Supplementary Online Content

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

eAppendix: Statistical Methods

Details on the Primary Analysis

Length of stay (LOS) was the primary outcome in the NIH grant application, study protocol, and on ClinicalTrials.gov. Although LOS was thought to be the most relevant outcome at the time of study initiation, experts now prefer length of treatment (LOT), because it excludes days spent in the hospital for non-medical reasons. We decided to keep LOS as the primary outcome because it was in the original protocol and was endorsed by the NIH, but we also report LOT.

Methods for count data are appropriate for LOS, because LOS is a count of days and because of the skewness of the LOS distribution. The analysis plan in the protocol called for Poisson regression for LOS, a method for count data that uses the log link, and therefore estimates and tests the relative mean LOS (mean LOS in the methadone group divided by mean LOS in the morphine group). Because the data were overdispersed (that is, variation was large relative to the mean), we used negative binomial regression, a generalization of Poisson regression that handles overdispersion. We report the relative mean LOS and the difference in adjusted means calculated from the negative binomial model.

The analysis plan in the NIH grant and study protocol stated that the primary analysis would adjust for the variables in the stratified randomization plan - mother's treatment and site. We adjusted for these two stratifying variables and no others.

Randomization

Randomization was 1:1 according to computer generated randomization sequences. Three randomization sequences were used for each center so enrollment could be stratified by site and antenatal exposure to buprenorphine, methadone, or pain medication. Each randomization sequence contained permuted blocks of size four and six, in random order. The study statistician (NT) designed the randomization model, which was implemented in the research data management tool StudyTrax. Assignments were obtained online from StudyTrax by the pharmacist. Only the pharmacist had access to the assignments.

Power calculation

The NIH grant was planned for short-term and long-term outcomes. The sample size of 184 was required to detect a 0.5 standard deviation difference between groups on a long-term neurodevelopmental measure, accounting for drop-out and multiple testing. (The long-term measure is still being collected). We used Poisson simulation to calculate the detectable difference in LOS, given total sample size N=184, since that was the number required for the long-term outcome. In the R-code below, lambda1 and lambda2 are the mean number of days in the 2 groups, n1 and n2 are the sample sizes per group, and alpha is the alpha-level. Applying the R-code, we determined that there would be at least 80% power to detect a difference in mean LOS of 2.3 days, if mean LOS in the shorter-LOS group was 30 days or less. The shorter the LOS, the higher the power and the smaller the detectable difference. In our data, mean LOS was shorter than expected and the adjusted difference between groups was greater than expected. That explains the statistically significant result, despite the inability to recruit the planned number of patients.

```
function (lambda1, lambda2, n1, n2, alpha)
{
   plogicvec <- 99
   iter <- 5000
   for (n in 1:iter) {
      ygp1 <- rpois(n1, lambda1)
      ygp2 <- rpois(n2, lambda2)
```

```
    xgp1 <- rep(0, n1)
    xgp2 <- rep(1, n2)
    yy <- c(ygp1, ygp2)
    xx <- c(xgp1, xgp2)
    glmfit <- glm(yy ~ xx, family = poisson)
    pvalue <- summary(glmfit)$coefficients[2, 4]
    plogic <- (pvalue < alpha)
    plogicvec <- c(plogicvec, plogic)
  }
  out <- (sum(plogicvec[-1])/iter) * 100
  out
}
```

Stopping Rules:
There were no plans to stop for statistical reasons, because insufficient numbers of infants would have had 18-month outcomes by the time enrollment was complete. Sequential boundaries were used to monitor the cumulative number of SAEs, but since there was only one SAE in the study, it was not necessary to stop.

eTable 1: Phenobarbital Dosing

Phenobarbital Loading

- Load with 20 mg/kg x 1 dose or 10 mg/kg q6-12 hours x 2 doses (should result in a plasma level of approximately 20-30 mg/ml).
- If infant continues to have scores >8, may administer 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 last loading dose.
- Give maintenance dose every 24 hours in the evening. Some infants may do better with daily dose divided every 12 hours.
- Phenobarbital steady state will be reached at approximately 7-10 days (5 half-lives).
- Maintenance phenobarbital dose depends on sum of total loading doses:

Cumulative Sum of Loading Doses	Maintenance Dose
20 mg/kg	5 mg/kg/day
30 mg/kg	6.5 mg/kg/day
40 mg/kg	8 mg/kg/day

Phenobarbital Serum levels

- The ideal serum level to control NAS is 20 - 30 mg/ml.
- Draw a trough level after 1 week of treatment unless a cumulative loading dose of greater than or equal to 30 mg/kg has been given, in which case draw a serum level prior to giving any further medication.
- 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.)

Phenobarbital Weaning

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. If the infant is still in the hospital, the phenobarbital can be weaned by 20% of the maximum dose every 3 days for average scores of $\leq$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.

eTable 2: Sensitivity analyses omitting the four infants exposed to maternal prescription opioids for chronic pain

	Methadone (n=58)	Morphine (n=54)	Unadjusted			Adjusted*		
	Mean ± SD Median (IQR)		Relative # days (Methadone: Morphine)	95% CI	*P* value	Relative # days (Methadone: Morphine)	95% CI	*P* value
LOS	21.8 ± 15.0 16 (14, 22)	23.7 ± 8.9 21 (16, 27) ‡	0.92	0.78, 1.09	0.3	0.86	0.74, 1.00	0.04
LOS due to NAS	18.9 ± 7.9 16 (14, 22)	21.5 ± 7.0 19 (16, 26) ‡	0.88	0.77, 1.00	0.05	0.86	0.77, 0.97	0.01
LOT	14.7 ± 7.9 11.5 (10, 17)	16.9 ± 7.0 15.5 (12, 20) ‡	0.87	0.74, 1.02	0.09	0.84	0.73, 0.97	0.02

CI – confidence interval; IQR – interquartile range; SD – standard deviation

*Adjusted for site and type of maternal opioid (methadone, buprenorphine, opioids for pain)

‡Difference in medians statistically significant (*P*<0.01)

eTable 3: Per Protocol Analysis: Primary and Secondary Outcomes

	Methadone (n=57)	Morphine (n=55)	Unadjusted			Adjusted*		
	Mean ± SD Median (IQR)		Relative # days (methadone: morphine)	95% CI	*P* value	Relative # days (methadone: morphine)	95% CI	*P* value
LOS	22.0 ± 15.1 16 (14, 22)	23.0 ± 8.9 20 (16, 27) ‡	0.96	0.81, 1.13	0.59	0.86	0.74, 1.00	0.047
LOS due to NAS	19.0 ± 8.0 16 (15, 22)	21.2 ± 6.6 19 (16, 25) ‡	0.90	0.79, 1.02	0.09	0.86	0.77, 0.97	0.01
LOT	14.8 ± 8.0 12 (10, 17)	16.7 ± 6.6 15 (12, 19) ‡	0.89	0.76, 1.04	0.13	0.84	0.73, 0.97	0.02
	N (%)		Odds ratio (methadone: morphine)			Odds ratio (methadone: morphine)		
Phenobarbital**	10 (17.5)	17 (30.9)	0.43	0.17, 1.04	0.06	0.42	0.17, 1.03	0.058
Infants needing a dose increase**	22 (38.6)	28 (50.9)	0.61	0.29, 1.28	0.19	0.44	0.19, 1.01	0.052
	Mean ± SD		Difference (MT - MS)			Difference (MT - MS)		
Weight gain, g/day	8.6 ± 14.0	11.2 ± 14.5	-2.7	-7.9, 2.6	0.3	-3.2	-8.2, 1.8	0.2

CI – confidence interval; IQR – interquartile range; MS – morphine solution; MT – methadone; SD – standard deviation

*Adjusted for site and type of maternal opioid (methadone, buprenorphine, opioids for pain)

**Does not include n=4 infants whose mothers were treated with opioids for pain due to sparse strata leading to problems with model fit

‡Difference in medians statistically significant (*P*<0.02)

eTable 4. Comparison to Non-randomized Infants: Primary and Secondary Outcomes

	Mean ± SD Median (IQR)		**Unadjusted**			**Adjusted***		
	Randomized (both arms) (N=116)	**Non-randomized (N=170)**	**Relative # days: Randomized all to Non-randomized**	**95% CI**	***P* value**	**Relative # days: Randomized all to Non-randomized**	**95% CI**	***P* value**
Length of hospital stay	22.5 ± 12.3 18 (15, 26)	24.4 ± 11.2 22 (16, 29)‡	0.92	0.83, 10.2	0.12	0.89	0.80, 0.99	0.03
LOS due to NAS (days)	20.0 ± 7.5 17 (15, 24)	23.3 ± 9.3 21.5 (16, 28)‡	0.86	0.78, 0.94	<0.001	0.87	0.80, 0.95	0.002
LOT (days)	15.6 ± 7.5 13 (11, 19)	18.9 ± 9.3 17 (12, 23)‡	0.83	0.74, 0.92	<0.001	0.84	0.75, 0.94	0.002
	Randomized (morphine arm) (N=58)	**Non-randomized (N=170)**	**Relative # days: Randomized morphine to Non-randomized**	**95% CI**	***P* value**	**Relative # days: Randomized morphine to Non-randomized**	**95% CI**	***P* value**
LOS (days)	23.2 ± 8.8 20 (16, 27)	24.4 ± 11.2 22 (16, 29)	0.95	0.84, 1.07	0.4	0.98	0.87, 1.11	0.7
LOS due to NAS (days)	21.1 ± 6.9 19 (16, 25)	23.3 ± 9.3 21.5 (16, 28)	0.90	0.81, 1.01	0.08	0.94	0.84, 1.05	0.3
LOT (days)	16.9 **±** 7.0 15 (12, 19)	18.9 ± 9.3 17 (12, 23)	0.88	0.76, 1.01	0.07	0.91	0.79, 1.05	0.2
	Randomized (both arms) (N=116)	**Non-randomized (N=170)**	**Odds ratio: Randomized all to Non-randomized**			**Odds ratio: Randomized all to Non-randomized**		
Use of other NAS medications** N (%)	27 (23.3)	60 (35.3)	0.56	0.33, 0.95	0.03	0.40	0.22, 0.75	0.004
	Randomized (morphine arm) (N=58)	**Non-randomized (N=170)**	**Odds ratio: Randomized morphine to Non-randomized**	**95% CI**	***P* value**	**Odds ratio: Randomized morphine to Non-randomized**	**95% CI**	***P* value**
Use of other NAS medications** N (%)	17 (29.3)	60 (35.3)	0.76	0.40, 1.45	0.4	0.59	0.28, 1.26	0.2

All non-randomized infants were treated with morphine.
CI, confidence interval; LOS, length of stay; LOT, length of treatment; NAS, neonatal abstinence syndrome;
*Adjusted for site and maternal opioid type (MT, burphenorphine, prescription opioids for pain)

**Phenobarbital for randomized infants; phenobarbital and/or clonidine for non-randomized infants (or treatment with phenobarbital for 19 non-randomized infants who received clonidine in addition to MS as first line treatment)
‡Difference in medians statistically significant ($p<0.01$)