MULTI-CENTERED TRIAL RESEARCH PROTOCOL

Version date: August 19, 2016

Principal Investigator: Jonathan Davis, MD

Study Title: Improving outcome in neonatal abstinence syndrome with included sub-study: Establishing Risk in Neonatal Abstinence Syndrome (NAS) Genomics

I. Aim and Hypotheses

1: **SPECIFIC AIM I:** To compare the short term efficacy of morphine and methadone for the treatment of NAS. One hundred eighty four term infants with a diagnosis of NAS requiring pharmacotherapy will be studied. Infants born to mothers receiving a minimum of 2 prenatal visits in the third trimester and maintained on opioid agonist medication during pregnancy will be eligible. Infants will be randomized to receive either morphine solution or methadone in a double blind, double dummy design. It is hypothesized that morphine treated infants will require significantly fewer days in the hospital compared to methadone treated infants. While the primary outcome is the total length of initial hospital stay (LOS), total LOS related to NAS, total duration of medical treatment for NAS, the need for a second drug to control symptoms, and infant growth will also be evaluated as important secondary outcomes by medication group assignment.

2. **SPECIFIC AIM II:** To evaluate the effects of NAS treatment on long-term neurodevelopmental outcome. Infants in both treatment groups will be evaluated at 18 months of age using the Bayley III Scales of Infant Development. It is hypothesized that morphine treated infants will have better neurodevelopmental outcomes at 18 months compared to methadone treated infants. It is also hypothesized that neurobehavioral abnormalities (from either treatment group) identified at hospital discharge using the NICU Network Neurobehavioral Scale (NNNS) will correlate with neurodevelopmental impairment detected with the Bayley III. Early identification of infants at highest risk for impaired development will facilitate therapeutic interventions to improve outcome and decrease resource utilization.

3: **SPECIFIC AIM III:** To determine if single nucleotide polymorphisms (SNPs) or epigenetic changes in genes controlling opioid pharmacodynamics contribute to the severity of NAS. SNPs in the multi-drug resistance (MDR1), mu opioid receptor (OPRM1), or catechol-O-methyltransferase (COMT) genes may be important pharmacogenetic modulators of opioid action (9-12). An association between these candidate genes and opioid addiction has been studied in adults (with twin studies indicating that heritability is high), but not in newborns. Preliminary data suggest that SNPs in the MDR1 (C3435T, G2677T, C1236T), OPRM1 (A118G), and COMT (A158G) genes do influence the incidence and severity of NAS. SNP genotyping from buccal cells will be obtained from all infants at study entry, at the time of discharge and at the 18 month visit in infants enrolled in the treatment portion of the study and correlated with short term outcomes (Aim 1) and neurodevelopment assessments (Aim 2) to confirm that genetic variation plays a major role in the severity and outcome of infants with NAS. Furthermore, at Tufts Medical Center, SNP genotyping from buccal cells will also be performed on all mothers at study entry and correlated with short term outcomes and neurodevelopment assessments. It is hypothesized that certain SNP polymorphisms in mothers will be predictive of the development and or severity of NAS in the neonate. In order to allow for comparison of the SNPs in infants enrolled in the treatment portion of the study and healthy infants, 50 healthy control infants will be included at the Tufts Medical Center site, with 100 health control infants being included across all sites.

II. Background and Rationale

Misuse of opioids and/or other psychoactive drugs during pregnancy is a significant problem in the US, affecting 5% of all pregnant women. Opioids may also be used during pregnancy to control pain for a variety of chronic conditions. The incidence of NAS among infants born to mothers who received opioids is 60-80% (1-3). Since the FDA has not approved any specific therapeutic approach to NAS, there is wide variability among centers with multiple different medications used (4). These drugs can have significant side effects including sudden death (prolonged QTc), respiratory depression, poor feeding, ileus, brain injury, hypotension, and lethargy (5). Although the use of opiates has been recommended for treatment of infants with NAS, only one

small retrospective study has been published comparing morphine to methadone which did not demonstrate any short term differences (6). However, there have been **no** large prospective, randomized, controlled trials comparing these two most commonly used treatments. Most importantly, longer term safety and efficacy of these medications has **never** been established. NICHD has recently prioritized medications needing to be studied in newborns in response to the Best Pharmaceutical for Children Act (BPCA). Optimizing treatment of infants with NAS was considered very high priority (http://bpca.nichd.nih.gov/prioritization/index.cfm).

Although weight-based or symptom based treatment strategies can be employed, there is little empirical evidence to support the use of one medication or one treatment strategy over the other, reflecting a paucity of randomized studies in this area. However, recommended dosing strategies have been published by a number of physicians based on extensive clinical experience. The current trial will employ a novel symptom based treatment approach coupled to the infant's weight to control symptoms and restore physiologic stability.

The relationship between the severity and treatment of NAS and neurodevelopmental outcome has not been adequately studied. Given the large number of infants affected, establishing the best treatments that not only reduce initial length of hospital stay (LOS), but also improve short and long term neurodevelopmental outcome is crucial. It would also be important to correlate abnormalities in neurobehaviors in the neonatal period with neurodevelopmental impairment in later childhood to identify infants who could benefit from early intervention, physical/occupational therapy, and neonatal follow-up.

It is also crucial to understand how genetic variations contribute to NAS severity and outcome. Specific candidate genes have been identified as important pharmacodynamic modulators of opioid therapy in adults, with heritability estimates being high (>70%) based on twin studies (7,8). The OPRM1 gene encodes the mu-opioid receptor which is the active site for opioids in the brain. This receptor is responsible for opioid efficacy, dependence, and tolerance (9,10). The A118G SNP is the most prevalent and causes a decrease in the number of mu opioid receptors, with lower mRNA and protein expression (11). The G allele has been associated with increased binding affinity of β-endorphin and an increased risk of substance abuse in adults (12). There is conflicting evidence regarding opioid requirements, with some studies demonstrating G allele carriers require less opioid analgesia than controls (13,14).

Morphine and methadone are substrates of the P-glycoprotein transporter 170 (P-gp 170), encoded in the multidrug resistance gene MDR1 (15,16). P-gp 170 is involved in opioid absorption, distribution, bioavailability, elimination and CNS regulation (13,,17,18). The G2677T, C1236T, and C3435T SNPs are prevalent (40 – 50%) and can alter methadone requirements in adults by decreasing P-gp expression and function (13,16,18,19). Catecholamines are known opioid modulators via regulation of the mu opioid receptor (15). Catechol-O-methyltransferase (COMT) is involved in catecholamine metabolism in the CNS. The A158G SNP results in a 3-4 fold reduction in COMT activity and has a frequency of 50% (15,20). Adult cancer patients with the minor allele present require significantly less morphine to control pain compared to those with the major allele (20). Variations in these genes could also influence treatment of infants with NAS, with compelling preliminary data supporting this hypothesis (see Section IIIC2). Potential pharmacogenetic factors associated with increased short and long term complications have never been studied in infants with NAS. Furthermore, the impact of maternal pharmacogenetics on neonatal outcomes with respect to NAS has not been well studied. Ultimately this could lead to very early identification of infants at highest risk for NAS (even in utero from cell free fetal DNA circulating in the maternal circulation) and facilitate targeted interventions for the pregnant mother, fetus, and/or newborn to significantly improve outcome.

Rationale: NAS is a very significant problem in the US. Many different medications are used to treat NAS and none are currently FDA approved. Although the short term safety and efficacy of many of these agents have been examined in a limited number of studies that enrolled small numbers of infants, the most commonly used medications have never been directly compared in a large, multicenter trial. Neonatal morphine solution (NMS) has a shorter half-life than methadone, making it easier to adjust to the infant's NAS display and theoretically a safer and more efficacious treatment. It is critically important to study the influence of these medications on neurodevelopmental outcome, since simple reductions in LOS may not be important if longer term outcome is compromised. Finally, genetic factors that could predict NAS need to be explored.

III. Research Plan

Experimental design: Aim I Studies: The study will be a randomized, double blind, double dummy "intent to treat" clinical trial comparing the safety and efficacy of oral neonatal morphine solution (NMS) compared to methadone in 184 infants with NAS. The trial is registered at clinicaltrials.gov and the reporting and analyses will follow CONSORT 2010. To maintain the blind, a "double dummy" design will be used. Infants will be randomized to receive either NMS (0.2 mg/ml) every four hours and placebo every 8 hours, or methadone (0.4 mg/ml) every eight hours and placebo every 4 hours. Times of administration may be adjusted plus or minus an hour based on the infant's schedule (i.e. sleeping). The syringes will be labeled as Morphine/Placebo Oral Solution 0.2mg/mL and Methadone/Placebo Oral Solution 0.4mg/mL for safety and verification purposes. The research pharmacist will be aware of the study drug assignment and will prepare the syringes accordingly. The nurse administering the drug will be blinded and will not know if the infant is receiving active methadone or morphine. All doses for a 24 hour period will be sent up and placed in a patient specific drug kit (detailed in the pharmacy manual with drug administration modifications at each study site).

Enrollment/Randomization: There are 2 consent forms - the parents can decide if they want to be in the genetics portion of the study, the treatment portion, or both. Within the genetics portion of the study there are three components: Genomics Study for Subjects also enrolled in the treatment portion, these subjects have an additional buccal swab at 18 months; the Genomics Study for Subjects not enrolled in the treatment portion, these subjects have buccal swabs done at admission and at discharge with no 18-month follow-up swabs; and Genomics Study for Healthy Control Subjects, with no opioid exposure, these subjects will have buccal swabs done at admission—50 healthy control subjects will be enrolled at the Tufts Medical Center site, with 100 total healthy control subjects enrolled across all sites. Each eligible subject whose parent or guardian provides informed consent for either portion of the study should be assigned a unique subject number. Subjects whose parents consent to the genetics portion but not the treatment portion will be enrolled, but not randomized.

Subjects whose parents consent to the treatment portion only or to both the treatment and genetics portions will be enrolled, but not randomized immediately. After birth, subjects will be assessed and only those needing treatment (about 70%) will be randomized. Enrollment will continue until 184 infants have been *randomized* (approximately 300 mother-infant dyads will need to be consented). Randomization will be 1:1 according to computer generated randomization sequences with blocks of size four and six, in random order. Three randomization sequences will be used for each center so enrollment can be stratified by antenatal exposure to BPH, methadone, or pain medication. Subjects who are randomized to a treatment will have their subject number linked to a corresponding drug kit.

NAS Scoring: Infants will be scored using the standard Finnegan scoring system every 4 hours with the provided scoring sheets (Appendix A). All nursing staff caring for study babies will have completed training on the Finnegan scale through the intra-observer reliability program organized by Vanderbilt. Gold-standard scorers will be identified at each study site to do reliability checks. Pharmacologic treatment will be initiated when the infant scores ≥8 on two consecutive Finnegan Scores (4 hours apart) or ≥12 on one score. The starting dose of the study medication will be based on the highest score in the previous 8-12 hour period

Dosing Guidelines for NMS:

Level	NAS Score	Starting dose - 0.2mg/ml
1	8-10	0.3 mg/kg/day divided every 4 h
2	11-13	0.5 mg/kg/day divided every 4 h
3	14-16	0.7 mg/kg/day divided every 4 h
4	17 +	0.9 mg/kg/day divided every 4 h

Dosing Guidelines for Methadone:

Level	NAS Score	Starting dose - 0.4mg/ml
1	8-10	0.3 mg/kg/day divided every 8 h

2	11-13	0.5 mg/kg/day divided every 8 h
3	14-16	0.7 mg/kg/day divided every 8 h
4	17 +	0.9 mg/kg/day divided every 8 h

Maintenance doses of study drugs will be ordered as:

Morphine/placebo study (0.2 mg/mL) ___mg (_____ mL) PO q4h to deliver ___mg/kg/day, Level _____ dosing
Methadone/placebo study (0.4mg/mL) __mg (_____ mL) PO q8h to deliver __mg/kg/day, Level _____ dosing

All doses will be based on the infant's birth weight. Preliminary testing has demonstrated that the NMS, methadone, and placebo are indistinguishable when placed in oral syringes.

The pharmacy system will prompt the nurse to give a dose of the study drug / placebo every 4 hours as outlined below:

Methadone Arm

Dose 1: Methadone Active Q8h + Morphine Placebo Q4h (2 syringes)

Dose 2: Morphine Placebo Q4h (1 syringe)

Dose 3: Methadone Active Q8h + Morphine Placebo Q4h (2 syringes)

Dose 4: Morphine Placebo Q4h (1 syringe)

Dose 5: Methadone Active Q8h + Morphine Placebo Q4h (2 syringes)

Dose 6: Morphine Placebo Q4h (1 syringe)

Morphine Arm

Dose 1: Methadone Placebo Q8h + Morphine Active Q4h (2 syringes)

Dose 2: Morphine Active Q4h (1 syringe)

Dose 3: Methadone Placebo Q8h + Morphine Active Q4h (2 syringes)

Dose 4: Morphine Active Q4h (1 syringe)

Dose 5: Methadone Placebo Q8h + Morphine Active Q4h (2 syringes)

Dose 6: Morphine Active Q4h (1 syringe)

Study Drug Dose Escalation:

Once the medication has been started at dosing levels 1, 2, or 3, and the infant continues to have NAS scores ≥ 8 for two consecutive scores or one score ≥12 (believed to be related to worsening NAS), the dose should be increased to the next level. The NICU physician will be notified by the nurse and a written order must be sent to the Investigational Pharmacy. Escalation doses should be ordered in addition to the scheduled doses each day to minimize the delay in increasing to the next level. The escalation dose is the difference between the treatment levels (0.2 mg/kg/day). Escalation doses will be kept in the pharmacy and released to the clinical unit to be administered in addition to the scheduled doses after the order is received in the pharmacy. These doses will be continued until a new drug kit is dispensed with the new level dosing. If the next scheduled Q8H Methadone/Placebo administration time is greater than 4 hours away, an additional one time escalation dose of Methadone/Placebo (0.07mg/kg/dose) will be ordered to be given at the next 4 hour interval as detailed below.
If first escalation dose is required between the Q8H interval:

Dose 1 of escalation doses: Methadone/Placebo escalation + Morphine/Placebo + Morphine/Placebo escalation.
Dose 2 of escalation doses: Methadone/Placebo + Methadone/Placebo escalation + Morphine/Placebo + Morphine/Placebo escalation.
Dose 3 of escalation doses: Morphine/Placebo + Morphine/Placebo escalation.

When adequate control of symptoms is achieved (e.g. NAS scores generally <8), the same dose will be maintained for 24-48 hours before weaning is initiated.

A second medication will be added if level 4 dosing is reached and scores are consistently ≥8. Even though some safety concerns exist (neurodevelopmental delays in infants receiving phenobarbital for longer term treatment of neonatal seizures) (28), phenobarbital will be administered in this trial if a second line medication is needed. This drug is being used more frequently as an adjuvant treatment in many hospitals in the US due to the need to control more severe withdrawal symptoms in neonates associated with maternal polysubstance exposure since clonidine is often not adequate to control symptoms of NAS in these cases (29). Phenobarbital is associated with a number of adverse reactions including CNS depression, GI disturbances, hypoventilation, bradycardia, and hypotension. The infant's cardiopulmonary status will be closely monitored during the treatment phase for any indication of adverse reactions. Despite a long safety history using opioids and phenobarbital concurrently to treat NAS, potential drug-drug interactions could occur. This will be closely monitored during the treatment phase for NAS.

Phenobarbital Loading
- Load with 20 mg/kg x 1 dose or 10 mg/kg every 6-12 hours x 2 doses (should result in a plasma level of approximately 20-30 mcg/ml).
- If the infant continues to have scores ≥8, may re-load with 10 mg/kg/dose every 8-12 hours as needed x 2 more doses, until the cumulative total of all loading doses reaches a maximum of 40 mg/kg.

Maintenance Phenobarbital
- Begin maintenance dosing (see below) 12-24 hours after the last loading dose.
- If a cumulative loading dose of 30 mg/kg or 40 mg/kg has been given, draw a serum level 12 hours after the last loading dose prior to giving any further medication. Start maintenance dosing if levels are < 30 mcg/mL. If the level is ≥ 30 mcg/mL, do not give the maintenance dose and re-check the level in 12 hours.
- Maintenance Phenobarbital will be given at 5 mg/kg/day, divided every 12 hours.
- Phenobarbital steady state will be reached at 7-10 days (5 half-lives).

Phenobarbital Serum levels
- The ideal serum level to control NAS is 20 - 30 mcg/ml.
- Draw a trough level after 1 week of treatment unless a cumulative loading dose of 30 mg/kg or 40 mg/kg has been given, in which case draw a serum level 12 hours after the last loading dose prior to giving any further medication. Continue maintenance dosing if levels are < 30 mcg/mL. If the level is ≥ 30 mcg/mL, do not give the maintenance dose and re-check the level in 12 hours.
- Additional serum levels may be drawn as clinically indicated (i.e., if infant's scores are ≥8 despite appropriate loading, or with symptoms of toxicity such as persistent scores <4, sedation, decreased respiratory rate, apnea, hypotension, etc.)
- Dose adjustment based on levels: For serum levels between 15 - 20 mcg/ml, increase the maintenance daily dose by 10% and re-check level after 1 week. For serum levels between 30 - 40 mcg/ml, decrease the maintenance daily dose by 10% and re-check level after 1 week. For serum levels > 40 mcg/ml, please hold phenobarbital and re-check the level in 24 hours before administering any further doses.

Replacement of Lost Doses: If an infant spits up or vomits the dose: In general, missed or vomited doses will not be replaced. It will be impossible to know how much of the drug was absorbed and we do not want to potentially over medicate the infant. Replacement may be considered at the discretion of the treating physician in consultation with the site Principal Investigator and the study Principal Investigator.

Weaning of Medication: If the infant is receiving both study drug and phenobarbital, then the study drug will be weaned first by 10% of the maximum dose when scores are generally ≤ 8 for 12-48 hours (i.e. if the dose was 0.9 mg/kg, it would be weaned to 0.8 mg/kg, then 0.7, 0.6, etc). Subsequent weans should occur every 12-48 hours if scores are generally <8. If scores increase following a wean necessitating an increase in medication, the last effective dose should be given (10% rescue dose). Rescue doses will be administered in addition to the scheduled doses until a new drug kit is dispensed with the new dosing level. For the methadone/placebo syringes, if the infant is not due to receive methadone for another 8 hours, then an additional one time 10% difference "rescue dose" will be given at the next 4 hour interval.

Once stable for 12-48 hours then weaning by 10% should continue every 12-48 hours. The study drug should be discontinued when the dose is 20% of the maximum dose with scoring continuing for 48 hours. If the infant has weaned off study drug and then requires further treatment within the next 7 days, the study drug can be re-started at the last effective dose and then escalated or weaned accordingly.

If the infant appears to be excessively sleepy during treatment (believed to be related to study drug administration), one or even two doses of the medication can be held and the infant can be re-started at a lower dose.

If the infant is receiving phenobarbital then this should be weaned only after the infant has been weaned off of the study drug. Phenobarbital weaning should begin 48 hours after the study drug has been stopped. Phenobarbital can be weaned by 20% of the maximum maintenance total daily dose every 3 days for scores generally ≤8. An infant may be discharged home 48 - 72 hours after the first wean. If an infant is on twice daily dosing, this should be switched to once daily dosing prior to discharge home. The remaining Phenobarbital wean will be outlined in the discharge prescription, and followed up on by study staff with phone calls to the mother and primary care pediatrician. With weaning every 3 days, the infant should be weaned off phenobarbital within a 2 week period to minimize any adverse long-term effects. If the infant starts to exhibits signs of phenobarbital withdrawal (hyperactivity, tremors, hyperreflexia), the dose should be increased to the last effective dose after discussion with the infant's pediatrician. Once stabilized, the wean can be resumed as described above.

The study drugs will only be administered while the infants are in the hospital. If an infant is transferred to another facility, they will exit the study, receive standard treatment for NAS at that hospital, but still be followed and have a Bayley examination performed at 18 months corrected gestational age.

Outcomes: The primary outcome will be total hospital LOS. An independent physician at each site not connected to the study will also review the infant's medical record to insure that days spent in the hospital after NAS medications are discontinued is related to NAS treatment and not to social or other unrelated medical factors. If a significant discrepancy (>2 days) is identified, the local PI will discuss the case with Dr. Davis and LOS related to NAS treatment will be determined. Total LOS related to NAS, total duration of medical treatment for NAS, maximum daily dose of NMS or methadone, maximum Finnegan score, the need for a second drug to control symptoms, and infant growth will also be evaluated as secondary outcomes by medication group assignment.

Sample size and statistical analysis(es) : All variables will be described overall and by treatment group and stratification variable. Mean, median, standard deviation, and inter-quartile range will be used for continuous variables. Frequencies and percents will be used for categorical variables. Boxplots, histograms, scatterplots, and smoothing will be used to visualize the data. The two treatment groups will be compared on baseline (pre-randomization) measures using the t-test or Wilcoxon rank-sum test for continuous variables and the chi-square test for binary and categorical variables, with alpha=0.05. Treatment comparisons will be performed based on intention-to-treat. Residual diagnostics will be used to check model fit. The statisticians will be blinded to treatment allocation, so that model selection will not be influenced by effect size or significance. Although the DSMB may stop the study for safety, there are no plans to stop for statistical reasons, because insufficient numbers of infants will have 18-month outcomes by the time enrollment is complete.

Analysis for Aim I: The primary outcome for Aim 1 is initial LOS. We will also analyze, days in the hospital related to NAS, days of use and maximum daily dose of morphine or methadone, the use (yes/no) and total dose of phenobarbital, maximum Finnegan score, and growth parameters (height, weight, and head circumference) at birth and hospital discharge. Complete follow-up is expected for outcomes measured during the initial hospitalization. LOS and days on treatment will be analyzed with Poisson regression (equivalent to the exponential proportional hazards model) as in the MOTHER study (25). If the Poisson distribution is not a good fit, another distribution such as lognormal, Weibull, or gamma, selected according to goodness of fit will be used. Growth parameters will be analyzed with linear regression. Phenobarbital use will be analyzed with logistic regression and will be considered exploratory, since there is less power for the binary outcomes. The independent variable of interest is treatment group (morphine or methadone). All analyses will be adjusted for mother's treatment (methadone or BPH) and site (analyzed as a fixed effect). The interaction between the infant's medication assignment and mother's treatment will be tested and included if significant. The rationale for testing maternal treatment and its interaction with infant treatment is that maternal methadone and BPH could be associated with different LOS. In addition, we will adjust for baseline variables found to differ between the treatment groups (e.g. the number and types of medications the fetus is exposed to - data from mother/infant toxicology). In sensitivity analyses, we will adjust for discharge to a level II hospital (about 5% are expected).

Power Calculation for Aim I: We simulated from a Poisson model for LOS, using alpha=0.05, and a 2-sided test. With a total sample size of 184, there is at least 80% power to detect a difference in mean LOS of 2.3 days, if mean LOS in the shorter-LOS group is 30 days or less. The shorter the LOS, the higher the power and the smaller the detectable difference. For example, there is 85% power to detect a treatment effect if the mean LOS is 19.0 days in one group and 21.0 in the other.

Aim II Studies: Short term neurodevelopmental assessments - NNNS Administration: The NNNS measures active and passive tone, primitive reflexes, physical maturity, social and behavioral functioning (including visual and auditory tracking), and stress. The NNNS will be administered by certified examiners blinded to the treatment group upon discharge home after the infant has been weaned off of methadone or neonatal morphine solution. The exam is conducted 60 to 90 minutes after a feeding, when the infant has been sleeping for 30 minutes. Factors such as age, time since the last feeding and time of the exam will be recorded and used as covariates as appropriate. Algorithms will be used to compute the summary scores and profiles, including adjustments for missing data. The results of each NNNS will be reviewed by Dr. Lester and his study personnel and cleaned data will be sent electronically to the CC at Tufts on a monthly basis. A well-established NNNS training program exists at Dr. Lester's site. The training includes web-based and interactive teaching modules, a practice requirement, and an "in vivo" certification session. A two day intensive training session involving the NNNS and Bayley III (see below) examinations will be conducted in Providence prior to patient enrollment. Developmental psychologists or physical therapists from each site with extensive experience in neonatal follow up will be trained by Drs. Lester. Maintaining reliability on the NNNS throughout the study will be achieved by review of video recordings every 12 months with feedback to each center by Dr. Lester.

Longer-term neurodevelopmental assessments - The Bayley III Scales of Infant Development: This assessment is the "gold standard" for evaluating developmental delay and will be performed at 18 ± 1 month of age. In addition to the MDI and PDI, the Bayley III assesses cognitive, language and motor domains, receptive and expressive communication, and fine/gross motor development. A parental questionnaire measures social-emotional and adaptive behavior. The Bayley III has been criticized for underestimating developmental delay (26). However, problems with this study included lack of concurrent controls and a questionable age correction used for these children. All studies from the NICHD Neonatal Research Network use the Bayley III to assess longer term neurodevelopmental outcome. Bayley III training will be conducted by Dr. Lester through web-based and interactive modules as well as the training session in Providence. The raw data will initially be sent to Dr. Lester at Brown. Scores will be computed for cognitive, language and motor domains, for scale scores of receptive and expressive communication, for fine and gross motor development, and for the parent report of social-emotional and adaptive behavior.

Longer-term neurodevelopmental assessments - Brief Symptom Inventory (BSI): This assessment is a measure of psychological distress. The 53-item self-report inventory in which participants rate the extent to which they have been bothered (0 ="not at all" to 4="extremely") in the past week by various symptoms. The

BSI has nine subscales designed to assess individual symptom groups: somatization (SOM, e.g., "Faintness or dizziness"), obsessive-compulsive (OC, e.g., "Having to check and double-check what you do"), interpersonal sensitivity (IS, e.g., "Feeling inferior to others"), depression (DEP, e.g., "Feeling no interest in things"), anxiety (ANX, e.g., "Feeling tense or keyed up"), hostility (HOS, e.g., "Having urges to break or smash things"), phobic anxiety (PHB, e.g., "Feeling uneasy in crowds, such as shopping or at a movie"), paranoid ideation (PAR, e.g., "Others not giving you proper credit for your achievements"), and psychoticism (PSY, e.g., "The idea that something is wrong with your mind").

Longer-term neurodevelopmental assessments - Quick Inventory of Depressive Symptomatology (QIDS): This assessment is a measure of depressive symptom severity. The 16 item Quick Inventory of Depressive Symptomatology (QIDS) is designed to assess the severity of depressive symptoms. The patient is asked to rate the severity and frequency of specific symptoms present over the last 7 days. Patients choose the item response (0, 1, 2, or 3) that best describes themselves over the last 7 days. The QIDS assess all the criterion symptom domains designated by the American Psychiatry Association Diagnostic and Statistical Manual of Mental Disorders - 4th edition (DSM-IV) to diagnose a major depressive episode. The total score (range 0-27) measures Severity of Depression. 0=None, 1=Mild, 2=Moderate, 3=Severe, 4=Very Severe.

Longer-term neurodevelopmental assessments - Parenting Stress Index (PSI): This assessment is a measure of the parenting system and issues that may lead to problems in the child's or parent's behavior. Focuses on three major domains of stress: child characteristics, parent characteristics and situational/demographic life stress. Short form (36 items) has 3 subscales: Parental Distress, Parent–Child Dysfunctional Interaction and Difficult Child. Child and Parent domains combine to form Total Stress Scale. Child subscales include: Distractibility/Hyperactivity, Adaptability, Reinforces Parent, Demandingness, Mood, and Acceptability. Parent subscales include: Competence, Isolation, Attachment, Health, Role Restriction, Spouse/Parenting Partner Relationship parents respond to each statement using a 5-point scale 1) SA (Strongly Agree) 2) A (Agree) 3) NS (Not Sure) 4) D (Disagree) 5) SD (Strongly Disagree) to indicate the degree to which that item has been disturbing to them in the past week. This instrument yields scores for several factors in addition to a Total Stress score. The Total Stress score, utilized is a composite score of the subscale scores. Parents who obtain a Total Stress score above a raw score of 90 are considered to experiencing clinically significant parenting stress.

Longer-term neurodevelopmental assessments - Child Behavior Checklist (CBCL): This assessment is a measure of behavior problems in the child. The checklist consists of 100 statements about the child's behavior, e.g. "Acts too young for his/her age." Responses are recorded on a scale: 0 = Not True, 1 = Somewhat or Sometimes True, 2 = Very True or Often True. Items are grouped into syndromes, e.g. Aggressive behavior, and their scores are summed to produce a score for that syndrome. Some syndromes are further summed to provide 2 overall summary scores, Internalizing and Externalizing problems. A total score from all questions is also derived. For each syndrome, problem scale and the total score, there are norms for normal, borderline, or clinically significant scores.

At the 18 month visit, growth parameters will be measured and a physical examination performed. A questionnaire will be given to the parents detailing demographics, post discharge environmental factors (e.g. maternal functioning, environment where the infant is living, maternal psychopathology, maternal drug use/treatment, domestic violence exposure) growth, feeding, infant development, and any medical illness that has occurred. These are factors that could potentially influence the Bayley III scores and will be evaluated in the analysis plan for this Aim. Since performance of the Bayley III at 18 ± 1 months of age is critical, adequate long-term follow-up is essential. Parents will be contacted monthly after discharge to maintain contact with the families and address any outstanding issues.

Analysis for Aim II: The primary outcomes are 4 domains of the NNNS (quality of movement, arousal, hypertonicity, and stress/abstinence) and 2 domains of the 18-month Bayley III (MDI and PDI). We will also analyze the other subscales of the NNNS and the Bayley, as well as 18-month growth parameters. All scales will be checked for floor and ceiling effects. If necessary, the data will be transformed to obtain approximate normality and we will analyze the scales using linear regression models. If that is not possible, generalized

linear models will be used with gamma or other distribution to obtain a good model fit. The independent variable of interest is treatment group and analyses will be adjusted for mother's treatment and site. Subjects with 18-month data will be compared to those lost to follow-up on all neonatal measures. To perform the analyses of the 18-month measures according to intention-to treat, multiple imputation will be used to handle data that are missing because of loss to follow-up. A regression-based approach will be applied to take advantage of key information collected during the hospitalization which may be predictive of 18-month outcomes. Inference will be based on ten draws from the predictive distribution, to adequately account for uncertainty. This method is more realistic and less biased than other forms of imputation. A recent article in the journal "Drug and Alcohol Dependence" endorses multiple imputation for intention-to-treat analyses (30). Although few deaths are expected, in the unlikely event that a death does occur prior to an assessment (NNNS or Bayley III), zero will be imputed. In the main analyses for this Aim, adjustment will be made for baseline variables only (mother's treatment, site, and any variables that are imbalanced between the study groups at baseline). However, in secondary analyses, we will adjust for information collected at 18 months that is related to the outcome and unlikely to be affected by treatment allocation, such as the environmental factors listed above. The treatment comparison will also be performed using only subjects with follow-up at 18 months. The correlation between all NNNS and Bayley III scales will be calculated for subjects with 18-month follow-up.

Power calculation for Aim II: At 18 months, at least 156 (85%) of the 184 enrolled subjects are expected to be retained. Power is based on n=184 for the NNNS (the 2-week measure) and n=156 for the Bayley (the 18-month measure). The calculations are adjusted for multiple comparisons via Bonferroni, with alpha = 0.0125 for 4 scales of the NNNS and alpha = 0.025 for 2 scales of the Bayley. There is 80% power to detect a difference of 0.5 standard deviation between groups for each of the 6 scales, based on the 2-sided two independent sample t-test.

Aim III Studies: DNA samples from buccal cells will be obtained from all infants and mothers consented for the genomics portion of this study (see Genomics consent form) that meet entry criteria (identified prenatally or on admission to Labor and Delivery). Mother-infant dyads who do not subsequently meet the criteria for NAS treatment (Aims I and II) will still have their DNA retained for genetic analysis and data collection will be performed from the infant and maternal charts prior to discharge. In cases where the parent(s) consent to having their infant participate in both the genomics and treatment portions of the study, three infant DNA samples will be collected: one initial sample, one at discharge with the NNNS will be performed, and one at the 18 month visit.

Buccal cell Collection: Buccal cells will be collected from the infants and mothers using the Isohelix (Boca Scientific) collection swabs. Two swabs will be used on each infant and each mother, taking approximately 60 seconds at enrollment (for the mothers), or at the beginning of treatment, a second time when the infant has weaned off study medication (for infants) and a third time at the 18 month visit . Samples will then be de-identified and delivered at room temperature to the Core Laboratory at the Tufts Clinical and Translational Research Center. Buccal specimens are stable for up to 3 years at room temperature before DNA extraction. All buccal samples will be extracted using the Isohelix kits and the manufacturer's protocol. Preliminary studies using buccal cell samples collected via this method have been shown to yield an average of 10ug of genomic DNA per infant.

DNA Isolation and SNP Genotyping: DNA will be isolated according to the manufacturer and analyzed by SNP genotyping using the Taqman or Biotrove platforms (recently obtained by two separate NIH equipment grants at Tufts, G. Huggins, PI). Specimens will be isolated and genotyped in batches every 12 months. Typically, 0.5µg of DNA is necessary to run 1 SNP. The C3435T, G2677T, and C1236T SNPs within the MDR1 gene, A118G SNP within the OPRM1 gene, and the A118G SNP in the COMT gene will be analyzed. Genotyping for additional SNPs and DNA methylation changes that may be important for NAS may also be performed during the study period on the existing DNA sample. These techniques are routinely performed in our laboratory and no difficulties are anticipated. DNA will be discarded after the completion of the study and final analysis is performed.

Analysis for Aim III: The primary outcome will be length of hospital stay (LOS). Need for treatment, maximum daily dose of NMS or methadone, need for a second medication to treat NAS, NNNS, and Bayley III will also be analyzed. Comparison of SNP genotype frequencies between those infants who require treatment and

those who do not will be performed. Comparison of maternal SNP genotype frequencies between those infants who require treatment and those who do not will also be performed. Epigenetic analyses will also be conducted to see how the genotype might change as a result of receiving treatment to control symptoms. In the Caucasian population, the frequency of A118G, G2677T, C1236T, C3435T, and A158G is 12-15%, 40%, 45%, 50% and 50%, respectively *(See Section I)*. Genotype frequency does vary with ethnicity, however the global minor allele frequencies for these SNPs are similar to that of the Caucasian population (*http://www.ncbi.nlm.nih.gov/projects/SNP*). According to data from the MOTHER study, 90% of infants in this study population are of Caucasian ethinicity (25).The A118G analysis will be exploratory because of low expected frequency. Unadjusted comparisons between treatment groups will use the two independent sample t-test or Wilcoxon rank-sum test, as appropriate. For the adjusted analyses, linear regression will be used for NAS score, maximum daily dose of NMS or methadone, NNNS and Bayley III. Poisson regression will be used for LOS. Chi-square will be performed to assess for differences in need for a second medication to treat NAS. There will be no true confounders because none of the demographic or clinical factors or potential exposures could influence the mother or baby's genotype. Thus the main analyses for all outcomes in this aim will be unadjusted. However, some variables that are related to the outcome may reduce noise and thereby increase the precision of the coefficient for the genotype. Therefore, covariates that meet the $P<0.10$ threshold in univariate analyses will be tested in the multivariable models provided they are not in the causal pathway between the SNP and the outcome. Backward elimination and AIC will be used for variable selection and residual diagnostics will be used to check model fit. Separate analyses will be performed for methadone and morphine.

Power Calculation for Aim III: With 184 subjects for the primary outcome measure, based on genotype frequencies of the COMT and MDR1 SNPs, we will have 80% power to detect a 3 day difference in LOS and 0.6 SD difference in neurodevelopmental outcomes. Adjustment for multiple comparisons via Bonferroni is for 3 SNPs (hence alpha=0.017). All tests are 2-sided and assume the SNP frequency is between 40% and 50%.

Genetic Evaluations – Subject Characteristics

*Note: Two separate consent forms exist for this study. Subjects may consent to the genetic testing portion of the study, the treatment portion, or both. Within the genetic testing portion, at Tufts Medical Center, they will be given the opportunity to consent for maternal genotyping, infant genotyping, or both. If the subject consents for the treatment portion but ultimately does not require treatment, they may still participate in the genetic testing but will not undergo the NNNS exam or any follow-up visits.

Inclusion criteria:

1. Mother receiving methadone or BPH from a licensed physician or drug treatment program, or an opioid prescribed by a licensed health care worker for treatment of chronic pain (urine toxicology will be performed on the mother and meconium on the newborn infant at the time of delivery to detect other antenatal drug exposure)
2. Gestational age $\geq$37 weeks at birth defined by best obstetrical estimate
3. Infant medically stable in the opinion of the Attending Physician with no serious medical illness
4. Singleton pregnancy
5. Mother able to provide informed consent

Exclusion criteria:

1. Gestational age < 37 weeks at entry as defined by best obstetrical estimate
2. Major congenital anomalies including genetic syndromes
3. Serious medical illness in the infant
4. Multiple gestation pregnancy

Healthy Control Portion – Subject Characteristics (100 infants to be enrolled at 2 study sites)

Buccal cell Collection: Buccal cells will be collected from the infants and mothers using the Isohelix (Boca Scientific) collection swabs. Two swabs will be used on each infant and each mother, taking approximately 60 seconds. Samples will then be de-identified and delivered at room temperature to the Core Laboratory at the Tufts Clinical and Translational Research Center. Buccal specimens are stable for up to 3 years at room temperature before DNA extraction. All buccal samples will be extracted using the Isohelix kits and the manufacturer's protocol. Preliminary studies using buccal cell samples collected via this method have been shown to yield an average of 10ug of genomic DNA per infant.

DNA Isolation and SNP Genotyping: DNA will be isolated according to the manufacturer and analyzed by SNP genotyping using the Taqman or Biotrove platforms (recently obtained by two separate NIH equipment grants at Tufts, G. Huggins, PI). Specimens will be isolated and genotyped in batches every 12 months. Typically, 0.5µg of DNA is necessary to run 1 SNP. The C3435T, G2677T, and C1236T SNPs within the MDR1 gene, A118G SNP within the OPRM1 gene, and the A118G SNP in the COMT gene will be analyzed. Genotyping for additional SNPs and DNA methylation changes that may be important for NAS may also be performed during the study period on the existing DNA sample. These techniques are routinely performed in our laboratory and no difficulties are anticipated. DNA will be discarded after the completion of the study and final analysis is performed.

Both buccal cell collection and DNA isolation and SNP genotyping will be performed in the same manner as for mothers and infants enrolled in the treatment portion of the study.

1.Mother NOT receiving methadone or BPH from a licensed physician or drug treatment program, or an opioid prescribed by a licensed healthcare worker for treatment of chronic pain(urine toxicology will be performed on the mother and meconium on the newborn infant at the time of delivery to detect other antenatal drug exposure)
2.No need for treatment of NAS
3.Gestational age ≥37 weeks at birth defined by best obstetrical estimate (NAS is rare in preterm infants; preterm infants also have longer LOS, more neurodevelopmental impairment, and respond to lower doses of medication than term infants which would increase the variability in outcomes and reduce the power for the present trial)
4.Medically stable in the opinion of the Attending Physician
5.Singleton pregnancy
6.Mother able to provide informed consent

Healthy Volunteer Exclusion Criteria:
1.Gestation <37 weeks at entry defined by best obstetrical estimate
2.Major congenital abnormalities including genetic syndromes
3.Serious medical illness such as sepsis, asphyxia, seizures, or respiratory failure
4.Mother abusing alcohol during pregnancy

Treatment Portion - Subject Characteristics (184 infants to be enrolled at 5 study sites)

Inclusion criteria:

1. Mother receiving methadone or BPH from a licensed physician or drug treatment program, or an opioid prescribed by a licensed health care worker for treatment of chronic pain (urine toxicology will be

performed on the mother and meconium on the newborn infant at the time of delivery to detect other antenatal drug exposure)
2. Need for treatment of NAS by Finnegan Scoring criteria (see below)
3. Gestational age ≥37 weeks at birth defined by best obstetrical estimate (NAS is rare in preterm infants; preterm infants also have longer LOS, more neurodevelopmental impairment, and respond to lower doses of medication than term infants which would increase the variability in outcomes and reduce the power for the present trial)
4. Medically stable in the opinion of the Attending Physician
5. Mother receiving a minimum of two prenatal visits care from a qualified physician or midwife.
6. Singleton pregnancy
7. Mother able to provide informed consent
8. Infant able to take oral medications

Exclusion criteria:
1. Gestation <37 weeks at entry defined by best obstetrical estimate
2. Major congenital abnormalities including genetic syndromes
3. Serious medical illness such as sepsis, asphyxia, seizures, or respiratory failure
4. Mother abusing alcohol during pregnancy since alcohol exposure can be toxic to the fetus and cause neurodevelopmental impairment (average of 3 or more drinks per week in the last 30 days) (27)
5. Multiple gestations (due to high heritability patterns)
6. Mother received less than one prenatal visits in the third trimester

Withdrawal/Termination criteria: The DSMB will develop the statistical modeling needed to analyze AEs and SAEs in conjunction with Dr. Norma Terrin, the senior Biostatistician on the trial from the Tufts CTSI. They may request data to be unblinded. SAEs and AEs specific to NAS treatment include: sudden death (possibly from prolonged QTc), lethargy, constipation, decreased reflexes and response to pain, hypotonia, obtundation, hypoventilation, apnea, hypotension, bradycardia, and hypothermia. The DSMB may stop the trial if one or more SAEs are increased in either treatment group – this may be clinically significant even if not statistically significant. In addition, the parents can withdraw the infant from the study at any time.

Risk/benefit assessment: The incidence of true SAEs in infants treated for NAS are extremely rare, despite the inherent risks that are stated. There are risks to using these medications as mentioned above. However, this is currently the standard of care for infants with NAS and we are performing this study to ensure these medications are safe and effective in treating NAS in order to obtain FDA approval for one or both medications. The study medication administration will be handled similarly to the local current standard of care practices at each site in an attempt to minimize the risk of dosing errors. Nursing staff and physician staff on the pediatric units will be in-serviced on the study protocol. Infants will benefit from the standardized scoring system, increased monitoring from both the clinical and research team, and will benefit from the standardized developmental testing performed at 18 months of age.

Physical risk: The only physical risks are from the medications which are both used routinely throughout the US for NAS. There is a possibility of mild discomfort when obtaining the saliva samples from the infant's inner cheek. Saliva samples will be obtained by trained study staff just prior to feeding and/or medication administration to optimize comfort.

QTc Prolongation Monitoring:
Prolongation of the QT interval normally occurs in 1/2500 newborns, but has also been recognized as a rare but potentially serious side-effect of methadone therapy. All subjects will be monitored continuously for cardiac arrhythmia as a matter of routine for patients in the NICU. Any abnormal rhythm will be evaluated fully with a 12 lead EKG with specific measurement of the QTc interval. A Pediatric Cardiology consultation may also be requested.

For the purpose of this study, the QTc will be considered to be prolonged if the interval is ≥500 msecs. If a prolonged QTc is confirmed, Pediatric Cardiology will be consulted and the blind broken by the local PI by contacting the site research pharmacist. The Chair of the DSMB will also be notified. If the infant was receiving morphine, the infant will continue in the study. If the infant was receiving methadone, this medication will be stopped, the patient will exit the study, and morphine will be started. Data on treatment and neurodevelopmental outcomes will continue to be followed with adjustments made in the final statistical analyses.

2. Psychological risk: There is always a potential for breach in confidentiality about patient information, particularly with maternal substance abuse history and genetic material in this vulnerable population. All efforts will be taken to prevent breach in confidentiality as outlined below in the confidentiality section.

3. Social risk: The mother and infant's participation in this study will be kept confidential. The study will not examine any genes that can definitively predict long term health outcome or risk of specific future disease. Therefore, this study will not affect any future employment or insurance coverage for the child or family. Care will be taken to prevent any breach in confidentiality as outlined below.

4. Economic risk: If the results of genetic testing or the maternal substance abuse history were inadvertently disclosed, this could negatively affect access to insurance or employment for the mother. All efforts will be made to protect mother-infant confidentiality according to hospital HIPPA guidelines as outlined below.

5. Benefits of participating in the study:

To individual subject and/or parent: These direct benefits include: a) receiving important neurodevelopmental testing information which will help direct any interventions that are needed if the testing is abnormal (that might not be picked up by a primary care provider at a routine visit). b) Improved monitoring and standardization of NAS scoring while during the inpatient hospitalization c) infants will be given a preservative free preparation of methadone or morphine, which is currently not standard of care.

To population from which subject is drawn: This is the first large scale study of NAS examining safety, efficacy, neurodevelopmental outcome and the genomics behind NAS and has the potential to significantly impact clinical care by identifying infants who may be at greater risk for a more severe course of NAS.

To science, society, and humanity in general: NAS is a growing problem in the United States leading to prolonged hospitalizations and extensive medical therapy. This study will examine safety, efficacy, neurodevelopmental outcomes and the genomics of NAS with the potential to significantly impact clinical care.

Specific methods and techniques used throughout the study:
Laboratory Tests: Maternal and infant DNA will be collected from buccal cells by use of the Isohelix DNA kits with the use of 2 SK-1 swabs inserted into the inner cheek. Buccal cell samples will be collected by our trained study staff.

Neurodevelopmental Assessments: NNNS Administration: The NNNS measures active and passive tone, primitive reflexes, physical maturity, social and behavioral functioning (including visual and auditory tracking), and stress. The NNNS will be administered by certified examiners blinded to the treatment group 24 to 48 hours prior to discharge after neonatal morphine or methadone has been discontinued. The exam is conducted 60 to 90 minutes after a feeding, when the infant has been sleeping for 30 minutes. Factors such as age, time since the last feeding and time of the exam will be recorded and used as covariates as appropriate. Algorithms will be used to compute the summary scores and profiles, including adjustments for missing data. The results of each NNNS will be reviewed by Dr. Lester and his study personnel and cleaned data will be sent electronically to the CC at Tufts on a monthly basis. A well-established NNNS training program exists at Dr. Lester's site. The training includes web-based and interactive teaching modules, a practice requirement, and an "*in vivo*" certification session. A two day intensive training session involving the NNNS and Bayley III (see below) examinations will be conducted in Providence prior to patient enrollment. Developmental psychologists or physical therapists from each site with extensive experience in neonatal follow up will be trained by Drs.

Lester. Maintaining reliability on the NNNS throughout the study will be achieved by review of video recordings every 6 months with feedback to each center by Dr. Lester.

The Bayley III Scales of Infant Development: This assessment is the "gold standard" for evaluating developmental delay and will be performed at 18 ± 1 month of age. In addition to the MDI and PDI, the Bayley III assesses cognitive, language and motor domains, receptive and expressive communication, and fine/gross motor development. A parental questionnaire measures social-emotional and adaptive behavior. The Bayley III has been criticized in one study for underestimating developmental delay (44). However, problems with this study included lack of concurrent controls and a questionable age correction used for these children. All studies from the NICHD Neonatal Research Network use the Bayley III to assess longer term neurodevelopmental outcome. Bayley III training will be conducted by Dr. Lester through web-based and interactive modules as well as the training session in Providence. The raw data will initially be sent to Dr. Lester at Brown. Scores will be computed for cognitive, language and motor domains, for scale scores of receptive and expressive communication, for fine and gross motor development, and for the parent report of social-emotional and adaptive behavior.

Questionnaires: Self report questionnaires will be completed by the mother, or primary caregiver, at the 18-month follow-up visit. The mother/primary caregiver will be told that if she doesn't understand or needs help filling out the questionnaires, the questions can be read to the mother/primary caregiver by the study coordinator. The questionnaires need to be completed at the visit. They cannot be taken home or mailed out ahead of time. Often the questionnaires are completed while the infant is being tested or after when the infant is in the room with the mother/primary caregiver. Questionnaires will assess maternal stress, infant growth, environment, behavior and development. The Child Behavior Checklist for Ages 1 ½-5: (CBCL) will be completed by the mother/primary caregiver to identify behavior problems in the child. Maternal health and well-being will be assessed using the Brief Symptom Inventory (BSI), Quick Inventory of Depressive Symptomatology (QIDS), Parent Stress Index (PSI), and a self-report questionnaire will collect information on the living environment, caretakers, maternal health and substance use since child's birth. The Bayley III assessment and questionnaires will take approximately 2 hours to complete. The mother or primary caregiver will be provided $100 clin-card incentive for their time and cooperation.

Parent and child questionnaire provides information from the child's date of birth to 18 months. Items gather information concerning child's health history, physical growth, and early intervention services (Occupational therapy, speech, and behavior). Items also collect information about the child's living environment, childcare, and home setting. Parental subscales include current maintenance medication and/or other substances, relapse, and domestic violence since child's date of birth. These questions are designed to evaluate the child's living environment and daily stress level.

Subject Timeline: The first 3 months of the study will involve finalizing the protocol, training methods, and obtaining IRB approvals. Then training of all study personnel will be conducted. Infants with NAS will be enrolled starting in month 4-5 and continued until all patients are enrolled (month 36). At Tufts Medical Center, mothers will also be enrolled. The follow up phase (month 29 to 46) will continue until the 18 month Bayley III examinations have been completed on all eligible infants (17 months after the final patient is enrolled). The final 3 months will involve closing out each center, finalizing the database, and performing all necessary analyses. After discharge but prior to the 18 month Bayley III examinations, subjects will be contacted every 3 months by telephone or by mailing questionnaires. This is to ensure adequate follow-up and retention of study subjects.

Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan: The DSMP has been reviewed and accepted by NIDA and the FDA for this trial.

Definition of Serious Adverse Event (SAE) and Adverse Event (AE) for this study: The FDA definition of SAEs and AEs will be used in the proposed study(Guidance document 2010), because these are the standard definitions in the field. An SAE is any adverse event occurring at any dose that results in:

- Death
- Is life-threatening
- Prolongation of the present hospitalization and any new hospitalizations
- A permanent, persistent or significant disability
- A medically significant event that may require medical or surgical intervention to prevent one of the outcomes listed here

Relationship to Study Drug: The determination of the likelihood that the test drug caused the AE will be provided by the Investigator. The Investigator's signature and date on the source document and CRF that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. The signed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the Investigator in assessing the likelihood of a relationship between the test drug and the adverse event. The following components will be used to assess this relationship; the greater the correlation with the components and their respective elements, the more likely the test drug caused the AE.

- Time Course: Did the SAE follow in a reasonable temporal sequence from administration of the test drug? Is the time of onset of the SAE compatible with a drug-induced effect?
- Likely Cause: Is the SAE not reasonably explained by another etiology such as underlying disease, other drug(s), or other host or environmental factors?
- Consistency with the study drug profile: Is the clinical and pathological presentation of the SAE consistent with previous knowledge regarding the study drug or drug class pharmacology or toxicology. The assessment of relationship will be reported by the investigator according to his/her best clinical judgment, including consideration of the above elements. The following scale of criteria may be used as a guidance (not all criteria must be present in order to be indicative of a drug relationship).

Probably related to test drug:

- There is evidence of exposure to the study drug
- The temporal sequence of the SAE onset relative to administration of the study drug is reasonable
- The SAE is more likely explained by the study drug than by another cause

Possibly related to test drug:

- There is evidence of exposure to study drug
- The temporal sequence of the SAE onset relative to administration of the study drug is reasonable
- The SAE could have been due to another equally likely cause

Unlikely related to test drug:

- There is evidence of exposure to the study drug
- There is another more likely cause of the SAE
- There is no temporal relationship to study drug administration

Reporting timeframe for SAEs and AEs: Any death due to any cause, which occurs to any subject in this study, whether or not related to the study medication, must be reported within 24 hours to Dr. Davis. All other SAEs must be reported to Dr. Davis within 5 days of the study team's knowledge of the event. SAEs will be reported to the lead IRB and the Coordinating Center (CC) at Tufts within 5 days of knowledge of the event. SAE's will then be reported to the FDA, NIDA, the DSMB and other site IRB's within 72 hours of the submitted report. An electronic system has already been set up with NIDA to report SAE's. All SAEs must be followed up for outcome. AEs must be reported for the entire duration of the trial, not just during administration of study drug (Period 1). As far as possible, each AE will also be described by: the duration (start and end dates), the severity grade (mild, moderate, severe), the relationship to the study drug (unlikely, possibly, and probably), the action(s) taken and the outcome. The 2010 FDA guidance document on SAE's and AE's will be used as a template for the present study.

Formal Trial Stopping Rules
Serious adverse events that are unexpected and related to the study drugs (abbreviated as "events") are defined as follows:

1. Death associated with one of the study drugs (methadone or morphine).
2. Episodes of significant cardiac arrhythmia requiring intervention thought to be related to the study drug (e.g. methadone and prolonged QTc syndrome).
3. Episodes of clinically significant respiratory depression, bradycardia, or hypotension related to one of the study drugs (methadone or morphine).

Medication errors associated with study drug administration due to differences in volume, concentration, and dosing intervals will be reported as protocol deviations, unless they result in harm to the baby. If the baby experiences a serious adverse reaction, then the medication error would be reported as an SAE. SAEs will not cause the study to be formally stopped unless they result in any of the three conditions listed above.

Since these drugs have been used routinely for 30 years and have a high safety profile, death is a rare occurrence. However, since a novel weight and score based dosing approach is being used for this study, any deaths will be examined very carefully in order to determine (with the IRB, DSMB, NIDA, and FDA) if there is a relationship to study drug. If any deaths occur, enrollment of new infants will be temporarily suspended (although treated infants can continue in the study) until the DSMB notifies the PI that the trial is open.

Sequential boundaries will be used to monitor the cumulative number of events. The premise is that an event rate of 0.10 or lower is acceptable, but higher rates are not. Monitoring will take place separately for each of the two arms. Planned enrollment is 92 subjects per arm. The DSMB will be notified and a temporary hold will be placed on enrollment, pending review, if excessive numbers are seen in either arm. That is, if the number of events is equal to or exceeds b_n out of n patients (Table 1) with follow-up through hospital discharge. This is a Pocock-type stopping boundary. The probability of crossing the boundary in a given arm is at most 0.10 if the true event rate is equal to 0.10 for that arm (Table 2). The probabilities of crossing the boundary, assuming event rates that are higher than 0.10, are also shown in Table 2. Because monitoring will take place separately in each arm, the overall probability of the temporary hold is higher. For example, if the true event rate is 0.10 in each arm, then the overall probability of the temporary hold is 0.19, and if the true event rate is 0.20, then the overall probability of the temporary hold is 0.98 (Table 2).

Table 1. A temporary hold will be placed on enrollment if, in either of the study arms, the number of serious adverse events that are unexpected and related to the study is equal to or exceeds b_n out of n patients, with follow-up through hospital discharge.

Number of Patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	2	3	3	3	3	4	4	4	4	4	4	5	5	5	5	5	6	6	6
Number of Patients, n	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	6	6	6	7	7	7	7	7	7	8	8	8	8	8	8	8	9	9	9	9
Number of Patients, n	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Boundary, b_n	9	9	9	10	10	10	10	10	10	10	11	11	11	11	11	11	11	12	12	12
Number of Patients, n	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Boundary, b_n	12	12	12	12	13	13	13	13	13	13	13	13	14	14	14	14	14	14	14	15
Number of Patients, n	81	82	83	84	85	86	87	88	89	90	91	92								
Boundary, b_n	15	15	15	15	15	15	15	16	16	16	16	16								

Table 2. Chance of a temporary hold, conditioned on the true rate of SAEs that are unexpected and related

True rate of SAEs that are unexpected and related	Probability of crossing the boundary in a given arm	Probability of a temporary hold
0.10	0.10	0.19
0.20	0.86	0.98
0.30	1.00	1.00

Subject Participation

Recruitment: 300 total mother-infant dyads will be enrolled at 8 sites in order to randomize a total of 184 infants.

Transportation: Parents will need to arrange for their own transportation to the study site for the 18 month visit. They will receive a $100 Clin Card payment to offset any costs at the 18 month visit.

Informed consent process and timing of obtaining of consent:

Prenatal Consent: An attempt will be made to identify pregnant women on methadone, buprenorphine, or narcotics administered for chronic pain prenatally. Study staff will work with the prenatal clinics to identify when eligible subjects come into the office for routine prenatal visits, and will then approach the mother in during the third trimester of pregnancy to see if she is interested in participating in the study. There is no time limit for which consent must be obtained – a mother may be re-visited at a later date if she would like time to think about participation. A consent form for the Genomics component will be given to all mothers meeting criteria for enrollment.

Consent on L&D and postnatally: If the mother is not identified and approached for consent prenatally, eligible mothers will be identified on admission to L&D, or in the Mother Infant Units (MIU) or NICU after delivery. If a mother is eligible and identified prenatally, study staff will be notified to obtain consent prior to the delivery of the infant. Mothers may also be consented to enroll their infant or theirselves (at Tufts)postnatally in the mother-infant units or NICU. If the infant is presenting with signs of NAS, primary consent (Improving Outcome in Neonatal Abstinence Syndrome NAS) will be obtained. Study staff will screen admission diagnoses for mothers in L&D, MIU, and the NICU on a daily basis. All efforts will be made to ensure that the effects of any sedating medications given during the delivery have worn off and that the mother's mental status has returned to baseline prior to being approached for consent. This will be determined by the mother's medical providers. Informed consent will take place in the privacy of the mother's L&D or MIU room, or in a private family conference room in the NICU.

Non-English speaking persons: All consent forms will be provided in English. If a mother does not speak English, then either a "short form" or a fully translated consent form will be presented to the mother according to institutional protocol. If using the short form, hospital translators will be present and will orally translate the entire English consent form for the mother. The mother will then be presented with a "short form" consent document in the language that is understandable to her. The mother will then be asked to sign both the English consent form and the "short form." The translator will be present throughout the entire consent process. The original signed consent form will be securely maintained in the study file record; a copy will be given to the family; and a copy will be kept in the maternal and infant medical records.

All study staff will be up to date in CITI training and will be trained by the PI as to how to obtain informed consent.

Location where study will be performed: Tufts Medical Center, Baystate Children's Hospital, Boston Medical Center, Maine Medical Center, Shands Jacksonville Medical Center, University of Pittsburgh Medical Center, Vanderbilt University Medical Center, and Women & Infants Hospital of Rhode Island

Subject fees: None

Study results: Neurodevelopmental assessments will be made available to parents after the testing is completed and any early intervention services recommended as part of the Neonatal Follow up program process. The results of the genetic testing will not be revealed to the parents.

Procedures to protect subject confidentiality: In keeping with the hospital's HIPAA regulations, the medical records will only be reviewed by study staff and will be kept confidential without any identifying information on any study materials. A unique study ID number will be assigned and will be used on all study materials. No master cord will link the list of subjects to the unique study number. An unlinked list will be maintained to help keep track of the records that have been reviewed and will be destroyed immediately afterwards. Mother and infant records will need to be reviewed in pairs for data collection and analysis purposes, and linked study ID numbers will need to be created. In order to further protect the privacy of our subjects, no dates will be recorded. All dates of birth and dates of therapy will be converted to Days of Life. No names or any other demographic data that could be used to identify individual patients will appear in reports or publications. All data forms / files will be kept in a locked drawer and/or a password protected computer in the PI's or study coordinator's office at each site. All paper forms will be kept for 5 years from the time of publication of the results. After the 5 year period, the data forms will be destroyed by the shredding of all paper files or erasing data from electronic files.

Buccal cell samples will be used for study purposes only. The results of the genetic testing will be kept in a locked drawer and/or password protected computer on a secure server in the office of the site PI or study coordinator with no identifying information. The samples will be marked only with the study ID number. The remaining samples will be discarded immediately after study testing is complete. No samples will be kept for tissue banking. The results of the genetic testing will not be part of the medical record and will not be shared with the physicians caring for the patient.

Confidentiality:

Certificate of Confidentiality: Has been obtained from the FDA and has been provided to the IRB.

How data will be coded, recorded, and stored to protect confidentiality: In keeping with the hospital's HIPAA regulations, the medical records will only be reviewed by study staff and will be kept confidential without any identifying information on any study materials. A unique study ID number will be assigned and will be used on all study materials. An unlinked list will be maintained to help keep track of the records that have been reviewed and will be destroyed immediately after the study ends. Mother and infant records will need to be reviewed for data collection and analysis purposes and linked study ID numbers created. In order to further protect the privacy of our subjects, no dates will be recorded. All dates of birth and dates of therapy will be converted to Days of Life. No names or any other demographic data that could be used to identify individual patients will appear in reports or publications. All data forms / files will be kept in a locked drawer and/or a password protected computer in the PI's or study coordinator's office at each study site. The data collection forms will be kept for 5 years from the time of publication of the results. After the 5 year period, the data forms will be destroyed by the shredding of all paper files or erasing data from electronic files.

Saliva samples will be used for study purposes only. The results of this genetic testing will be kept in a locked drawer and/or password protected computer in the office of the site PI or study coordinator with no identifying information. The samples will be marked only with the study ID number. The remaining samples will be discarded immediately after study testing is complete. No samples will be kept for tissue banking. The results of the genetic testing will not be part of the medical record and will not be shared with the physicians caring for the patient.

Parties who will have access to the data, including the key to the identity code: All study staff will have access to the study data for their particular study site. The overall study PIs and statistician will be able to view

data entered from all study sites for the purpose of monitoring and data analysis. **Parties who will have access to research records:** All study staff will have access to research records. The IRB or any federal agencies will have access to research records upon request if investigating an adverse event. No one else will have access to research records.

Collaboration: This is an NIH sponsored multisite clinical trial with collaborations with Baystate Children's Hospital, Boston Medical Center, Maine Medical Center, Shands Jacksonville Medical Center, University of Pittsburgh Medical Center, Vanderbilt University Medical Center, and Women & Infants Hospital of Rhode Island, with Dr. Barry Lester at Women & Infants being a Co-PI with Dr. Davis.

Alternatives: To not participate in the study and have the infant receive one of the medications being tested anyway.

How new information will be conveyed to the study subject and how it will be documented: If the study protocol changes or significant results occur that could benefit the patient, the PI will inform the mother within 5 days and this will be documented in the study records. Also, if significant adverse events occur, the mother will be notified within 48 hours depending on the severity of the event, and this will be documented in the study records.

Payment, including a prorated plan for payment: Since the Bayley examination is such an integral art of the study, it is essential to closely track these patients after hospital discharge and maintain contact between the parents/legal guardians and the study team in order to optimize follow-up. Subjects will be contacted every three months (3, 6, 9, 12, and 15 months) to maintain contact via a telephone call, email, text, or in person if patient is already coming to the hospital for visit unrelated to the study. $10 dollars will be added to a Clin-card each time a follow up is completed. An additional $100 will be added to the Clin-card and given to the parent/guardian at the time of completion of the neurodevelopmental assessment at approximately 18 months to cover expenses related to the hospital visit.

Payment for a research-related injury: None

Outcome: The primary outcome will be total hospital LOS. An independent physician at each site not connected to the study will also review the infant's medical record to insure that days spent in the hospital after NAS medications are discontinued is related to NAS treatment and not to social or other unrelated medical factors. If a significant discrepancy (>2 days) is identified, the local PI will discuss the case with Dr. Davis and LOS related to NAS treatment will be determined. Total LOS related to NAS, total duration of medical treatment for NAS, the need for a second drug to control symptoms, and infant growth will also be evaluated as secondary outcomes by medication group assignment. In addition, infants in both treatment groups will be evaluated at 18 months of age using the Bayley III Scales of Infant Development. It is hypothesized that neurobehavioral abnormalities identified at two weeks of age using the NICU Network Neurobehavioral Scale (NNNS) will correlate with neurodevelopmental impairment detected with the Bayley III. Finally, SNP genotyping from saliva will be obtained from all consented mothers and infants and correlated with short term outcomes (Aim 1) and neurodevelopment assessments (Aim 2) to confirm that genetic variation plays a major role in the severity and outcome of infants with NAS.

Tissue banking considerations: There will be no banking of specimens in this study.

VULNERABLE POPULATIONS: This study involves pregnant women with a history of drug dependence and their infants. However, this study involves little risk and has the potential for significant benefit for these infants in the future. The confidentiality of the mothers and infants will be protected at all times according to hospital HIPAA guidelines. No identifiable information will be collected on any study materials.

As infants are unable to provide informed consent, the mother will consent on his/her behalf. The physicians, nurses, and social workers carrying for the mother will determine if the mother is capable of providing informed consent. If the mother is unable to provide informed consent, the infant will not be enrolled. Mothers who are

less than 18 years of age are able to provide consent for their infants provided that they meet all other criteria demonstrating capacity for informed consent.

References:

1. U.S. Department of Health and Human Services, Substance Abuse and Mental Health Administration. Results from the 2008 National Survey on Drug Use and Health: National Findings. 2008.

2. Jones HE, Johnson RE, Jasinski DR, et al. Buprenorphine versus methadone in the treatment of pregnant opioid-dependent patients: Effects on the neonatal abstinence syndrome. Drug Alcohol Depend 2005;79:1-10.

3. Velez M, Jansson LM. The opioid dependent mother and newborn dyad: non-pharmacologic care. J Addict Med 2008;2:113-20.

4. Sarkar S, Donn S. Management of neonatal abstinence syndrome in the neonatal intensive care units: A national survey. J Perinatol 2006;26:15-7.

5. Neofax, 24th edition, Young TE, Mangum B, eds., Thomas Reuters, 2011.

6. Lainwala S, Brown ER, Weinschenk NP, Blackwell MT, Hagadorn JI. A retrospective study of length of hospital stay in infants treated for neonatal abstinence syndrome with methadone versus oral morphine preparations. Adv Neo Care 2005;5:265-72.

7. Kendler KS, Karkowski LM, Neale MC, Prescott CA. Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. Arch Gen Psych 2000;57:61-9.

8. Goldman D, Oroszi G, Ducci F. The Genetics of Addiction: Uncovering the Genes. Focus 2006;6:401-15.

9. Hoehe MR, Kopke K, Wendel B, et al. Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. Human Mol Genetics 2000;9:2895-908.

10. Garrido MJ, Troconiz IF. Methadone: a review of its pharmacokinetic/pharmacodynamics properties. J Pharmacol Toxicol 1999;42:61-6.

11. Zhang Y, Wang D, Johnson AD, et al. Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G. J Biol Chem 2005;280:3618-24.

12. Bond C, LaForge KS, Tian M, et al. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. Proc Natl Acad Sci 1995;95:9608-13.

13. Lotsch J, Starke C, Liefhold J, Geisslinger G. Genetic predictors of the clinical response to opioid analgesics: Clinical Utility and Future Perspectives. Clin Pharmacokinetics 2004; 43983-1013.

14. Oertel, B.G., Kettner, M., Scholich, K., Renne, C., Roskam, B., Geisslinger, G. et al. A common human mu-opioid receptor genetic variant diminishes the receptor signaling efficacy in brain regions processing the sensory information of pain. J Biol Chem 2009; 284: 6530-5.

15. Klepstad P, Dale O, Skorpen F, et al. Genetic variability and clinical efficacy of morphine. Acta Anaesthesiol Scand 2005;49:902-8.

16. Coller JK, Barratt DT, Dahlen K, et al. ABCB1 genetic variability and methadone dosage requirements in opioid-dependent individuals. Clin Pharmacol Ther 2006;80:682-90.

17. Yuferov V, Levran O, Proudnikov D, et al. Search for genetic markers and functional variants involved in the development of opiate and cocaine addiction and treatment. Ann NY Acad Sci 2010;1187:184-207.

18. Somogyi AA, Barratt DT, and Coller JK. Pharmacogenetics of opioids. Clin Pharm & Ther 2007;81:429-44.

19. Levran O, O'Hara K, Peles E, et al. ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence. Human Mol Gen 2008;17:2219-2217.

20. Reyes-Gibby CC, Shete S, Rakvag T, et al. Exploring joint effects of genes and the clinical efficacy of morphine for cancer patients: OPRM1 and COMT gene. Pain 2007;130:25-30.

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

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

23. Bio LL, Siu A, Poon CY. Update on the pharmacologic management of neonatal abstinence syndrome. J Perinatol 2011; 1-10.

24. Forcelli PA, Janssen MJ, Stamps LA, Sweeney C, Vicini S, Gale K. Therapeutic strategies to avoid long-term adverse outcomes of neonatal antiepileptic drug exposure. Epilepsia 2010 51(suppl)3:18-23.

25. Jones HE, Kaltenbach K, Heil S, et al. Neonatal abstinence syndrome following methadone or buprenorphine exposure. New Engl J Med 2010;363:2320-31.

26. Anderson PJ, De Luca CR, Hutchinson E, Roberts G, Doyle LW. Underestimation of developmental delay by the new Bayley III scale. Arch Pediatr Adolesc Med 2010;164:352-6.

27. Riley EP, Infante MA, Warren KR. Fetal alcohol spectrum disorders: an overview. Neuropsychol Rev 2011;21:73-80.

28. Bittigau P, Sifringer M, Ikonomidou C. Antiepileptic drugs and apoptosis in the developing brain. Ann NY Acad Sci 2003;993:103-14.

29. Bio LL, Siu A, Poon CY. Update on the pharmacologic management of neonatal abstinence syndrome. J Perinatol 2011;31:692-701.