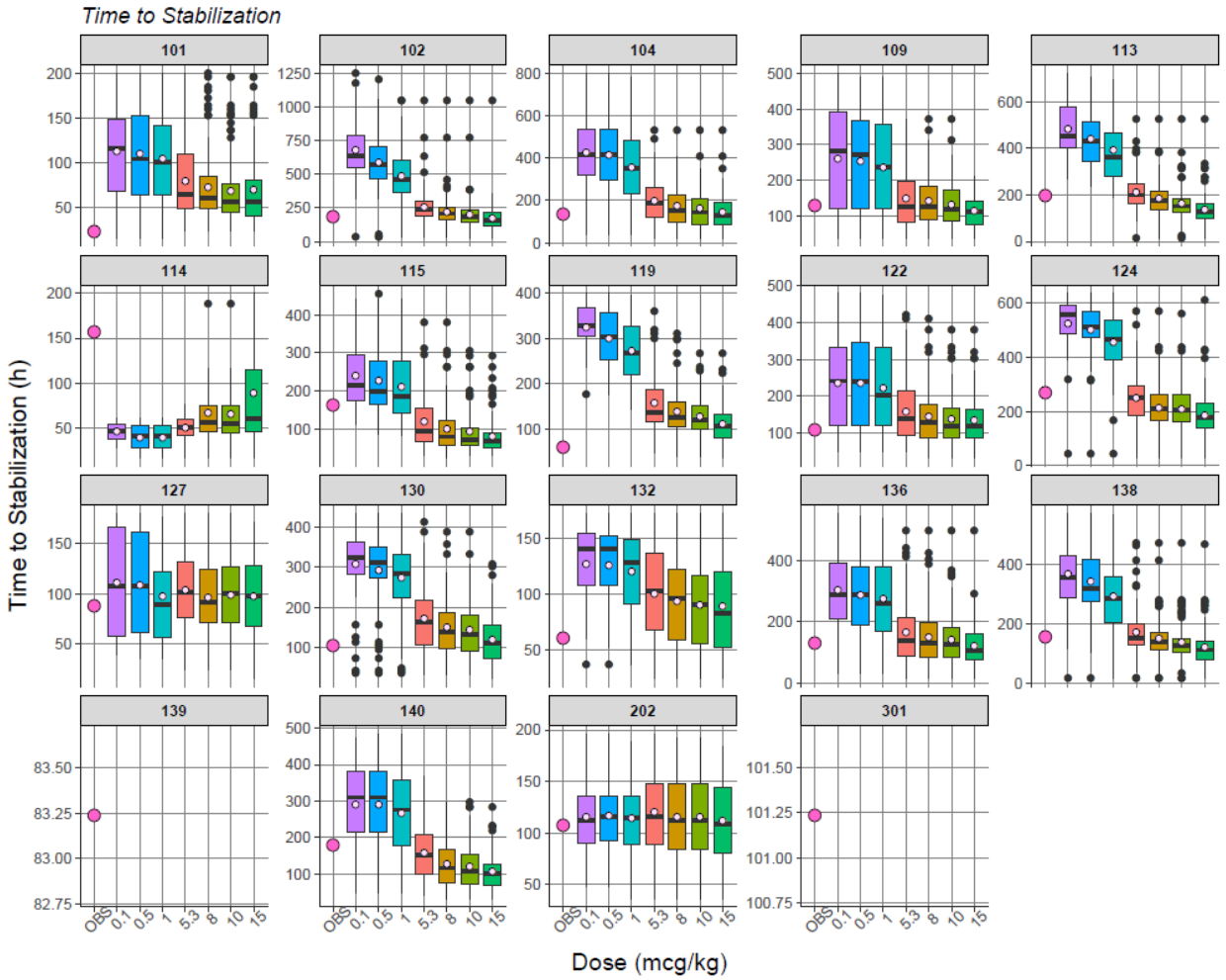
variability. However, residual variability was incorporated to account for random effects. This was modeled by assuming a normal distribution and randomly sampling from the distribution from the final PK/PD model with 100 simulations per subject, resulting in a probability distribution for the expected exposure for each simulated dose.

1.3.4.2 Initial Buprenorphine Dose

A simulation using initial doses of 0.1, 0.5, 1, 5.3, 8, 10 and 15 mcg/kg was run for each individual patient and compared to actual observed time to stabilization. (Figure 6)

Figure 6: Modeled Time to Stabilization Using BBORN Uptitration (25%) and Weaning (10%) Rates.

Grey boxes identify individual subjects in the BBORN trial. Simulated doses are listed, with mean time (light pink dot), interquartile ranges and actual observed (OBS) values in dark pink circle.



Time to stabilization was defined as the time after initiation of treatment at which there were no up-titrations in dose for 48 hours and the patient had first weaning dose. Specific parameters used to define this in the

simulation were based upon the weaning algorithm (Figure 2), which was the time point at which there was a single Finnegan score <6, cumulative score over previous 3 was < 17, weaning dose was employed, and no up titration. These simulations suggested there was limited efficacy advantage beyond an initial dose of 8 mcg/kg. This dose represents an only modest increase over that used in clinical trials and a treatment setting, and as such would likely to be accepted by clinicians involved in the proposed trial.

1.3.4.3 Simulated Uptitration

The BBORN trial used an uptitration rate of 25%. A 33% and 50% up-titration rate were simulated (Table 4, reference in shaded box) at the 8 mcg/kg q8 initial dose. The endpoint was time to stabilization, defined as 48 hours without need for up-titration of dose.

Table 4: Simulated Time to Stabilization (hours) with Varied Uptitration Rate

	Time to stabilization (hours)			**Δ time to stabilization from reference 25% uptitration rate (hours)**	
Initial dose (mcg/kg)	**Titration rate**			**Titration rate**	
	25%	**33%**	**50%**	**33%**	**50%**
5.3	153.8	139.2	126.0	-14.6	-27.8
8.0	137.3	131.5	118.0	-5.8	-19.3

Shaded box represents the dose regimen used in the BBORN trial.

Both 33% and 50% provide advantages over the 25% uptitration rate in meeting the time to stabilization. However, as a higher initial dose is being used, the use of a 50% uptitration rate would entail large, rapid increase in exposures. In the BBORN trial there were 6 titrations before maximum dose was reached. Even with increasing the maximum dose, the number of titrations before need for a secondary agent drops. At a 50% increase, maximum dose is reached after only three uptitrations. (Table 5)

Table 5. Uptitration Steps to Maximum Dose

		Uptitration step						
Uptitration rate	**Initial dose (mcg/kg)**	1	2	3	4	5	6	**Max daily dose (mcg/kg)**
25%	5.3	6.6	8.3	10.4	12.9	16.2	20.0	60.0
25%	8	10.0	12.5	15.6	19.5	25.0		75.0
33%	8	10.6	14.2	18.8	25.0			75.0
50%	8	12.0	18.0	25.0				75.0

Shaded row represents the dose regimen used in the BBORN trial.

Based on the favorable simulated efficacy advantages, combined with a conservative approach to rate of increase and ability to titrate before reaching maximum dose and need for adjunctive phenobarbital, a uptitration rate of 33% will be used.

1.3.4.4 Maximum Buprenorphine Dose

The maximum dose employed in all reported studies has been 20 mcg/kg q8. The primary safety concern with an opioid treatment is respiratory depression. No respiratory depression was noted clinically in either buprenorphine or morphine arms in either the phase 1 or BBORN studies, though in BBORN the morphine patients in had on average a 4.4 breath per minute slower respiratory rate. In the PK/PD model, there was no relationship between buprenorphine exposure and respiratory rate. Respiratory depression using morphine and methadone for NAS in an inpatient setting is unheard of. Maximum doses of morphine vary across units. The Thomas Jefferson University Hospital maximum is 1.25 mg/kg, though doses as high as 1.5 mg/kg have been used on occasion in difficult to control cases. These values are similar to that described by O'Grady in a survey of units in the United Kingdom.[25] Of interest, one third of surveyed units had no stated maximum dose used but instead described a dose driven by symptoms. The safety margin of buprenorphine is expected to be much wider than morphine or methadone. It is a partial rather than full mu opioid receptor agonist and has superior respiratory safety profile in adults and adolescents. As such, an increase in the maximum dose to 25 mcg/kg q8 will be tested. Use of the higher dose will allow a fuller exploration of the pharmacokinetics and exposure of buprenorphine, and will strengthen the ability to simulate dose regimens to be subsequently tested in a phase 2 study.

Exposures of buprenorphine will be well below those anticipated to incur any safety issues. The buprenorphine exposure response relationship varies with specific pharmacodynamic endpoint measured, but for respiratory rate depression the plateau occurs at 16 mg of sublingual solution in adult, opioid naïve subjects. This dose is associated with a Cmax of ~10 ng/ml.[21] At that exposure a decrease in respiratory rate was observed, but none of the opioid naïve volunteers had clinically significant respiratory depression. Recent investigations confirm that the ceiling effect is pharmacodynamic and not pharmacokinetic, as there is linear dose to exposure relationship, with serum concentration of >170 ng/ml well tolerated in opioid experienced volunteers.[26] In infants with opioid exposure in utero, extrapolation from opioid experienced compared to naïve adult volunteers is more applicable. The highest recorded serum concentration in either BBORN or the phase 1 trial was 18 ng/ml without any observed respiratory depression or excess somnolence. The mean concentration in BBORN of those above the limit of quantification was 0.5 ng/ml.

1.3.4.5 Simulated Wean

A 15% and 25% weaning rate was simulated compared to the current standard of 15% (Table 7, reference in shaded box) at the 8 mcg/kg q8 dose. The endpoint was time to wean, defined as the time in hours from start of treatment to last dose (including any rescued doses).

Table 7: Simulated Time to Wean (hours) with Varied Wean Rate

	Time to wean (hours)			**Δ time from reference 10% weaning rate (hours)**	
Initial Dose (mcg/kg)	**Weaning rate**			**Weaning rate**	
	10%	**15%**	**25%**	**15%**	**25%**
5.3	166.1	125.1	102.4	-41.1	-63.7
8	155.7	112.8	97.5	-42.9	-58.2

Shaded box represents the dose regimen used in the BBORN trial.

1.3.4.6 Cessation Buprenorphine Dose

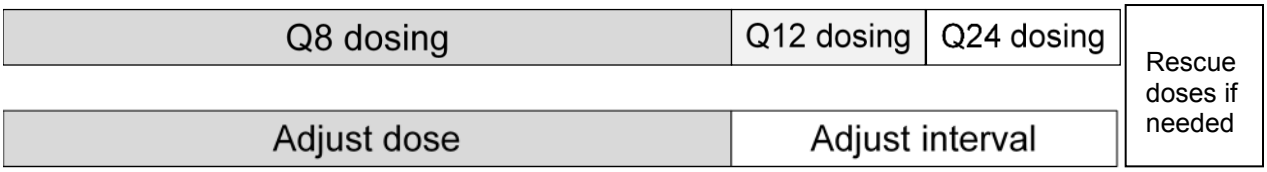
In the BBORN trial, the cessation dose was within 10% of the initial dose. The mean dose at time of cessation was 5.3 mcg/kg, based upon actual weight at the time of cessation. No infants required readmission following discharge, however 44% required a rescue dose after scheduled dosing was completed. The current trial will define the cessation dose as the dose that is equal or less than the initial dose in mcg (and not a weight normalized dose of mcg/kg). Most infants will regain their birthweight at about the time for cessation. A simulation was performed to estimate the impact of the proposed cessation dose (Table 8). This simulation was based upon cessation of all dosing at the cessation dose without any extension of interval. Despite a higher cessation dose, the model predicted less of a need for rescue doses. Symptom driven administration of rescue doses ensure that there will not be inadequate treatment of symptoms, even if the precision of the modeled response is less than expected.

Table 8: Simulated Time to Wean (hours) with Varied Wean Rate

	BPHORE	BBORN
Initial dose (mcg/kg)	8	5.3
Cessation dose	≤ 100% of the initial dose	≤ 110% of the initial dose
Initial mean infant weight used in simulation (gm)	2988	2988
Simulated weight at cessation (gm)	2940	3010
Simulated dose at cessation (mcg)	27.1	18.1
Simulated normalized dose at cessation (mcg/kg)	9.3	6.1
Simulated % of infants who would require post cessation rescue dose	25%	39%
Actual % of infants in BBORN requiring post cessation rescue dose	N/A	44%
Actual median birthweight in BBORN (gm)	N/A	3040

1.3.4.7 Terminal Extension of Dosing Interval

In BBORN 44% of infants required a post cessation rescue dose of buprenorphine. The last administered dose was used for determination of the primary endpoint of length of treatment in the BBORN trial. Though the model suggests a lower rate of post dose cessation, the cessation dose is higher than in BBORN and remains relatively high. Rescue dose after a patient has cessation of medication is often seen as weaning "failure" by parents and some clinicians, rather than part of the weaning process. As the goal of this protocol is to explore alternate dosing regimens, the protocol will at the cessation or "bottom" dose switch over to a weaning of dose interval. This is represented in Figure 3 and below. The impact of this change in interval has not been formally simulated, but it is anticipated that this will reduce the number of post cessation rescue doses. The sample size of this trial will make formal assessment of this approach on clinical endpoints of length of treatment not possible, but the exercise will assist with exploration of regimen optimization.



1.3.4.8 Final Dose Regimen Selection

Simulated regimens suggest additional efficacy when uptitration rate is extended from 30 to 50%, as well as decreased treatment duration when weaning rate is increased from 15 to 25%. In both cases, the larger

jumps reduce the discriminatory power of the regimen to avoid higher buprenorphine doses in infants who would have either been managed with lower dose of buprenorphine afforded by a slower uptitration, or those who would have benefited from a slower down titration rate. In addition, the incorporation of a new maximum dose is challenging to accurately capture using modeling techniques. As the goal of this protocol is to gather information to refine an existing model, it is prudent to undertake more modest dose and regimen changes.

1.3.5 *Efficacy Assessment of Length of Treatment*

Buprenorphine efficacy has been demonstrated in infants even with concentrations below the 0.7 ng/ml considered to be the level at which relief of adult abstinence symptoms begins.[14] The model of Moore and Ng suggest the value in infants to be 0.8 ng/ml. Using the pharmacometric model used to describe the BBORN data, a simulation was created to predict the range of expected exposures associated with the proposed regimen. Compared to 5.3 mcg/kg, an initial dose of 8 mcg/kg would did not substantially change the mean number of titrations needed to reach stabilization, but did reduce the time to stabilization. The small sample size and lack of comparator group make an assessment of the endpoints of length of treatment, length of stay, or need for phenobarbital rescue within this study to be at best exploratory.

1.3.6 *Safety*

There has been no indication of idiosyncratic or dose-related toxicity. Respiratory related toxicity is of the largest concern with dosing of an opioid. In the BBORN trial there were no episodes of clinical respiratory depression. Model based analysis implied that at any time during the treatment period, the mean respiratory rate in morphine treated infants was lower by 4.4 breaths per minute (95% CI: 0.7, 8.1; p=0.020) as compared to buprenorphine treated infants in the same strata. Furthermore, in the pharmacometric modeling analysis there was no relationship between observed concentration of buprenorphine and respiratory rate. Hysteresis plots and patient-level data were also analyzed but showed no relationship between buprenorphine or norbuprenorphine concentration and respiratory rate. Considering the wide therapeutic index of buprenorphine in multiple populations, the modest increase in maximum dose is considered to have low risk for dose-dependent adverse effects.

1.4 Pharmacogenetics Sample

Pharmacogenetic variants can lead to differences to drug exposure. Since the primary endpoint of the trial is pharmacokinetic, a blood sample for pharmacogenetics will be obtained from infants enrolled. Pharmacogenetic variation in the neonatal abstinence syndrome has been primarily based upon differences in receptor polymorphisms such as OPMR1 or genes associated with neurotransmitter disposition such as

prepronociceptin (PNOC), catechol-O-methyltransferase (COMT) genes.[27-30] There has not been demonstration of pharmacogenetic-linked drug metabolism or transport on outcomes in NAS. For this reason there will not be an a priori list of genes to be surveyed. In addition, the small number of patients to be enrolled will allow very limited power to differentiate drug exposure according to genotype. However, as the genetic analysis of the BBORN trial is currently underway, it is possible that there will be an emergent determinant of drug disposition based upon genotype.

2 STUDY OBJECTIVES

2.1 Primary Objective

To define buprenorphine pharmacokinetic exposure in infants treated with buprenorphine for neonatal abstinence syndrome (NAS) using a model-based optimized dose.

2.2 Exploratory Objectives

1) To examine the safety of a model-based optimized dose of buprenorphine in neonates.

2) To estimate efficacy of length of treatment of a model-based optimized dose of buprenorphine for infants treated for NAS.

3) To evaluate possible developmental trajectory of urinary glucuronidated metabolites of buprenorphine.

3 OVERALL DESIGN AND PLAN OF THE STUDY

3.1 Overview

This is a single-site, open label clinical trial. Potential patients will be identified in the prenatal period by staff of the Thomas Jefferson University Family Center and by daily screening of the inpatient census at Thomas Jefferson University Hospital. Infants at risk for NAS will have abstinence assessed using the MOTHER scoring instrument,[31] which is based upon Finnegan Score and will hereafter be called the “NAS score” (Appendix 1). This is the standard instrument used at Thomas Jefferson University Hospital. 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 (Figure 2). When the threshold for initiation of treatment is reached, a re-review of inclusion and exclusion criteria will take place prior to dose administration. NAS scores will be obtained every 4 hours. Dose assessment will take place daily. If the three previous NAS scores are ≥24, a dose advancement will take place (at the discretion of the neonatologist). Buprenorphine dose will be increased by 33% per uptitration. After two days of stability, patients will have weaning of dose until initial dose is reached.

3.2 Endpoints

3.2.1 *Pharmacokinetic Endpoint*

Sparse sampling will be used to generate an estimate of pharmacokinetic parameters and intersubject variability of sublingual buprenorphine. Population PK generated exposure profile of the revised dose schema will be compared to model simulations.

3.2.2 *Developmental Glucuronidation Endpoint*

Diaper cotton will be used to collect urine every 5-7 days. Glucuronidated buprenorphine metabolites will be assayed. The relative fraction of each glucuronidated metabolite will be reviewed as a function of post-natal age. Developmental phase 2 metabolic processes will be estimated on an exploratory basis using glucuronidated metabolites collected from spot urine.

3.2.3 *Safety Endpoint (exploratory)*

3.2.3.1 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 graded by an investigator according to a severity score (mild, moderate, severe).

3.2.3.2 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, and the FDA. An independent safety monitor will review all serious adverse events.

3.2.4 *Efficacy endpoints (exploratory)*

3.2.4.1 Length of 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. If feasible, hours of treatment from first until last dose of buprenorphine will be recorded.

3.2.4.2 Length of Stay

Length of stay is defined as the number of calendar days from date of birth to date of discharge from the hospital.

3.2.4.3 Need for Supplemental Phenobarbital Use

Phenobarbital is used as a rescue therapy when maximum opioid replacement therapy dose is reached without adequate resolution of symptoms. The number of infants requiring phenobarbital rescue will be recorded.

3.3 Justification of Inclusion Criteria

3.3.1 ***Preterm Infants***

Preterm infants have a well-described natural history of NAS and a need for treatment that differs from term infants.[32,33] The Finnegan score does differ in preterm infants,[34] but it remains the standard instrument for gauging withdrawal severity. The preterm population thus appears to differ from term infants in manifestations of disease. However, treatment approaches are similar and ~30% of infants at risk for NAS are premature. Barrett described the intravenous use of buprenorphine in 12 infants between the ages of 27-31 weeks with no safety issues identified.[35] Hall reported infants 34 weeks and older in his descriptions of infants treated with buprenorphine in NAS.[8,9] The buprenorphine treated infants in BBORN had a mean gestational age of 37.9 (SD 1.9). While inclusion of premature infants appears safe, this is a dose finding study with expected differential clearance based upon developmental stage. For this reason, the inclusion criteria will be widened slightly to $\geq$36 weeks. This will serve to expand the generalizability of data. Given the small sample size and few infants enrolled between 36-37 weeks, there likely will be only modest ability to detect maturation associated differences in drug exposure.

3.3.1.1 Benzodiazepine Exposure

A retrospective study at Thomas Jefferson University Hospital demonstrated that the length of NAS treatment for benzodiazepine exposed infants between 2000-2006 was prolonged,[32] a finding confirmed in other reports.[36] Hall reported 40% of buprenorphine treated infants as having been polysubstance exposed, though the exact break out of benzodiazepine exposures were not included. The unpublished MOP Plus trial at Thomas Jefferson University Hospital enrolled specifically infants with benzodiazepine exposure. [NCT01671410] No safety issues were identified in any of the six infants enrolled. There is not an anticipated pharmacokinetic interaction from in utero exposure. Inclusion of infants with benzodiazepine

exposure is thus safe and will increase the generalizability of findings.

4 STUDY POPULATION

4.1 Inclusion Criteria

Patients eligible for participation include:

1. ≥ 36 weeks gestation
2. Exposure to opioids in utero
3. Demonstration of signs and symptoms of neonatal abstinence syndrome requiring treatment

4.2 Exclusion Criteria

Patients ineligible for participation include:

1. Major congenital malformations and/or intrauterine growth retardation, defined as birth weight <2000 gm
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. Inability of mother to give informed consent due to co-morbid psychiatric diagnosis

4.3 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 4001. Patients who require treatment and are allocated to will receive a three-digit allocation number that begins with sequence 501.

5 DRUG ADMINISTRATION REGIMEN

5.1 Identity

All study drug will be prepared in bulk solution, distributed to the patient ward, and unit dosed by nursing in 1 mL pediatric dispensing vials (Healthcare Logistics Cat. # 7870 or equivalent). Preparation and stability is listed in Appendix 2.

5.2 Calculation of Dose and Administration

5.2.1 *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. The patient weight at the initiation of dosing will be used for the calculation of all subsequent doses of buprenorphine. The calculation of maximum dose will be based upon weight at time of administration. Dose will be communicated with the primary team by way of a dose sheet. The primary team will order the buprenorphine using the electronic medical record. This includes protocol specified maximum of 75 mcg/kg/day for buprenorphine. To allow for alterations in sleeping and feeding schedules, each dose of buprenorphine 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. 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.

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5.2.1.1 Starting doses

The starting daily dose for buprenorphine will be 24 mcg/kg/day (8 mcg/kg/q8 hours).

5.2.1.2 Cessation dose

The cessation dose will be ≤ initiation dose.

5.2.2 *Phenobarbital*

The hospital standard of care phenobarbital elixir formulation (20 mg/5 ml or similar equivalent) will be used. Birth weight will be used for the calculation of phenobarbital loading and maintenance dose. A loading dose 20 mg/kg followed by daily oral dose of 5 mg/kg/day will be used. When buprenorphine is weaned to 50% of highest dose, phenobarbital is decreased to 2.5 mg/kg/day. After three additional opioid weans, phenobarbital will be discontinued and the buprenorphine weaned until cessation dose was met.

5.3 Drug Product Quality Control

5.3.1 *Drug Preparation Procedures*

Preparation of stock solution for study drug will take place in the pharmacy of Thomas Jefferson University Hospital.

5.3.2 *Stability*

Buprenorphine solution will be used within 30 days of stock drug preparation. Buprenorphine has stability in plastic syringes for at least 7 days at room temperature.[37]

5.3.3 *Investigational New Drug (IND) Certification*

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

6 STUDY PROCEDURES

6.1 Identification of Potential Study Subjects

Potential subjects will be identified through the outpatient treatment clinics and by review of all infants at risk for NAS based on maternally identified use of opioid therapy. All infants at risk for NAS have standard care NAS scoring conducted every 4 hours. Consented infants with the sum of three scores $\geq$24 or more or a single score of $\geq$12 will be eligible for allocation. The ultimate decision to initiate treatment will be that of the treating pediatrician. A child will be allocated 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.

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 $\geq$18 but <24 can be observed additional days as inpatients at the discretion of the attending physician.

6.2 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.

6.3 Dose Administration

From initiation through reaching bottom dose, buprenorphine is administered every eight hours. After reaching bottom dose (≤ initial dose), the next weaning step is q12 dosing for at least two doses, and then following next weaning step q24 hour dosing interval. 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.

6.4 Dose Escalation

Dose escalation of 33% of the previous dose will take place if

- the sum of 3 NAS scores is ≥24 or a single score is ≥12

  OR
- a rescue dose was administered [section 6.5].

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.

6.5 Rescue Dose

If, between scheduled doses, a child has a single score of ≥12, a rescue dose may be administered at the discretion of the treating physician. The rescue dose will be same as the previous dose. A rescue dose must be given at least 1 hour after and 1 hour before the next scheduled dose.

6.6 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 ≥8.
- Dose reductions will occur at a rate of 15% per wean until reaching the bottom dose (≤ initial dose).
- After bottom dose is reached, next weaning step is from q8 to q12 hours, followed by a step from

q12 to q24 hours.

- If the sum of the previous three scores is ≥ 28 and at the discretion of the treating physician, the standing dose will revert to the previous dose or dose interval 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 ≥ 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.

6.7 Buprenorphine Dose Cessation and Observation

The cessation dose of buprenorphine is at or below the initial dose. Following wean of interval at the bottom dose, buprenorphine dosing will cease. Infants will be observed in an inpatient setting for at least 2 days following last dose, 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. If a post cessation rescue dose is given, patients must be observed at least 1 day following the last rescue dose.

6.8 Maximum Dose and Use of Adjunctive Phenobarbital

Rescue doses of buprenorphine cannot be given when at maximum dose of 25 mcg/kg. Maximum dose will be defined by the actual weight at the time of dose administration. When the maximum dose of buprenorphine (75 mcg/kg/day) has been achieved, phenobarbital will be initiated with a loading dose of 20 mg/kg followed by 5 mg/kg/day. 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 has 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 buprenorphine dose 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.[38] As such, phenobarbital will be continued for three dose or interval titrations. When the sum of the previous three scores is < 18 and no single score is ≥ 8, the attending physician may discontinue phenobarbital. The buprenorphine will not be

weaned on the step when phenobarbital is discontinued.

6.9 Study Data

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

- mode of birth
- gestational age
- gender
- Apgar scores
- NAS scores
- birth weight
- daily weight
- daily intake (cc/kg/day) and type of feed
- stool number and characteristics
- head circumference
- concomitant medication
- urine drug screen 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

6.10 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. Where possible, blood draws would be 2 or 3 samples after an individual dose.

One or two samples collected for pharmacokinetic analysis may be retained to examine ethanol pharmacokinetics for infants on maximum dose buprenorphine and phenobarbital. 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 9: 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

6.11 Efficacy

Length of treatment and length of stay will be collected in units of days. A day is defined as calendar day (rather than 24 hour blocks).

6.12 Safety

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

6.13 Urine Samples for Metabolite Analysis

Urine collections will take place while on treatment every 5-7 days (or within on day of treatment cessation). Urine will be collected by means of absorbent cotton balls in the diaper. These will be squeezed into collection tubes. A description of urine processing is outlined in Appendix 3. Due to the difficulty of reliably collecting urines from infants, and the role of this assessment as an exploratory goal, failure to collect urine will not be considered a protocol deviation or violation.

6.14 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.

7 STATISTICAL/ANALYTICAL METHODS AND POWER ANALYSIS

7.1 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 will also be analyzed using advanced modeling software (metrumrg package on RStudio, www.R-project.org) to better support modeling and simulation efforts. Based on established population PK models, parent-metabolite relationship will be used to further understand buprenorphine 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 ($p < 0.001$).

Developmental changes in newborns with NAS will be evaluated using NONMEM and SIMCYP (Version 8.1, SIMCYP Inc, Sheffield, UK). 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.

7.2 Power Analysis

No formal power analysis is performed. This analysis is primarily descriptive and will build upon existing buprenorphine neonatal models.

7.3 Efficacy, Safety and Pharmacogenetics

Efficacy, safety, and pharmacogenetic analysis are expected to be exploratory and descriptive. There will be no power analysis for these endpoints. Pharmacogenetic analysis will primarily be to correlate externally identified variants which may impact buprenorphine pharmacokinetic disposition. Pharmacogenetic analysis may also assist in assessing the biologic basis for extreme outliers of buprenorphine concentration.

8 SAFETY PARAMETERS

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 until stabilization 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.

8.1 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.

Table 10: 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
Buprenorphine assay	Yes	20*	0.4	8.0*
Maximum amount of blood drawn per female patient				10.6*
Circumcision blood loss (estimated)				0.2
Maximum amount of blood drawn per male patient				10.8*

* maximum

8.2 Stopping Rules

No further enrollment will take place following a SAE judged to be probably or definitely related to study treatment. Following a related SAE, the independent safety monitor will review the adverse event and assess investigator designated causality. The safety monitor can request pharmacokinetic analysis for the affected infant if there is concern that the event was exposure related. Depending upon the clinical situation, options could include 1) continuation of study treatment 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. This decision will be a consensus approach with the

investigator, with the input of the IRB in case of conflict between investigators and the safety monitor.

If an infant is withdrawn from the study, treatment will follow clinician guidance and will not be protocol-driven. However, such infants will be followed and will be included in safety analysis. There is no prespecified stopping rule for trial cessation. There is no predefined interim look for safety or efficacy.

8.3 Data Safety Monitoring Plan

An independent medical monitor will evaluate each serious adverse event judged by the investigator to be possibly, probably, or definitely related to study drug. The monitor will provide recommendations to the investigators.

8.4 Certificate of Confidentiality

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

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 STOCK SOLUTION AND STABILITY

	Stability of Stock
Buprenorphine 0.075 mg/mL <u>Composition of neonatal stock solution</u> • 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	7 days

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.[39] In urine, there is stability for 16 hours at 22 C, 72 hr at 4 C and through 3 freeze/thaw cycles.[40]

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.

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.

9 REFERENCES

1. Hudak ML, Tan RC, COMMITTEE ON DRUGS, COMMITTEE ON FETUS AND NEWBORN, American Academy of Pediatrics. Neonatal drug withdrawal. Pediatrics 2012;129:e540-60.

2. Jansson LM, Velez M, Harrow C. The opioid-exposed newborn: assessment and pharmacologic management. J Opioid Manag 2009;5:47-55.

3. Osborn DA, Jeffery HE, Cole MJ. Opiate treatment for opiate withdrawal in newborn infants. Cochrane Database Syst Rev 2010;(10):CD002059.

4. Patrick SW, Kaplan HC, Passarella M, Davis MM, Lorch SA. Variation in treatment of neonatal abstinence syndrome in US children's hospitals, 2004-2011. J Perinatol 2014;34:867-72.

5. Kraft WK, Dysart K, Greenspan JS, Gibson E, Kaltenbach K, Ehrlich ME. Revised dose schema of sublingual buprenorphine in the treatment of the neonatal opioid abstinence syndrome. Addiction 2010;.

6. Kraft WK, Gibson E, Dysart K, et al. Sublingual Buprenorphine for Treatment of Neonatal Abstinence Syndrome: A Randomized Trial. Pediatrics 2008;.

7. 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.

8. Hall ES, Isemann BT, Wexelblatt SL, et al. A Cohort Comparison of Buprenorphine versus Methadone Treatment for Neonatal Abstinence Syndrome. J Pediatr 2016;170:39,44.e1.

9. Hall ES, Rice WR, Folger AT, Wexelblatt SL. Comparison of Neonatal Abstinence Syndrome Treatment with Sublingual Buprenorphine versus Conventional Opioids. Am J Perinatol 2017;.

10. Ng CM, Dombrowsky E, Lin H, et al. Population Pharmacokinetic Model of Sublingual Buprenorphine in Neonatal Abstinence Syndrome. Pharmacotherapy 2015;35:670-80.

11. Moore JN, Gastonguay MR, Ng CM, et al. The Pharmacokinetics and Pharmacodynamics of Buprenorphine in Neonatal Abstinence Syndrome. Clin Pharmacol Ther 2018;.

12. Allegaert K, van den Anker J. Neonatal drug therapy: The first frontier of therapeutics for children. Clin Pharmacol Ther 2015;98:288-97.

13. Kraft WK. Buprenorphine in Neonatal Abstinence Syndrome. Clin Pharmacol Ther 2018;103:112-9.

14. Kuhlman JJ,Jr, Levine B, Johnson RE, Fudala PJ, Cone EJ. Relationship of plasma buprenorphine and norbuprenorphine to withdrawal symptoms during dose induction, maintenance and withdrawal from sublingual buprenorphine. Addiction 1998;93:549-59.

15. Greenwald MK, Johanson CE, Moody DE, et al. Effects of buprenorphine maintenance dose on mu-opioid receptor availability, plasma concentrations, and antagonist blockade in heroin-dependent volunteers. Neuropsychopharmacology 2003;28:2000-9.

16. Greenwald M, Johanson CE, Bueller J, et al. Buprenorphine duration of action: mu-opioid receptor availability and pharmacokinetic and behavioral indices. Biol Psychiatry 2007;61:101-10.

17. Bogenschutz MP, Abbott PJ, Kushner R, Tonigan JS, Woody GE. Effects of Buprenorphine and Hepatitis C on Liver Enzymes in Adolescents and Young Adults. J Addict Med 2010;4:211-6.

18. Hayes BD, Klein-Schwartz W, Doyon S. Toxicity of buprenorphine overdoses in children. Pediatrics 2008;121:e782-6.

19. Kraft WK, Stover MW, Davis JM. Neonatal abstinence syndrome: Pharmacologic strategies for the mother and infant. Semin Perinatol 2016;40:203-12.

20. Jansson LM. Neonatal abstinence syndrome. Acta Paediatr 2008;97:1321-3.

21. Walsh SL, Preston KL, Stitzer ML, Cone EJ, Bigelow GE. Clinical pharmacology of buprenorphine: ceiling effects at high doses. Clin Pharmacol Ther 1994;55:569-80.

22. Yassen A, Kan J, Olofsen E, Suidgeest E, Dahan A, Danhof M. Pharmacokinetic-pharmacodynamic modeling of the respiratory depressant effect of norbuprenorphine in rats. J Pharmacol Exp Ther 2007;321:598-607.

23. Alhaddad H, Cisternino S, Decleves X, et al. Respiratory toxicity of buprenorphine results from the blockage of P-glycoprotein-mediated efflux of norbuprenorphine at the blood-brain barrier in mice. Crit Care Med 2012;40:3215-23.

24. Brown SM, Campbell SD, Crafford A, Regina KJ, Holtzman MJ, Kharasch ED. P-glycoprotein is a major determinant of norbuprenorphine brain exposure and antinociception. J Pharmacol Exp Ther 2012;343:53-61.

25. O'Grady MJ, Hopewell J, White MJ. Management of neonatal abstinence syndrome: a national survey and review of practice. Arch Dis Child Fetal Neonatal Ed 2009;94:F249-252.

26. Huestis MA, Cone EJ, Pirnay SO, Umbricht A, Preston KL. Intravenous buprenorphine and norbuprenorphine pharmacokinetics in humans. Drug Alcohol Depend 2013;131:258-62.

27. Wachman EM, Hayes MJ, Sherva R, et al. Association of maternal and infant variants in PNOC and COMT genes with neonatal abstinence syndrome severity. Am J Addict 2017;26:42-9.

28. Wachman EM, Hayes MJ, Brown MS, et al. Association of OPRM1 and COMT single-nucleotide polymorphisms with hospital length of stay and treatment of neonatal abstinence syndrome. JAMA 2013;309:1821-7.

29. Wachman, EM. Brown, MS. Paul, JA. Logan, BA. Heller, NA. Harvey-Wilkes, KB. Kasaroglu, HO. ,Marino, T. Davis, JM. Aranda, JV. Hayes, J.. Single Nucleotide Polymorphisms and Variability in Severity of Neonatal Abstinence Syndrome. Pediatric Academic Societies; April 28-May 1, 2012; Boston, MA 2012;.

30. Wachman EM, Hayes MJ, Sherva R, et al. Variations in opioid receptor genes in neonatal abstinence syndrome. Drug Alcohol Depend 2015;155:253-9.

31. Kaltenbach K, Jones HE. Neonatal Abstinence Syndrome: Presentation and Treatment Considerations. J Addict Med 2016;10:217-23.

32. Seligman NS, Salva N, Hayes EJ, Dysart KC, Pequignot EC, Baxter JK. Predicting length of treatment for neonatal abstinence syndrome in methadone-exposed neonates. Am J Obstet Gynecol 2008;199:396.e1,396.e7.

33. Dysart K, Hsieh HC, Kaltenbach K, Greenspan JS. Sequela of preterm versus term infants born to mothers on a methadone maintenance program: differential course of neonatal abstinence syndrome. J Perinat Med 2007;35:344-6.

34. Allocco E, Melker M, Rojas-Miguez F, Bradley C, Hahn KA, Wachman EM. Comparison of Neonatal Abstinence Syndrome Manifestations in Preterm Versus Term Opioid-Exposed Infants. Adv Neonatal Care 2016;16:329-36.

35. Barrett DA, Barker DP, Rutter N, Pawula M, Shaw PN. Morphine, morphine-6-glucuronide and morphine-3-glucuronide pharmacokinetics in newborn infants receiving diamorphine infusions. Br J Clin Pharmacol 1996;41:531-7.

36. Kocherlakota P. Neonatal abstinence syndrome. Pediatrics 2014;134:e547-61.

37. Anagnostis EA, Sadaka RE, Sailor LA, Moody DE, Dysart KC, Kraft WK. Formulation of buprenorphine for sublingual use in neonates. J Pediatr Pharmacol Ther 2011;16:281-4.

38. Pitlick W, Painter M, Pippenger C. Phenobarbital pharmacokinetics in neonates. Clin Pharmacol Ther 1978;23:346-50.

39. Huang W, Moody DE, McCance-Katz EF. The in vivo glucuronidation of buprenorphine and norbuprenorphine determined by liquid chromatography-electrospray ionization-tandem mass spectrometry. Ther Drug Monit 2006;28:245-51.

40. Kacinko SL, Concheiro-Guisan M, Shakleya DM, Huestis MA. Development and validation of a liquid chromatography-tandem mass spectrometry assay for the simultaneous quantification of buprenorphine, norbuprenorphine, and metabolites in human urine. Anal Bioanal Chem 2008;392:903-11.