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**Ondansetron to Reduce Neonatal Opioid Withdrawal Severity**  
*A Randomized Clinical Trial*

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**Clinical Trial Registration**

Clinicaltrials.gov NCT01965704

## **Abstract**

### *Objective*

To determine if treatment with a 5-HT<sub>3</sub> antagonist (ondansetron) reduces need for opioid therapy in infants at risk for neonatal opioid withdrawal syndrome (NOWS).

### *Study Design*

A multicenter, randomized, placebo controlled, double blind clinical trial of ninety (90) infants. The intervention arms were intravenous ondansetron or placebo during labor followed by a daily dose of ondansetron or placebo in infants for five days.

### *Results*

Twenty-two (49%) ondansetron-treated and 26 (63%) placebo-treated infants required pharmacologic treatment ( $p>0.05$ ). The Finnegan score was lower in the ondansetron-treated group (4.6 vs. 5.6,  $p=0.02$ ). A non-significant trend was noted for the duration of hospitalization. There was no difference in need for phenobarbital or clonidine therapy, or total dose of morphine in the first 15 days of NOWS treatment.

### *Conclusions*

Ondansetron treatment reduced the severity of NOWS symptoms; and there was an indication that it could reduce the length of stay.

## INTRODUCTION

*In utero* exposure to opioids predisposes infants to a postnatal opioid withdrawal syndrome, which is referred to as the neonatal opioid withdrawal syndrome (NOWS). Pharmacotherapy is the standard of care when withdrawal symptoms cannot be controlled by supportive non-pharmacologic approaches. While meta-analysis(1) and expert opinion(2) have identified opioids as the primary pharmacologic therapy for reducing NOWS symptoms, there are concerns about the long-term consequences of their use as the primary therapy for NOWS.(3) We previously examined 18 inbred mouse strains that were physically dependent on opioids. The inbred strains exhibit a highly heritable level of variability in the extent of experimentally induced (i.e., naloxone-precipitated) opiate withdrawal (NPOW) symptoms, which can range from nonexistent to maximal. Computational genetic analysis of the measured severity of NPOW identified allelic variation within the gene (*Htr3a*) encoding the 5-HT<sub>3</sub> receptor as most highly correlated with the response pattern. Consistent with this genetic finding, administration of a 5-HT<sub>3</sub> antagonist (ondansetron) significantly decreased NPOW in mice.(4) Subsequent studies in healthy human volunteers demonstrated that NPOW symptoms were greatly reduced by prior administration of ondansetron,(4) or the structurally unrelated 5-HT<sub>3</sub> antagonist palonosetron.(5) These findings led to a dose finding study that characterized ondansetron pharmacokinetics in pregnant women and placental transfer to neonates.(6) The dose identified in that study was used in this clinical trial, which was designed to produce ondansetron blood levels that were shown to be sufficient for its anti-emetic effect. Our underlying hypothesis is that a short course of ondansetron treatment administered in the perinatal period could reduce the NOWS severity in at risk infants.

## METHODS

This study (NCT01965704) was a double-blind, placebo-controlled, multisite clinical trial that tested the hypothesis that a brief period of ondansetron treatment could reduce the severity of withdrawal symptoms in infants who had *in utero* opioid exposure. The primary endpoint was the fraction of infants requiring pharmacologic therapy with morphine. The secondary endpoints were NOWS severity as defined by duration of hospitalization, severity of symptoms as measured by Finnegan scores, the total dose of morphine required in the first 15 days of treatment, and the need for adjunctive phenobarbital or clonidine therapy for NOWS treatment.

After providing informed consent, pregnant women and their infants were randomized in a 1:1 fashion to the ondansetron or placebo treatment arms using a RedCap allocation. Research staff, subjects and treating providers were blinded to group status. Unblinded pharmacists at each site managed drug preparation. Both mother and infant received the same treatment allocation. In the active treatment arm, mothers received an 8 mg dose of ondansetron intravenously (IV) within 4 hours prior to delivery. This dose could be repeated one time if the delivery did not occur within the 4 hr time frame. Infants had an electrocardiogram (ECG) before receiving the first dose of the study drug. If the QTc was not elevated and no contraindications for participation were identified, infants born to mothers who received active treatment received a daily oral dose of ondansetron 0.1 mg/kg daily for five days, a duration at which symptoms of NOWS requiring opioid therapy are manifest. Mothers in the placebo arm received a saline infusion and their infants received placebo oral medication. All other treatments and procedures were identical. The first infant dose of ondansetron or placebo was initiated within 4-8 hours of delivery. An ECG was performed on the infants within 2-5 hours after each dose of study drug. **Figure 1**

Maternal ondansetron (Zofran, Sandoz or generic equivalent) or saline placebo was administered as an IV infusion. Infant ondansetron was a commercially available oral formulation (Apotex generic or equivalent). Oral placebo for infants was simple syrup USP (Humco, Sucrose 85% W/V; Purified Water; Citric Acid; 0.1% Methylparaben or equivalent). If infants could not take oral medication, they received the ondansetron 0.04 mg/kg or placebo intravenously. Maternal inclusion criteria were daily opioid use for at least three weeks prior to delivery and known or estimated gestational age of  $\geq 37$  and  $< 42$  weeks. Exclusion criteria were ingestion of ondansetron within 24 hours prior to delivery, known prior QTc prolongation, or other medical concern of the investigator. Infants were ineligible if they had a prolonged QTc; a medical condition, or administration of a concomitant drug that would impact the ondansetron metabolism. All infants were provided non-pharmacologic care delivered according to the local hospital protocols. NOWS symptoms were monitored using the local site's modified Finnegan Scoring instrument. The threshold for pharmacologic treatment with morphine was according to local protocols with standardized rater assessments. Phenobarbital or clonidine was added as an adjunct medication according to local protocol when symptoms were not adequately controlled at a maximum morphine dose. Morphine treatment approaches remained uniform during the time period the study was conducted. If infants were discharged before day 15 of life, mothers received daily follow-up telephone calls to assess for NOWS symptoms and adverse events using a standardized study questionnaire. A final follow-up phone call was made 30 days after the baby's last dose of study medication. The study was approved by the IRB at each of the enrolling sites and informed consent was obtained for each mother and infant enrolled in the study. The study was performed in accordance with the Declaration of Helsinki. A certificate of confidentiality protected the privacy of subject research data.

## Statistics

All infants who received at least one dose of study medication and met the inclusion criteria were included in the efficacy analysis. All infants and mothers who were allocated and received at least one dose of study medication in any form were included in the safety analysis. Power analysis effect size was based upon NPOW response data that was obtained from the healthy opioid-naïve adult volunteers under controlled conditions(4). In that study, there was a difference of 2.38 between the means of the control and ondansetron-treated withdrawal responses, with standard deviations of 2.23 and 0.89, respectively. Based upon these numbers, 13 patients per treatment arm would have 90% power to ensure that a treatment difference (ondansetron vs. control) could be detected at the  $p=0.05$  level. However, because of the very large of differences between opioid-naïve adults analyzed under experimentally controlled conditions and infants that are exposed *in utero* to opioids, the actual relative effect size of ondansetron treatment was assumed to be 25-33% of that estimated from the pilot data in normal subjects. This revised effect size estimate provided 45 infants in each arm to provide the same statistical power. For categorical outcomes (the fraction of infants requiring pharmacologic therapy with morphine and the need for adjunctive phenobarbital or clonidine therapy), Fisher's Exact test was used to perform the analysis of independence. Because the need for morphine was not impacted by the sex of the neonates or by the race/ethnicity of the mother, these stratification factors were not corrected for in the analysis of primary outcome (**Table S1**).

The null hypothesis for the Fisher's Exact test was that the need for morphine treatment and study drug allocation were independent. The continuous outcomes of secondary endpoints were examined using the two-sided Wilcoxon Rank-Sum test (equivalent to the Mann-Whitney test), where the null hypothesis is stated as the outcomes in the ondansetron and placebo arms are equal.



QTc values in the safety population were analyzed by two sample t-test to determine if the mean QTc of two groups were equal. All tests were two-sided, and the p-values obtained from statistical tests were compared with the significance level ( $\alpha=0.05$ ) to determine whether null hypothesis could be rejected or retained. Statistical analyses were performed using R software (version 3.6.1).

## RESULTS

The study took place between September 2014 and August 2020. Ninety-eight mothers provided consent to reach our goal of 90 infants receiving at least a single dose of ondansetron/placebo. Twelve infants were removed from the efficacy analysis. In the ondansetron group, three mother-neonate pairs were excluded because 1 mother delivered at home and 2 mothers withdrew consent prior to receipt of the study drug. In the placebo group, 9 mother-neonate pairs were excluded because: 1 mother revealed that she was not taking any opioids, 1 mother and her baby did not receive study drug due to a prolonged QTc, 1 mother withdrew consent for her baby prior to study drug administration, 1 mother refused her study drug but allowed her infant to be in the study, however, this infant was excluded after delivery due to respiratory concerns; 1 neonate was withdrawn due hypoxic encephalopathy, and 4 mother-neonate pairs did not meet inclusion criteria (3 received off-trial ondansetron that was ordered by the clinical teams within 24 hours of delivery, and one mother had opioid use but none in the 8 days before delivery). The study flow is diagrammed in **Figure 2** and subject demographic data are provided in **Table 1**. As described in the **supplemental note**, the ondansetron dosing regimen produced plasma ondansetron concentrations that are consistent with those required for anti-emetic efficacy (**Fig. S1**).

## Efficacy

For the primary endpoint, 22 (49%) ondansetron-treated and 26 (63%) placebo-treated infants required pharmacologic treatment for NOWS. While the number of infants requiring opioid pharmacotherapy for NOWS was reduced in the ondansetron-treated group, this difference was not statistically significant. However, the mean modified Finnegan score (defined as the mean of all modified Finnegan scores recorded for that infant) was significantly reduced in the ondansetron-treated versus the placebo group ( $p=0.02$ ) (**Figure 3, Table 2**). Given the high cost of treating infants with NOWS, we investigated whether ondansetron treatment would reduce the duration of hospital stay among the at-risk neonates. A graphic analysis of the duration of hospital stay revealed that the number of ondansetron-treated neonates whose duration of hospitalization was  $\leq 5$  days or 6-10 days in duration was increased (vs. placebo) and was decreased among those with hospital stays  $\geq 16$  days (vs. placebo) (**Fig. 4**). A clear reversal in the ratios of the number of placebo and ondansetron-treated subjects became readily apparent among those whose length of stay was  $\leq 10$  days or  $\geq 16$  days. There was a trend toward a reduction in the overall mean length of hospital stay for the ondansetron-treated group (15.5 days) versus the placebo group (17.4 days), but this difference did not achieve statistical significance ( $p=0.56$ ) (**Table 2**). However, it was possible that the large number of subjects whose hospital stay was  $\geq 16$  days in the ondansetron (33%) and placebo-treated (56%) groups could have obscured the change in the mean number of hospital days, which was seen in the graph in the subjects with a shorter duration of hospitalization. Therefore, when the maximum hospital stay was capped at 15 days, the calculated p-value ( $p=0.07$ ) for the difference in mean hospital stay between the two groups was very near to the threshold for statistical significance. There was not a statistically significant difference between the placebo and treatment arms in the amount of morphine administered to infants in the first 15 days of treatment,

or in the need for adjunctive phenobarbital or clonidine therapy (**Figure 3, Table 2**). Among the infants who did not require pharmacologic therapy, the maximum Finnegan scores in the ondansetron-treated group were below those in the placebo-treated group, but these reductions did not reach statistical significance. Three infants that were assigned to the placebo arm, but whose mother had received ondansetron for clinically indicated reasons within 24 hours of birth were excluded from the analysis. In a sensitivity analysis, when these infants were included in an as-treated comparison, 59% of placebo treated infants required pharmacologic treatment, compared to the 49% in the ondansetron group ( $P>0.05$ ).

## **Safety**

Any infant exposed to study drug, either indirectly via administration to the mother or directly to the infant, was included in the safety cohort. Six infants had a serious adverse event (SAE); 2 were in the ondansetron group and 4 were in the placebo group. There was 1 maternal serious event in each treatment arm (**Table 3**). No SAEs were judged to be due to ondansetron treatment. One neonate had a SAE (hypoxic encephalopathy) and four additional AEs (seizure, sepsis, laryngomalacia, and left brachial plexus injury). This neonate was excluded from the per-protocol analysis due to complications during the birth. One infant allocated to ondansetron had recurrent seizures graded as possibly related to the study drug; and another had late onset NOWS symptoms (the mother reported irritability and the infant's weight was below the expected trajectory). Two infants in the placebo group were readmitted for NOWS symptoms, but both were managed without the need for pharmacologic therapy. Fourteen (29%) infants in the ondansetron arm and 12 (27%) in the placebo arm were withdrawn from the study due to a prolonged QTc interval. The initial protocol-specified that the maximum QTc for infants was 440 milliseconds (ms) based upon FDA input. A prespecified safety and pharmacokinetic interim analysis took place in May 2016

after 27 infants had been enrolled. The average QTc was 418 ms in the ondansetron group and 437 ms in the placebo group, with 26% of infants having a QTc >440 ms. After discussion with the Data Safety Monitoring Board and subsequent approval by the FDA, the maximum allowed QTc value was increased to 480 ms. Of particular importance, no clinically significant arrhythmias were noted and there was no significant difference in the QTc of infants in the ondansetron and placebo groups. (**Table 4, Figure S2**) The baseline QTc in infants whose mothers received at least one dose of ondansetron in labor was 424 ms and compared to 419 ms in those who did not for a difference of 5 ms (95% CI -9.4 to 19.4, P =0.49).

## **DISCUSSION**

NOWS treatment, which is focused on reducing symptom severity in at-risk infants, initially involves the use of non-pharmacologic approaches. However, opioids are administered to many of these infants because their symptoms are not controlled by the non-pharmacologic measures. While NOWS symptoms can be controlled by opioid administration, there are concerns about the potential detrimental effects that opioid treatment, which is associated with a prolonged and costly period of hospitalization, can have on infant development and maternal bonding. (7-11) It is therefore essential to develop non-opioid therapies that can reduce or prevent NOWS symptoms. Based on the striking results obtained in rodents(4) and in opioid naïve adults,(5) we investigated whether ondansetron could provide a non-opiate based treatment for reducing NOWS symptoms and incidence. Of importance, the internal validity of this trial was good with a low risk of bias from randomization, robust local protocols were used for standardization of scoring, and there were few deviations from intended therapy or missing outcomes. The external validity of this trial was strengthened by a multicenter design at high functioning sites serving a variety of patient populations. Since ondansetron treatment produced a statistically significant reduction in the

severity of NOWS symptoms, this study demonstrates that ondansetron treatment reduces NOWS symptom scores. While the impact of a symptom reduction of a 20% magnitude (4.6 vs. 5.6) on an individual patient may be uncertain, the importance of this decrease is magnified when viewed across the NOWS patient population. Since symptom scores determine whether opioid therapy is initiated, a 20% decrease in symptom scores would in many patients alter a protocol-driven decision about whether to initiate opioid treatment. It was also noteworthy that there was a trend toward a reduction in the duration of hospitalization in the ondansetron treated group, which was observed in the decreased number of ondansetron-treated subjects requiring a longer hospital stay and an increase in the number of ondansetron-treated subjects with a short hospital stay.

Ondansetron treatment caused a statistically significant decrease in the Finnegan scores but not in the total dose of morphine administered. This divergence is not surprising since the Finnegan score is an elastic measurement (i.e., it is determined at each time point without other constraints), while the morphine dose administered is constrained by the treatment protocol, and it is altered in a stepwise fashion based upon the prior morphine dose. Thus, a change in the morphine dose will be less responsive to a change in NOWS expression than will a change in the Finnegan score and so a change in the morphine dose is likely less likely to differ between treatment groups than will the Finnegan scores. It was also noteworthy that ondansetron's efficacy in this clinical trial was far less than what we observed previously in mice or human subjects.(4),(5) There are several potential reasons for this. First, unlike that during experimentally induced opioid withdrawal, NOWS expression in any infant can be influenced by factors that are known and measurable and by others that are unknown. While there could be differences in the adult versus neonatal opioid withdrawal physiology, the similarities in buprenorphine ED50 for amelioration of opioid

withdrawal symptomatology in adults and infants(12) argues against this possibility. Another factor that could reduce the efficacy of ondansetron in this trial could be that a suboptimal level of ondansetron was achieved with the ondansetron dosing regimen used here. While the ondansetron exposures in this trial were adequate for achieving anti-emetic efficacy, its anti-emetic effect is mediated by drug action at sites (vagal afferent nerves in the gastrointestinal tract) outside of the CNS.(13,14) Our intra-ventricular injection study in mice demonstrates that ondansetron's effect on opioid withdrawal occurs within the CNS.(4) Moreover, a peripherally acting opioid antagonist (methylnaltrexone), which is used to treat opioid-induced constipation, does not precipitate withdrawal.(15,16) In addition, ondansetron has a slow rate of CNS entry.(17) Although ondansetron has moderate levels of protein binding and ionization at plasma pH and good lipid solubility, its rate of blood brain barrier penetration is below that predicted by its intrinsic properties.(17) Ondansetron CNS kinetics have not been established in either neonates or adults. Thus, it is likely that if a sufficient level of ondansetron binding to its target within the CNS could be achieved, it could have an even larger effect on reducing opioid withdrawal symptomatology and incidence which is consistent with our observation that intrathecal ondansetron administration in an animal model demonstrated such marked efficacy in reducing opioid withdrawal behavior.(4)

This clinical trial did not identify any safety issues. These results suggest that it may be possible to administer ondansetron to the mother for a longer period prior to delivery or possibly to the neonate at higher doses after delivery. This would increase the concentration of ondansetron that is present at its site of action in the CNS for a period of time, which may generate a more marked reduction in NOWS symptoms. If so, this would impact a significant and costly public health problem that arises from the prevalence of opioid use disorder, which generates a severe clinical

condition that could have a downstream effect on the infant's subsequent development. Of course, the effect of a more prolonged period of ondansetron treatment of the expectant mother prior to giving birth must be tested in a subsequent clinical trial.

### **Conflict of interest disclosure**

The authors have no conflicts of interest relevant to this article to disclose.

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### **Contributors Statement**

Dr Peltz conceptualized and designed the study, carried out the initial analyses, assisted with the initial draft, and reviewed and revised the manuscript

Dr Kraft drafted the initial manuscript, collected data, carried out the initial analyses, and reviewed and revised the manuscript

Drs Wang conducted statistical analysis, generated figures and tables, assisted with the initial draft, and reviewed and revised the manuscript

Drs Jansson and Adeniyi-Jones collected data, contributed to the initial analyses, and reviewed and revised the manuscript

Drs Drover and Shafer, and Ms Cohane coordinated and supervised data collection and review, contributed to the analyses, and reviewed and revised the manuscript

Dr Wu developed and conducted the analytical assay, and reviewed and revised the manuscript

Drs Govindaswami, Jegatheesan, Argani, and Kahn collected data, and reviewed and revised the manuscript

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### **Participating sites (number of subjects enrolled)**

Thomas Jefferson University (42)

Johns Hopkins Bayview Medical Center (31)

Santa Clara Valley Medical Center (13)

University of Louisville (4)

University of Tennessee Health Science Center (3)

Lucile Packard Children's Hospital (2)

University of Utah (2)

University of California, San Francisco General Hospital (1)

### **Data availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.



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## **Tables**

Table 1. Characteristics of the infants and mothers (Per-Protocol Population).

Table 2. Efficacy endpoints

Table 3. Safety

Table 4. QTc values

**Table 1.** Characteristics of the infants and mothers (Per-Protocol Population).

<b>Characteristic</b>	<b>Ondansetron (N=45)</b>	<b>Placebo (N=41)</b>
<b>Infants</b>		
Male sex - # (%)	23 (51%)	22 (54%)
Median birth weight (range) - grams	3053 (1940 to 4017)	3060 (2017 to 4120)
Median gestational age (range) - weeks/days	39/1 (36/0 to 41/2)	39/0 (37/0 to 40/6)
Median Apgar scores at 1 minute (range)	8 (2 to 9)	8 (5 to 9)
Median Apgar score at 5 minutes (range)	9 (6 to 9)	9 (5 to 10)
Receipt of any breastmilk in hospital (%)	18 (40%)	17 (41%)
<b>Mothers</b>		
Ethnicity and race - # (%)		
White non-Hispanic	35 (78%)	34 (83%)
Black non-Hispanic	6 (13%)	4 (10%)
White Hispanic	3 (7%)	3 (7%)
Black + other non-Hispanic	1 (2%)	0 (0%)
<b>Maternal substance exposure</b>		
Methadone (%)	38 (84%)	34 (83%)
Heroin (%)	23 (51%)	20 (49%)
Methamphetamine (%)	7 (16%)	5 (12%)
Tobacco use (>5 cigarettes day) (%)	24 (53%)	18 (44%)
Marijuana (%)	10 (22%)	7 (17%)
Cocaine (%)	8 (18%)	9 (22%)
Benzodiazepine (%)	10 (22%)	7 (17%)
PCP (%)	1 (2%)	0 (0%)
<b>Mother did not receive study drug during labor (%)</b>	5 (11%)	7 (17%)

**Table 2.** Primary and Secondary Outcomes

<b>Outcome</b>	<b>Ondansetron (N=45)</b>	<b>Placebo (N=41)</b>	<b>Difference (95% CI)<sup>a</sup></b>	<b>P Value</b>
<b>Primary outcome</b>				
Need for morphine to treat NOWS – #. (%)	22 (49%)	26 (63%)	-4	0.2 <sup>c</sup>
<b>Secondary outcomes</b>				
Mean hospital stay (range)	15.5 (2 to 64)	17.4 (3 to 63)	-1.9 (-8.0 to 4.3)	0.56 <sup>d</sup>
Mean hospital stay (range) with a 15-day maximum <sup>e</sup>	9.3 (2 to 15)	11.2 (3 to 15)	-1.9 (-4.0 to 0.2)	0.07
Median dose of morphine in 15 days (range) – mg	9.3 (0.8 to 37.4)	11.1 (0.8 to 31.1)	-1.8 (-8.8 to 2.7)	0.31 <sup>d</sup>
Mean modified Finnegan score <sup>b</sup> (standard deviation)	4.6 (1.6)	5.6 (2.1)	-1.0 (-1.8 to -0.2)	0.02 <sup>f</sup>
Use of adjunctive medication <sup>g</sup> to treat NOWS – # (%)	5 (11%)	5 (12%)	0	1 <sup>c</sup>

<sup>a</sup> The 95% confidence interval (CI) between the ondansetron group and the placebo group was calculated with the use of Hodges-Lehmann estimator.

<sup>b</sup> The modified Finnegan score of a neonate is represented by the mean of all modified Finnegan scores recorded for that neonate. The mean modified Finnegan score by ondansetron/placebo allocation reported in the table is the mean of all neonates' scores in the corresponding allocation.

<sup>c</sup> Fishers exact test.

<sup>d</sup> Wilcoxon Rank-Sum test

<sup>e</sup> By capping the maximum stay at 15 days, the statistical significance of the treatment effect is improved.

<sup>f</sup> Two-sample t-test.

<sup>g</sup> phenobarbital or clonidine

**Table 3.** Adverse Events.

Category (number of patients in safety analysis)	Ondansetron (n=48)	Placebo (n=44)
	no. of events	
<b>Serious Adverse Events (SAEs)</b>		
<i>Maternal</i>		
Suicidal ideation	1	
C-section superficial wound infection		1
<i>Neonatal</i>		
Readmission for NOWS observation		2
Hyperbilirubinemia		1
Late onset NOWS	1	
Intermittent seizures	1	
Hypoxic encephalopathy		1 <sup>b</sup>
<b>Adverse Events (AEs)</b>		
<i>Maternal</i>		
Subcutaneous hematoma drainage from C-section incision		1
Vaginal bleeding	1	
<i>Neonatal</i>		
Prolonged QTc	14 <sup>a</sup>	12
Short QTc	3	2
Cyanotic episode	1	
Seizure		1 <sup>b</sup>
Sepsis		1 <sup>b</sup>
Laryngomalacia		1 <sup>b</sup>
Left brachial plexus injury		1 <sup>b</sup>
Secundum atrial septal defect	1 <sup>a</sup>	
<b>Total AE and SAE</b>	23	24

<sup>a</sup> One neonate had two AEs (prolonged QTc and secundum atrial septal defect).

<sup>b</sup> One neonate had one SAE (hypoxic encephalopathy) and four AEs (seizure, sepsis, laryngomalacia, and left brachial plexus injury). The neonate was excluded for per-protocol analysis due to complications during the birth.

**Table 4.** Infant QTc values in the safety population.

Outcome	Ondansetron (N=48)	Placebo (N=44)	Difference (95% CI)	P Value
Mean number of ECG (range)	3.3 (0 to 5)	3.6 (0 to 5)	-0.3 (-1.1 to 0.5)	0.46 <sup>b</sup>
Baseline QTc (in ms)	423.6	418.9	4.7 (-9.7 to 19.1)	0.52 <sup>b</sup>
Mean QTc after receipt of first infant dose <sup>a</sup> (in ms)	435.3	429.1	6.2 (-9.9 to 22.3)	0.45 <sup>b</sup>
Mean QTc change from baseline (initial QTc – mean of all post dose values)	-15.7	-17.7	2.0 (-15.8 to 19.8)	0.83 <sup>b</sup>

<sup>a</sup> This refers to mean of all QTc values measured for post doses

<sup>b</sup> Two-sample t-test.

Figure 1. Study design

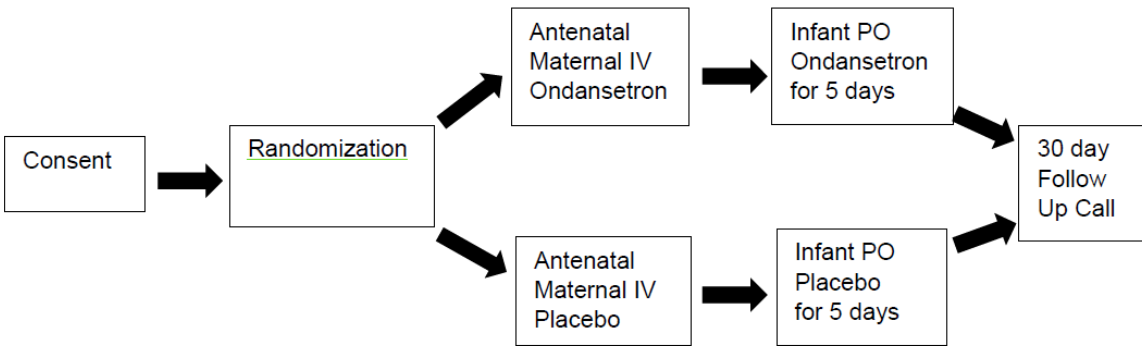


Figure 2. Patient flow CONSORT diagram

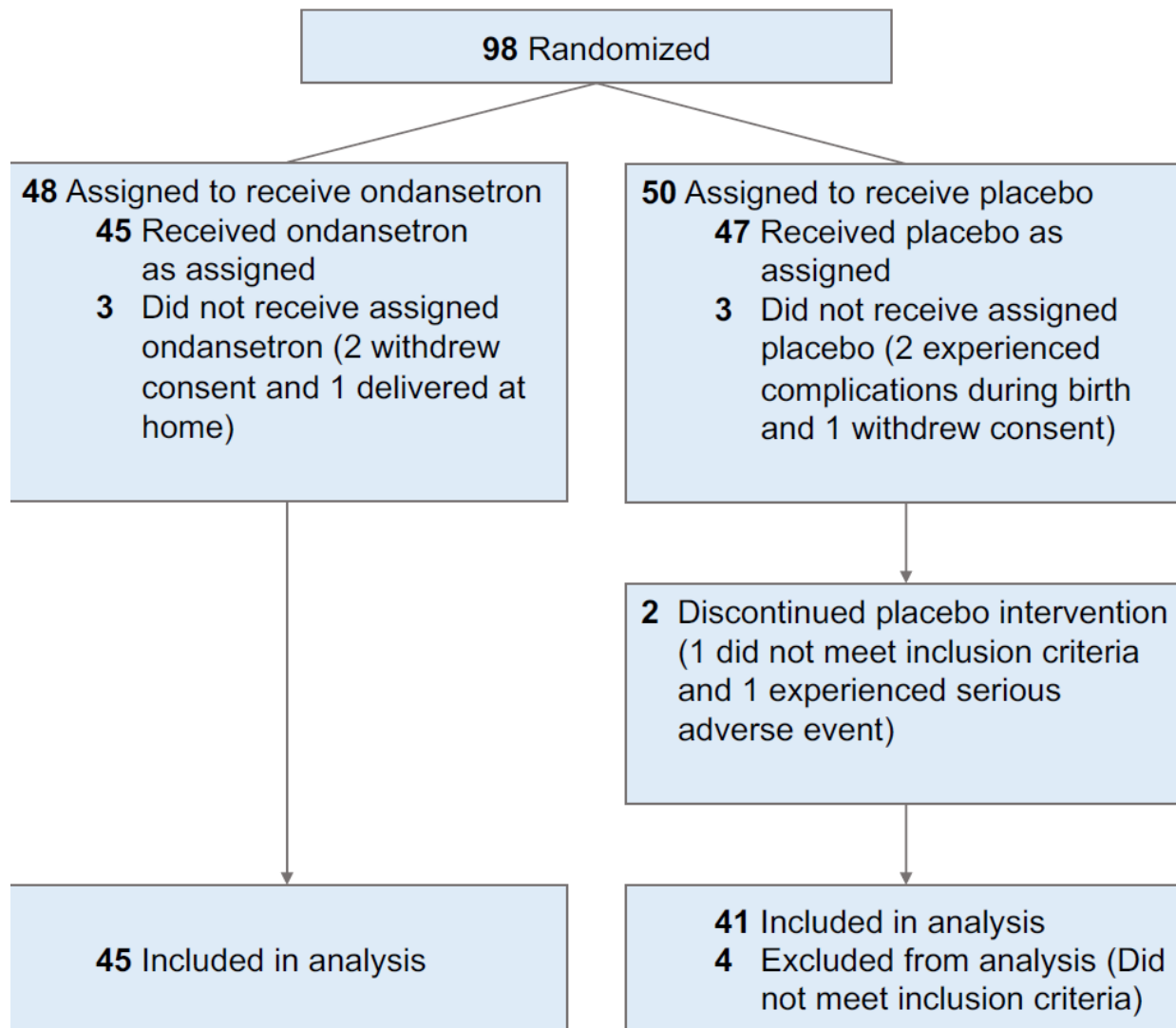


Figure 3. These graphs compare the secondary outcomes of total dose of morphine administered and the average modified Finnegan score in the placebo and ondansetron-treated groups.

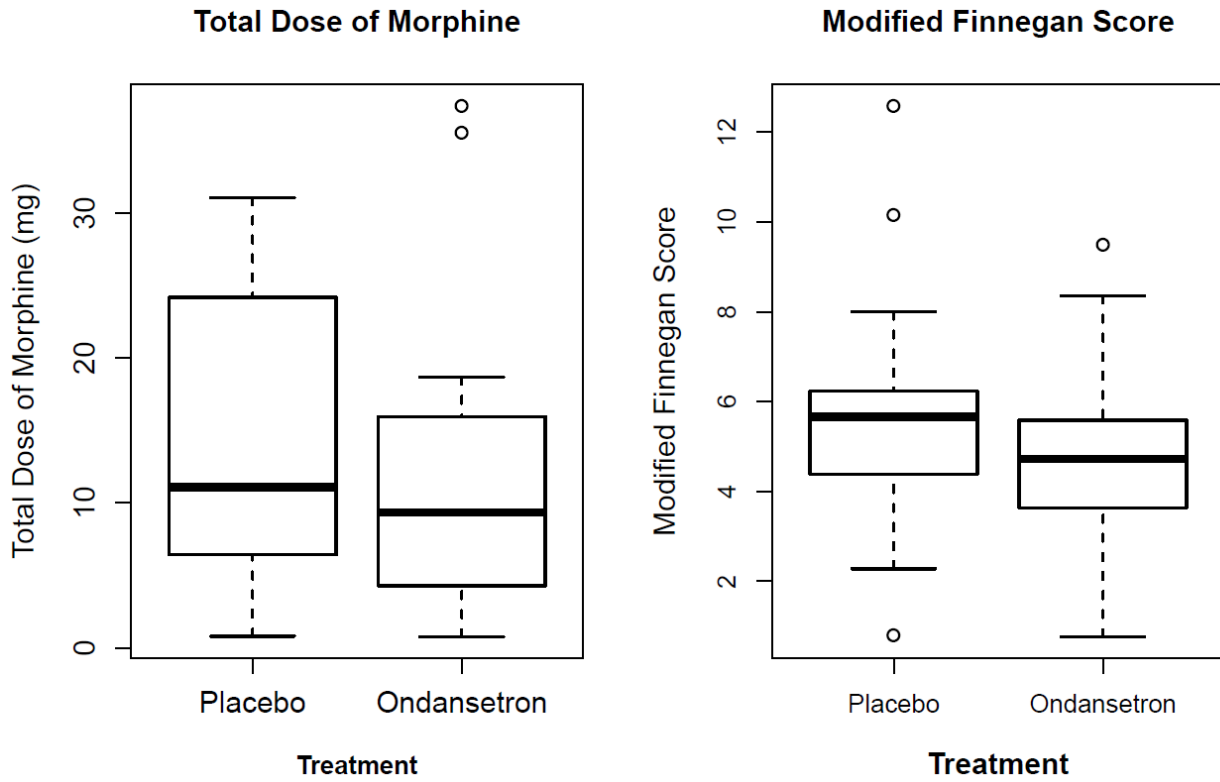
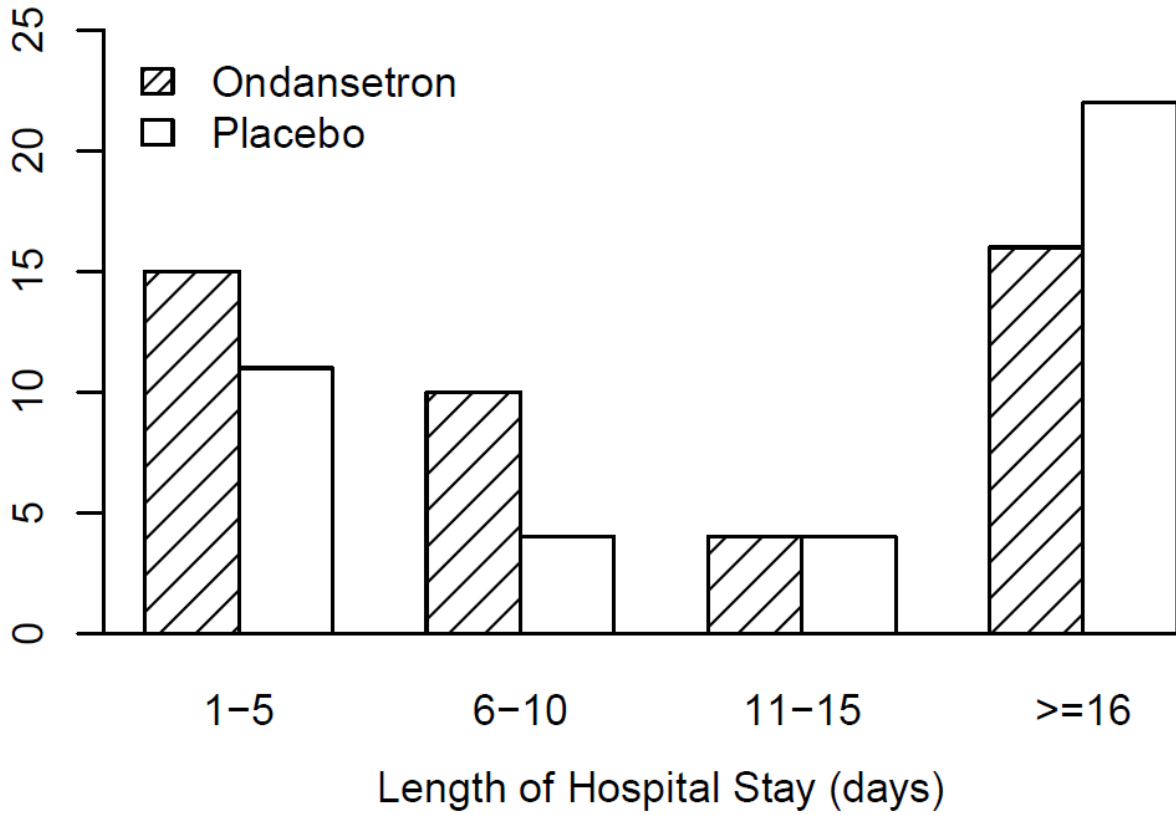


Figure 4. This bar graph shows the number subjects in the placebo and ondansetron-treated groups with a duration of hospitalization for each indicated time. It is noteworthy that the number of ondansetron-treated subjects with a duration of hospitalization that was less than  $\leq 5$  days or 6-10 days was increased but was decreased among those whose hospital stays was  $\geq 16$  days (vs. placebo).





Supplemental Material

**Supplemental Tables**

**Table S1.** Primary and secondary outcomes of confounding factors in neonates

**Table S2.** Primary and secondary outcomes of confounding factors in mothers

**Supplemental Figure**

**Figure S1.** Ondansetron Concentration in Infants

**Figure S2.** The fraction of infants with the QTc values above the threshold of the screening ECG and after each dose of study drug.

**Supplemental Note**

Pharmacokinetic Methods and Results

**Table S1.** Potential confounding factors in primary outcomes in neonates.

Sex of neonates

Primary outcome	Male (N=45)	Female (N=41)	P value
Number of infants requiring morphine to treat NOWS – # (%)	25 (56%)	23 (56%)	1*

\*Fishers Exact test.

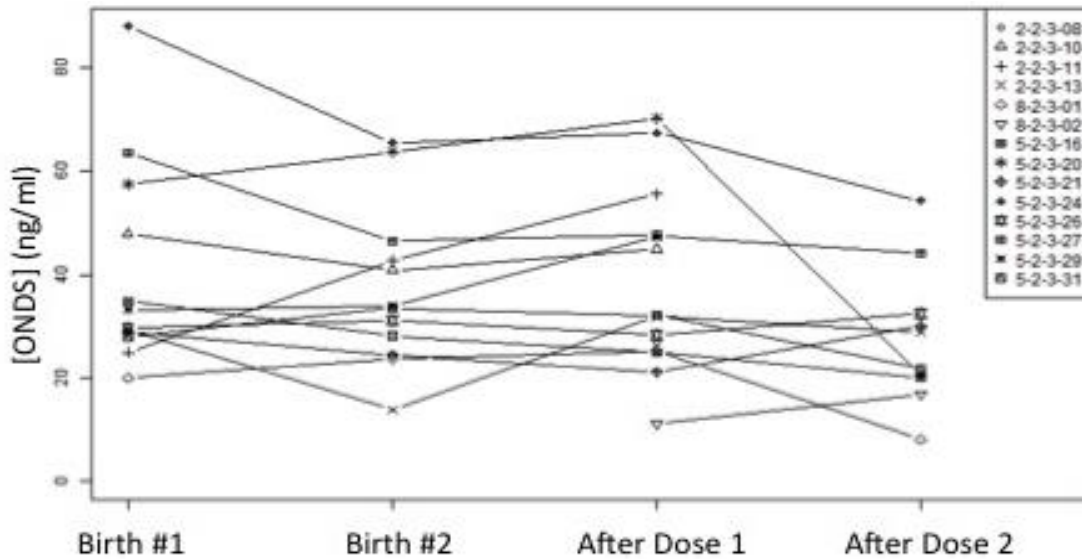
**Table S2.** Potential confounding factors in primary outcomes in mothers.

Race/ethnicity of mothers

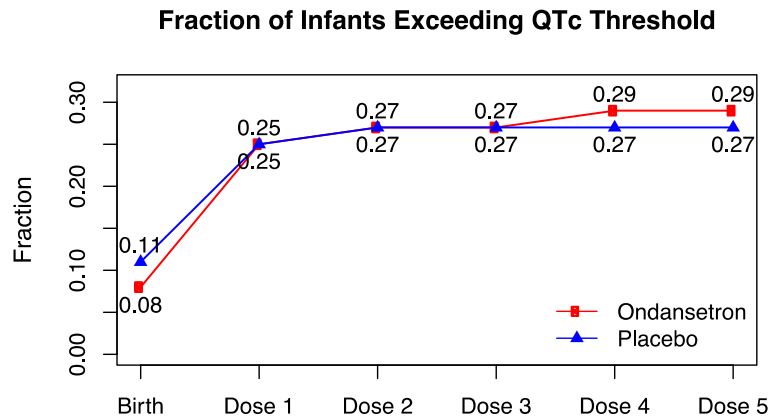
Primary outcome	NH/W (N=69)	NH/Black (N=10)	Hisp/W (N=6)	NH/Black + other (N=1)	P value
Need of morphine to treat NOWS – # (%)	39 (57%)	5 (50%)	3 (50%)	1 (100%)	0.80*

\*Pearson's Chi-squared test.

**Figure S1.** Ondansetron Concentration in Infants. The plasma ondansetron concentration [ONDS] was sequentially measured in 14 neonates. The measurements performed before a baby received a dose of ondansetron are labeled as Birth #1 and Birth #2, and reflect placental transfer from the mother. Birth #1 was cord blood and Birth #2 was heel stick. The Dose 1 and Dose 2 measurements were performed after the 1<sup>st</sup> or 2<sup>nd</sup> dose of ondansetron, respectively, was administered to the baby.



**Figure S2.** The fraction of infants with the QTc values above the threshold of the screening ECG and after each dose of study drug.



## Ondansetron Concentration Measurements

Ondansetron HCl hydrate (Sigma) and the internal standard ondansetron-D3 (Santa Cruz Biotechnology) were used in these analyses. High-performance, liquid-chromatography (HPLC)-grade water, methanol, and acetonitrile, which were used for sample extraction and as the mobile phase. The calibration curve ranged from 219.6 to 0.429 ng/ml. Three quality control samples were also prepared at concentrations of 109.8, 10.98 and 1.098 ng/ml. Plasma samples were mixed with 3 volumes of acetonitrile containing the internal standard, dried and reconstituted in 50  $\mu$ l of 0.1% formic acid, 5% acetonitrile in HPLC water. Dried blood spot samples were used for ondansetron measurements in neonates.<sup>1</sup> The calibration curve range for ondansetron ranged from 219.6 ng/mL to 0.429 ng/mL. The extracts were analyzed using an LCMS system according to previously described methods.<sup>2</sup> Briefly, 10  $\mu$ L of reconstituted supernatant for plasma samples or 18  $\mu$ L for dried blood spot samples was injected onto an analytical column (Phenomenex) C18 2.6  $\mu$  100 x 2.1 mm. A gradient was run from 95% solvent A (0.1% formic acid in water) to 95% solvent B (0.1 % formic acid in acetonitrile) over 30 min at solvent B: flow rate of 0.5 mL/min. An Agilent 1290 Infinity UHPLC system was interfaced with an Agilent QTOF accurate mass 6520 mass spectrometer using a positive electrospray ionization source. Full scan (m/z 110–1000) spectra were collected. For ondansetron, the ion was monitored: 294.1601 [M+H]<sup>+</sup>. For the ondansetron D3 internal standard, the ion was monitored: 297.1789 [M+H]<sup>+</sup>.

## Ondansetron concentration in neonates

Our ondansetron dosing regimen was based upon results obtained from a prior population pharmacokinetic analysis.<sup>3</sup> To assess ondansetron exposure in the treated neonates, plasma ondansetron concentrations were measured in 14 neonates that received ondansetron and had a blood drawn for purposes unrelated to the study. The interval between ondansetron dosing and the sample acquisition time varied because sample acquisition was only obtained when blood was required for other purposes. Nevertheless, the plasma ondansetron concentrations measured after birth (but before the baby received a dose of ondansetron) averaged  $41 \pm 20$  ng/ml (range 20 to 88 ng/ml) and after receiving one or more ondansetron doses averaged  $38 \pm 18$  ng/ml (range 11 to 77 ng/ml) (**Fig S1**). Of note, the ondansetron concentrations in the neonates were very similar to those measured in non-pregnant women (mean 33 ng/ml) and in pregnant mothers (mean 46 ng/ml) in our prior pharmacokinetic study,<sup>3</sup> and are within the therapeutic range found after oral ondansetron dosing in several different adult populations.<sup>4</sup> Thus, this dosing regimen produced plasma ondansetron concentrations required for anti-emetic efficacy.

1. Xu X, Bartlett MG, Stewart JT. Determination of ondansetron and its hydroxy metabolites in human serum using solid-phase extraction and liquid chromatography/positive ion electrospray tandem mass spectrometry. *J Mass Spectrom.* 2000;35(11):1329-1334.
2. Spooner N, Lad R, Barfield M. Dried blood spots as a sample collection technique for the determination of pharmacokinetics in clinical studies: Considerations for the validation of a quantitative bioanalytical method. *Anal Chem.* 2009;81(4):1557-1563.
3. Elkomy MH, Sultan P, Carvalho B, et al. Ondansetron pharmacokinetics in pregnant women and neonates: Towards a new treatment for neonatal abstinence syndrome. *Clin Pharmacol Ther.* 2015;97(2):167-176.
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