MEDICAL OFFICER REVIEW

Division of Pulmonary, Allergy and Rheumatology Drug Products (HFD-570)

Application #:	20-845	**Application Type:**	NDA
Sponsor:	INO Therapeutics	**Proprietary Name:**	INOmax
Reviewer:	K. Witzmann, MD	**USAN Name:**	Nitric Oxide
		Route of Administration:	Inhalational
		Review Date:	November 19, 2010

SUBMISSIONS REVIEWED IN THIS DOCUMENT

Document Date	CDER Stamp Date	Submission Type	Comments
June 24, 2010	June 25, 2010	Response to Written Request	

REVIEW SUMMARY:

INOmax is an inhaled nitric oxide product produced by INO Therapeutics, initially approved in 1999. INOmax is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated "for the treatment of term and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension".

INO Therapeutics has also been studying the use of iNOmax for the prevention of chronic lung disease (Bronchopulmonary Dysplasia, BPD) in preterm infants with a gestational age < 26 weeks to 34 weeks, with respiratory insufficiency. The functional hypothesis, based on experimental in vitro and animal data, is that inhaled nitric oxide in the preterm neonate will act by decreasing airway muscularization and improve pulmonary angiogenesis and alveolarization and, thus impact the development of chronic lung disease associated with premature birth.

After discussions between the Sponsor, the Division of Cardiovascular and Renal Products, and the Division of Pulmonary, Allergy, and Rheumatology Products regarding the data required to support the issuance of a written request for studies of iNO in premature infants for the prevention of BPD, a Written Request (WR) was issued to the Sponsor on April 30, 2010. INO Therapeutics subsequently responded to the WR on June 25, 2010. On November 2, 2010, after discussion with DPARP, the Pediatric Exclusivity Board determined that the Sponsor had fairly responded to the WR.

The Sponsor has submitted full study reports for studies INOT27 and BALLR1 in response to the WR, and has also included the study data for an additional study of iNO in premature infants, INOT25. Below are clinical reviews of the three studies submitted to the FDA in support of the WR followed by FDA-proposed changes to the INOmax label.

Medical Reviewer: K. Witzmann, M.D.

Medical Team Leader: Anthony Durmowicz, M.D.

1. Introduction and Regulatory Background

Product Information

INOmax is an inhaled nitric oxide product produced by the Sponsor INO Therapeutics, initially approved in 1999. INOmax is a vasodilator, which, in conjunction with ventilatory support and other appropriate agents, is indicated for the treatment of term and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension, where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation. It is commercially available in the United States and Europe.

INO Therapeutics has been studying the use of iNOmax for the prevention of chronic lung disease (Bronchopulmonary Dysplasia, BPD) in preterm infants with a gestational age < 26 weeks to 34 weeks, with respiratory distress. The functional hypothesis, based on experimental in vitro and animal data, is that inhaled nitric oxide in the preterm neonate will act by decreasing airway muscularization and improve pulmonary angiogenesis and alveolarization and, thus impact the development of chronic lung disease associated with premature birth.

Regulatory Activity Related to Submission

INO Therapeutics has previously submitted a New Drug Application for INOmax, with an indication for respiratory failure in preterm to term neonates with evidence of pulmonary hypertension which was approved in 1999, by the Division of Cardiovascular and Renal Products. After discussions between the Sponsor, the Division of Cardiovascular and Renal Products, and the Division of Pulmonary, Allergy, and Rheumatology Products (DPARP) regarding the data required to support the issuance of a written request for studies of iNO in premature infants for the prevention of BPD, a Written Request (WR) was issued to the Sponsor on April 30, 2010 requesting the following 2 clinical studies:

> *Study 1*: *Double-blind, placebo-controlled, randomized efficacy and safety trial to assess the effect of nitric oxide on survival without bronchopulmonary dysplasia (BPD) in approximately 800 preterm neonates > 500 grams, < 24 hours old, and 24-28 weeks gestational age with respiratory distress who require ventilatory assistance.*
>
> *Study 2: Double-blind, placebo-controlled, randomized efficacy and safety trial to assess the effect of nitric oxide on reducing the risk of chronic lung disease (CLD) in approximately 587 preterm infants < 32 weeks gestational age and 500 to 1250 grams at birth who are 7-21 days old at high risk of developing CLD.*

INO Therapeutics responded to the WR with a submission received on June 25, 2010. In addition to the study reports requested for the 2 studies specified in the WR letter, the Sponsor also submitted the full study report for an additional large clinical study of the use of INOmax in premature infants designated study INOT25. Of note, based on the findings of the studies submitted, the Sponsor is not seeking an indication for the use of INOmax for the prevention of BPD in premature infants.

Table 1 below compares patient enrollment, study patient population, drug dose and duration for the 3 studies submitted by the Sponsor.

Table 1: Comparison of iNO Study Populations

Study	ITT #/safety (iNO/Placebo)	GA /wt	Patient Age at tx	iNO dose	Duration of treat.
INOT27	800 ITT 792 safety (395/ 397)	24+0 wk to 28+6 wk	2hrs of life to 26 hrs of life	5ppm	7-21 days
BALLR1	587 ITT 584 safety (295/ 289)	<34wk, 500-1,250g	7-21 days of life	20ppm x 48-96hr, taper by 50% weekly until d/c	Minimum 24 days
INOT25	793 ITT 793 safety (398/395)	<34 wk, 500-1,250 g	<48 hrs of life	5ppm	21 days or until extubation

Following are reviews of each of the 3 studies of INOmax for the prevention of BPD in premature infants followed by FDA labeling changes based on the study results.

Reviewer's Comment:
The three studies described below use the terms gestational age (GA), corrected gestational age, post-menstrual age (PMA), and post-conceptual age (PCA) in some form, and the Sponsor's study report also uses these terms somewhat interchangeably. For the purposes of this review, I have used gestational age (GA) to denote age at the time of birth, and postmenstrual age (PMA) to denote the age at which the infant would equal 36 weeks, corrected for their early birth. These are the terms currently favored in the neonatal literature, which have evolved since the writing of these protocols/ publications.

2. STUDY INOT27

Title

The Effects of Nitric Oxide for Inhalation on the Development of Chronic Lung Disease in Preterm Infants

Protocol

Administrative

Study Initiated: May 29, 2005
Study Completed: March 16, 2008
Clinical Centers: Multicenter; 35 study centers enrolled subjects throughout Europe
Study Report Dated: June 25, 2009
Study Sponsor: INO Therapeutics, LLC

INOT27 was carried out at multiple centers throughout Europe, data was managed by INO Therapeutics (Clinton, NJ) throughout the course of the trial, and a statistical analysis plan was in place before unblinding of data.

The original protocol was issued on 06 July 2004 and 6 subsequent protocol amendments were issued. Amendment 1 was issued on 25 August 2004, and Amendment 2 was issued 08

September 2004 (no subjects had yet enrolled). Amendment 3, issued on 01 November 2004 increased the planned total number of subjects and sites, and amended primary and secondary outcome variables. Amendment 4 was issued on 06 June 2005, with minor protocol clarifications. Amendment 5 was issued on 08 December 2005 to clarify primary endpoint measurements to include serious adverse events (SAEs) rather than AEs, and to include head ultrasounds. Amendment 6 was issued on 16 October 2006 (halfway through the enrollment period) to add infants requiring use of pulmonary surfactant or ventilatory support via nasal CPAP within 24 hours of birth for possible participation in the study.

Objectives

The primary objective for INOT27 was to assess the safety and efficacy of iNO to reduce the risk of chronic lung disease (CLD) in preterm infants with respiratory distress, and to assess the long-term effects of the therapy on the development of these children over seven years, with clinical follow-up. Secondary objectives were to measure other parameters of morbidity, including number of days on assisted ventilation, length of hospitalization, and survival without intracranial insult, as well as parameters for safety.

Reviewer's Comment:
For the purposes of the WR, only data for the primary endpoint were required, and therefore long-term data were not submitted at this time.

Study Design

This trial was a prospective, multicenter, randomized, blinded, placebo-controlled trial to assess the effects of nitric oxide for inhalation in preterm infants with respiratory distress. All infants were inborn at the investigational site, 24 $^{+0\ days}$ weeks' to 28$^{+6\ days}$ weeks' GA. Infants requiring use of pulmonary surfactant or ventilatory support via nasal continuous positive airway pressure (CPAP) within 24 hours of birth were offered participation in the study.

Subjects were randomized to either 5 ppm inhaled nitric oxide (iNO) or nitrogen (N2) placebo. Randomized subjects were stratified by site and gestational age at birth (24 $^{+0\ days}$ to 25 $^{+6\ days}$). Study drug was the approved INOmax product, delivered via the CDRH-approved inhaled nitric oxide delivery system, the INOvent. Therapy was initiated as soon as possible after consent was obtained (always within 24 hours of birth). Therapy was continued for a minimum of 7 days, or until the end of ventilatory support (whichever was later), and up to 21 days maximum. Subjects who required less than 7 days of assisted ventilation could complete the minimum duration of therapy via face mask or nasal cannula. The protocol did not mandate specific subject medical management. However, each participating center was required to develop and utilize standard management guidelines throughout the trial in order to provide internal consistency and minimize the effects of confounding factors. These included:

- Alarms and settings for oxygenation and CO2 monitoring
- Surfactant dosing
- Indication for tracheal Intubation
- Intermittent Mechanical Ventilation guidelines
- High-frequency Oscillatory Ventilation guidelines
- Criteria for extubation
- Use of nasal intermittent ventilation
- Use of CPAP
- Oxygen supplementation

- Wean for study drug
- Criteria for oxygen weaning
- Criteria for re-intubation
- Use of corticosteroids and bronchodilators

Duration

Subjects received iNO 5 ppm or placebo for a minimum of 7 days, and a maximum of 21 days in a blinded fashion. Subjects who required less than 7 days of assisted ventilation completed the minimum duration of therapy via face mask or nasal cannula. Subjects otherwise remained on study treatment as long as respiratory support was required, or until 21 days of therapy were completed.

Study Population

800 subjects (400 subjects per group) were planned; 800 were randomly assigned to drug (intent-to-treat), with 792 safety subjects. The 800 intent-to-treat (ITT) preterm infants were $24^{+0\ days}$ weeks' to $28^{+6\ Days}$ weeks' gestational age who required the use of surfactant or CPAP within 24 hours of birth.

Inclusion Criteria

For inclusion into the trial, subjects were required to fulfill the following criteria:

- Inborn, preterm infants $24^{+0\ days}$ weeks to $28^{+6\ days}$ weeks' GA (defined by first trimester ultrasound or, if not available, based on the last menstrual period) who required the use of surfactant within 24 hours of birth (either prophylactically, or for signs of developing respiratory distress), or who required the use of CPAP (fraction of inspired oxygen concentration [Fi02] $\geq$ 0.30 on a mean airway pressure $\geq$ 4 cm H20) within 24 hours of birth in order to maintain an oxygen saturation by pulse oximeter (Sp02) $\geq$ 85%
- Informed consent of the parent or legal guardian

Pertinent Exclusion Criteria

- Outborn infants
- Infants > 29 weeks' GA
- Infants with birth weight < 500 g
- Infants requiring Fi02> 0.5 to maintain Sp02 > 85%, on a sufficient mean airway pressure (e.g., > 8 cm H20 on controlled mechanical ventilation [CMV]) in order to achieve adequate chest inflation (8 to 9 ribs on chest x-ray) 2 hours after the proper administration of exogenous surfactant
- Any suspected congenital heart disease other than patent ductus arteriosus or atrial septal defect
- Any infant with suspected lung hypoplasia associated with congenital·diaphragmatic hernia
- Any infant with severe bleeding and/or coagulation abnormalities at high risk of diathesis (e.g., platelets < 50,000/mm_3 fibrinogen < 0.5 g/L, other clotting factors <10%)
- Any infant in whom a decision had been made not to provide full treatment, (e.g., chromosomal abnormalities, severe multiple abnormalities, severe birth asphyxia)
- Use of another investigational drug or device before or during the active study period

Withdrawal Criteria

Study drug was discontinued for the following reasons:

- If NO2 levels exceeded 3ppm on 2 samples 6 hours apart
- If methemoglobin levels exceeded 5% on 2 samples 6 hours apart

- If NO2 exceeded 5ppm on a single reading
- If methemoglobin levels exceeded 7.5% on a single reading
- If parent withdrew consent
- If Investigator deemed it in the best interest of the subject

Disallowed Therapies

Subjects who had received treatment with investigational medications were excluded from this trial. The use of pulmonary vasodilators (including nitrates, PDE-5 inhibitors, and prostacyclin analogues) was prohibited at any time during the trial.

The use of commercially available iNO for preterm infants outside this study protocol was "strongly discouraged", but not an expressed exclusion.

Concomitant Treatments

Concomitant medications were recorded on the case report form (CRF).

Study Treatments/ Blinding

All study drug cylinders were randomized prior to shipment to the investigative site. Personnel responsible for the set-up and monitoring of the delivery system had the ability to unblind the delivery system during treatment and were therefore asked to not be involved in patient care decisions. The "blinded" version of the INOvent® delivery system blanks out the NO and NO2 monitor displays, and the device was covered by an opaque faceplate and sealed with a plastic lock. If the decision was made to unblind a subject, the subject was removed from the trial but included in the intent-to-treat (ITT) analysis.

Efficacy Evaluations

Primary Endpoint

The primary efficacy variable for this study was a binary endpoint determined by the assessment at 36 weeks post-menstrual age (PMA). An infant who was alive without BPD at 36 weeks PMA was counted as a 'success'. An infant who had died, or who had BPD at 36 weeks PMA would be counted as a 'failure'. BPD is defined as the need for supplemental oxygen at 36 weeks post-menstrual age.

The assignation of BPD was based on a physiologic assessment published by Walsh and colleagues [Pediatrics 2004, 114 (5): 1305-1311], evaluated at 36 $^{+1}$ weeks' PMA. Infants on positive pressure support or requiring >30% supplemental oxygen with saturations at 90-96% were considered to have "BPD," and were not tested further. If the infant had oxygen requirement less than or equal to 30%, or if >30% but saturations were over 96%, infants were challenged on room air per protocol.

Reviewer's Comment:

"Need for supplemental oxygen at 36 weeks' PMA" was not explicitly defined in the body of the study protocol, and it was not clear if the specific protocol to test this was determined prospectively. The protocol did suggest that each center have a management plan for "O2 supplementation" and for "O2 weaning," but more detail regarding these definitions is not provided. The publication of these results by Mercier, et al, reports using the specific criteria for challenge set by Walsh and colleagues, as does the Statistical Analysis Plan.

The requirement for oxygen at 36 weeks postmenstrual age was used as a surrogate endpoint for the development of chronic lung disease or BPD. While the use of oxygen requirement as a surrogate endpoint for BPD has been commonly used in other clinical trials, its validity as such is debatable.

Secondary Endpoints

Secondary endpoints include:

- Number of days of assisted ventilation
- Length of hospitalization
- Survival without severe IVH and PVL (added to SAP in October 2006)
- Total number of days in-hospital from 36 weeks' PMA to 1 year and 2 years corrected age
- Average number of days in-hospital for respiratory illness from 36 weeks' PMA to 1 year and 2 years corrected age.

Safety Evaluations

The safety of iNO in premature infants was assessed by:

- Incidence of methemoglobinemia and elevated nitrogen dioxide concentrations
- Incidence and types of reported adverse events (AEs) including those related to intraventricular hemorrhage.
- Incidence of medically treated and/or surgically ligated ductus arteriosus
- Incidence of retinopathy of prematurity (ROP)
- Incidence of death after 36 weeks' GA to 1 year and 2 years corrected age, stratified by GA at birth
- Incidence of death after 36 weeks' PMA to 7 years corrected age, stratified by GA at birth
- Long-term neurodevelopmental outcome assessed by a validated, age appropriate developmental assessment at 2 and 7 years corrected age
- Pulmonary function testing at 7 years corrected age.

Schedule of Evaluations

The Schedule of Study Assessments is found in the table that follows.

Table 2: INOT27 Study Assessments

Assessment	Baseline	60 Minutes	24 Hours	Days 2-6	Day 7	Day 14	Day 21 or End of Ventilatory Support	36 Weeks' GA	1, 2, 7 Years
Window		± 15 minutes	± 2 hours		± 1 day	± 1 day	± 1 day	± 3 day	± 1 month
	Study Period							**Follow-up**	
Informed Consent	X								
Prenatal History & Demographics	X								
Physical Exam	X							X	X[a]
Vital Signs	X	X	X	X	X	X	X	X	X
Arterial O_2 Saturation by Pulse Oximetry	X	X	X	X	X	X	X	X	
Arterial PaO_2	X								
Chest X-ray	X[b]								
Head Ultrasound	X[c]				X		X[d]	X	
Mechanical Ventilation Support Settings [e]	**Throughout the Study Period**								
Assess Survival and Need for Supplemental Oxygen								X	
Study Drug Treatment	**Throughout the Study Period**								
Methemoglobin Level	X		X	X	X	X	X		
Concomitant Therapies		X	X	X	X	X	X		X[f]
Concomitant Medications		X	X	X	X	X	X		X[f]
Adverse Events		X	X	X	X	X	X		
Survival								X	X
Developmental Assessments									X[g]
Pulmonary Function Test									X[h]

[a] To include height and weight
[b] At least 4 hours after birth
[c] Within the first 72 hours of life, but preferably prior to initiation of study drug
[d] Between Day 21 and Day 28
[e] Including ventilatory mode, FiO_2, airway pressure (cm H_2O), tidal volume, respiratory rate
[f] Within 1 month of follow-up visit
[g] At 2 years and 7 years of age ONLY
[h] At 7 years of age ONLY

[INOT27 Clinical Study Report, section 9.1, Table 2]

Data Analysis

Data analysis was described in the Statistical Plan, as follows:
"The sample size for this study was determined using assumed rates based on a consensus of experts and are considered to be a realistic expectation. The sample size was chosen to provide at least 80% power of detecting a 20% improvement in the primary outcome of iNO compared to placebo. The rate of death due to prematurity in the intended population is estimated to be 20% across the allowable age range, and the rate of CLD is expected to be 20%. Using these estimates (80% survival and 80% without CLD), the percentage alive without CLD would be 80% x 80% or 64%. The minimum improvement in outcome to be detected is 20% or 76.8% survival without CLD. Expecting a 20% improvement in the iNO group (76.8% alive without CLD) gives a sample size of 214 (.05 alpha, 80% power). Given the uncertainty around these assumptions, we intend to randomize a maximum of 800 patients, and use a group sequential analysis approach, with one interim analysis at 400 patients, in order to limit enrollment once a threshold has been crossed."
The primary efficacy variable was analyzed by logistic regression with treatment group as the main effect and GA as the covariate. The secondary efficacy variables were evaluated using either analysis of variance (ANOVA) model or logistic regression adjusted by appropriate factors or covariates. Additional statistical methods included Kaplan-Meier method and log-rank test. Exploratory analyses were performed on both primary and secondary efficacy variables to evaluate the potential influence of selected baseline and demographic variables on the efficacy outcomes.
[INOT27 Statistical Analysis Plan, section 1.3]

Reviewer's Comment:
It is not clear from the review of the Clinical Study Report whether co-gestational siblings were included in the study and whether they were treated independently or dependently (i.e. same treatment assignment similar to Study BALLR1). Please refer to Dr. Buenconsejo's Statistical Review for further details.

Results

Study Population

Disposition
A total of 800 subjects were enrolled and randomly assigned to study treatment. Of the 800 subjects enrolled, 800 subjects were included in the ITT Population and 792 subjects were included in the Safety Population. Of the 792 subjects in the Safety Population, 676 (85.4%) subjects completed the study according to the protocol, 338 (85.6%) of those in the iNO group and 338 (85.1 %) of those in the placebo group (Table 3). A total of 116 of 792 (14.6%) subjects did not complete the study (withdrawn for any reason): 57 of 395 (14.4%) in the iNO group and 59 of 397 (14.9%) in the placebo group. A total of 33 (8.4%) iNO subjects and 31 (7.8%) placebo subjects did not complete the study due to death.

Table 3: INOT27 Subject Disposition

Status	iNO (N = 395) n(%)	Placebo (N =397) n(%)	Total (N =792) n(%)
Completed Study According to	338 (85.6%)	338 (85.1%)	676 (85.4%)

Status	iNO (N = 395) n(%)	Placebo (N =397) n(%)	Total (N =792) n(%)
Protocol			
Did Not Complete Study	57 (14.4%)	59 (14.9%)	116 (14.6%)
Delivery Device Failure	1 (0.3%)	3 (0.8%)	4 (0.5%)
Consent Withdrawn	2 (0.5%)	1 (0.3%)	3 (0.4%)
Request of Investigator	0(0.0%)	1 (0.3%)	1 (0.1 %)
Protocol Violation	1 (0.3%)-	2 (0.5%)	3 (0.4%)
Adverse Event	15 (3.8%)	12 (3.0%)	27 (3.4%)
Death	33 (8.4%)	31 (7.8%)	64(8.1%)
Other	5 (1.3%)	9 (2.3%)	14 (1.8%)

[INOT27 Clinical Study Report, section 10.1, Figure 1, and Table 3]

Protocol Violations

Eight subjects were noted to have violated inclusion/exclusion criteria: 6 subjects in the iNO group and 2 subjects in the placebo group. Other protocol deviations included missed assessments, assessments performed outside of the acceptable time windows, and late SAE reporting. These protocol deviations are not considered to have affected the interpretation of the study results; patients were included within the safety analysis.
[INOT27 Clinical Study Report, section 10.2]

Demographics

In general, the demographic and baseline characteristics for subjects and mothers were similar between the iNO and placebo treatment groups for the 800 subjects in the ITT Population. The treatment groups were comparable in GA, race, age of mother, Apgar scores at 5 and 10 minutes, length, head circumference, oxygenation index, and intubation/CPAP. The use of prenatal corticosteroids was quite high in subjects in both treatment groups; 89.5% of mothers of subjects of the iNO group and 90.3% of mothers of subjects in the placebo group. There were slightly more females (48.1% vs. 45.1%) and a slightly lower mean birth weight (851.5 grams vs. 864.1 grams) in subjects in the iNO group compared with subjects in the placebo group, respectively. Chorioamnionitis was more common in the mothers of subjects in the iNO treatment group compared with mothers of subjects in the placebo group (27.6% vs. 23.4%, respectively).

Table 4: INOT27 Racial Demographics

Variable	Parameter	iNO (N=395)	Placebo (N=397)	Total (N=792)
RACE				
	White	325 (82.3)	326 (82.1)	651 (82.2)
	Black	39 (9.9)	48 (12.1)	87 (11.0)
	Asian	4 (1.0)	2 (0.5)	6 (0.8)
	Other	27 (6.8)	21 (5.3)	48 (6.1)

[INOT27 Clinical Study Report, section 11.2, Tables 7 & 8]

Efficacy Results

Analysis of Primary Endpoint

In this study, treatment with iNO 5 ppm and less for 21 days was no better than placebo (p = 0.7340) with regard to survival without BPD in preterm infants with respiratory distress. There were 258 (65.3%) successes in the iNO group and 262 (65.5%) in the placebo group. The odds ratio of iNO compared with placebo was 1.05 (Table 5).

Table 5: INOT27 Primary Efficacy Analysis

Outcome	iNO (N =395) n(%)	·Placebo (N = 400) n (%)	Total (N = 795) n (%)	Odds Ratio	p value[b]
Success: Alive Without BPD	258 (65.3)	262 (65.5)	520 (65.4)	1.05	0.7340
Failure: Death or BPD	137 (34.7)	138 (34.5)	275 (34.6)		
Subjects with no efficacy Information were not included in the analysis. a iNO vs. placebo b Based on Wald Chi-square					

[INOT27 Clinical Study Report, section11.4.1.1, Tables 9-14]

For subjects with at least 21 days of treatment, a higher success rate was observed in the iNO group compared with placebo, but this did not reach significance; there were 98 (70.0%) successes in the iNO group and 90 (60.8%) in the placebo group (p =0.0839).

In addition, no treatment difference was observed for the primary outcome by BPD severity.

The primary efficacy variable was also analyzed in the ITT population by various strata of demographic characteristics using Wald Chi-square. In all stratifications (GA, race, birth weight, and type of ventilation) there were no statistical differences between infants treated with iNO compared to placebo.

Reviewer's Comment:

Outcomes varied greatly by country, despite the attempt to provide treatment paradigms in the protocol for common neonatal issues. For example, in Sweden, the success rate for subjects in the iNO group was 85.7% compared with 39.1% for subjects in the placebo group. However, in Spain the success rate for subjects in the iNO group was 50.0% compared with 61.3% for subjects in the placebo group. Variations in success rates were also observed from hospital to hospital.

Analysis of Secondary Endpoints

The Sponsor performed analyses on a number of secondary endpoints. In general, the secondary endpoints also failed to demonstrate significant treatment differences in favor of iNO compared to placebo. Outcomes for several secondary endpoints are shown in Table 8 below.

Table 6: INOT27 Descriptive Secondary Data

Outcome	Category	iNO (N = 399)	Placebo (N = 401)
Length of Assisted Ventilation (Days)	Mean (SD)	44.4 (26.1)	45.2 (28.7)
	Median	44.0	41.5
	Range	(2.0, 190.0)	(2.0,265.0)
Length of Hospitalization (Days)	Mean (SD)	94.2 (36.5)	93.0 (34.8)
	Median	88.0	86.0
	Range	(36.0,375.0)	(35.0, 366.0)
Alive without Brain Injury	n(%)	181 (69.3)	188 (75.5)

[INOT27 Clinical Study Report, section 11.4.1.2, Table 15, and sections 11.4.1.2.1, 11.4.1.2.2, 11.4.1.2.3]

Safety Results

Exposure

Of the 800 enrolled subjects, 792 subjects (395 in the iNO group and 397 in the placebo group) received at least 1 dose of study drug and had safety data available for analyses. The mean number of days of exposure to treatment was similar between treatment groups in all subjects and in only subjects who survived. The mean number of days of exposure for all subjects was 16.3 days for subjects in the iNO group and 16.4 days for subjects in the placebo group. In only subjects who survived, the mean number of days of exposure was 17.3 days for subjects in the iNO group and 17.4 days for subjects in the placebo group. No dose-response data was collected for INOT27.

[INOT27 Clinical Study Report, section 12.1, Table 16]

Overview of Adverse Events

Adverse Events

An overview of AEs is presented in the table below. A total of 380 of 395 (96.2%) and 365 of 397 (91.9%) subjects in the iNO and placebo groups, respectively, experienced at least 1 AE during the study. In both treatment groups, subjects aged 26 weeks or older GA at birth experienced slightly fewer AEs than subjects aged less than 26 weeks GA. A total of 243 of 255 (95.3%) and 234 of 264 (88.6%) subjects aged 26 weeks or older GA at birth in the iNO and the placebo groups, respectively, experienced an AE. In the iNO and placebo groups, subjects weighing 1000 or more grams at birth experienced fewer AEs compared with subjects weighing 500 to 749 grams and subjects weighing 750 to 999 grams at birth. A total of 38 (9.6%) subjects in the iNO group and 31 (7.8%) subjects in the placebo group experienced an AE that led to the modification of the dose of study drug. Study drug dose modification was considered to be: dose decreased, dose previously stopped, drug permanently stopped, and drug stopped temporarily.

Table 7: INOT27 Overview of AEs (safety)

	Category	iNO N=395 (n%)	Placebo N=397 (n%)
Any Adverse Event		380 (96.2)	365 (91.9)
Any Adverse Event by Gestational Age at Birth	< 26 weeks	137/140 (97.9)	131/133 (98.5)
	> 26 weeks	243/255 (95.3)	234/264 (88.6)
Any Adverse Event by Birth Weight Category	500 -749 g	133/138 (96.4)	126/130 (96.9)
	750 -999 g	166/171 (97.1)	153/167 (91.6)
	> 1000 g	81/86 (94.2)	86/1 00 (86.0)
Any Adverse Event When Action Taken was Dose Modified[a]		38 (9.6)	31 (7.8)
Any Adverse Event When Relation to Study Drug was Suspected[b]		133 (33.7)	138 (34.8)
Severe Adverse Events		134 (33.9)	128 (32.2)
Serious Adverse Events		158 (40.0)	164 (41.3)
Fatal Adverse Events		39 (9.9)	32 (8.1)

Subjects with multiple occurrences for an event are counted only once.
a= Dose modified includes 'Dose Decreased', 'Dose Previously Stopped', 'Permanently Stopped' and 'Stopped Temporarily.'
b= Suspected includes 'Remote (Unlikely)', 'Possible', 'Probable', and 'Highly Probable (Definitely).'

[INOT27 Clinical Study Report, section 12.2.1, Table 17]

Deaths

A total of 99 subject deaths were recorded during the study; 53 in the iNO group and 46 in the placebo group. A total of 95 of the 99 subject deaths occurred during and after treatment, are included in the clinical data base, and are presented in all summary tables and listings. The additional 4 subject deaths (4 subjects randomly assigned to the placebo group) were captured in the safety data base; these 4 subject deaths are not included in the clinical data base.

A summary of the 95 subject deaths (53 subjects in the iNO group and 42 subjects in the placebo group) that occurred during the study (during and after treatment) by treatment group and time period of occurrence is presented in the table below. The observed percentages of subjects in the Safety Population who died during treatment and after treatment were slightly higher in the iNO group compared with the placebo group. During-treatment deaths were 8.9% and 7.6% in the iNO and the placebo groups, respectively. After-treatment deaths were 4.6% and 2.8% in the iNO and placebo groups, respectively. The observed percentages of subjects who died by Week 36 PMA were higher in the iNO group compared with the placebo group: by Week 36 PMA, deaths in the Safety Population were 13.4% (53 of 395 subjects) and 10.6% (42 of 397 subjects), respectively.

Table 8: INOT27 Deaths by Grouping

Period	iNO n(%) N=395	Placebo n (%) N=397
By Week 36 PMA	53 (13.4)	42 (10.6)
During Treatment	35 (8.9)	30 (7.6)
After Treatment	18 (4.6)	11 (2.8) .
Missing Date of Death	0 (0.0)	1 (0.3)

[INOT27 Clinical Study Report, section 12.3.1.1, Table 20, 21]

A total of 35 subjects in the iNO group and 30 subjects in the placebo group died during the treatment period.

Reviewer's Comment: Throughout the safety population analyses, number of deaths was higher for the iNO-treated group (overall, during, and after treatment).

Nonfatal Serious Adverse Events

The percentage of subjects that experienced a nonfatal SAE was similar in the 2 treatment groups: 158 (40.0%) subjects in the iNO group and 164 (41.3%) subjects in the placebo group (see table below). The most frequently (more than 7%) reported SAE in both treatment groups was patent ductus arteriosus, followed by intracranial hemorrhage and sepsis. The table below presents SAEs that occurred in 3% or more of subjects in either treatment group.

Table 9: INOT27 SAEs Occurring in ≥ 3% of Safety Population

Preferred Term	iNO (N=395) n (%)	Placebo (N=397) n (%)
Subjects experiencing any SAE	158 (40)	164 (41.3)
Patent Ductus Arteriosus[a]	59 (14.9)	45 (11.3)
Intestinal Perforation	16 (4.1)	13 (3.3)
Sepsis[b]	32 (8.1)	31 (7.8)
Intracranial Hemorrhage[c]	49 (12.4)	41 (10.3)
Pneumothorax	12 (3)	13 (3.3)
Pulmonary Hemorrhage	12 (3)	14 (3.5)

a= PDA, PDA repair
b= sepsis, sepsis neonatal, septic shock, or Candidal, enterobacter, enterococcal, escherichia, serratia, or staphloccocal
c= cerebellar hemorrhage, cerebral hemorrhage, cerebral hemorrhage neonatal, hemorrhage intracranial, or intraventricular hemorrhage neonatal

[INOT27 Clinical Study Report, section 12.3.1.2, Table 22 and section 12.2.2, Table 18]

In addition to the overall SAEs seen during this trial, the Sponsor evaluated SAEs of interest separately. See below.

SAEs and AEs leading to Discontinuation/Dropout

Study drug was permanently stopped due to an AE for 46 subjects, 24 (6.1%) subjects in the iNO group and 22 (5.5%) subjects in the placebo group. A total of 28 subjects had study drug stopped temporarily due to an AE; 16 (4.1%) subjects in the iNO group and 12 (3.0%) subjects in the placebo group had study drug temporarily stopped. The most frequently reported AE leading to a subject permanently stopping study drug was intraventricular hemorrhage neonatal in the iNO group (5 subjects) and sepsis or septic shock in the placebo group (6 subjects). Two subjects in the iNO group and two subjects in the placebo group permanently stopped study drug due to non-serious AEs; the remainder of the subjects discontinued study drug due to serious AEs.
[INOT27 Clinical Study Report, section 12.3.1.4 and 12.3.3, Table 25]

Common Adverse Events

A total of 745 (94.1 %) subjects experienced an AE during the study, 380 of 395 (96.2%) and 365 of 397 (91.9%) subjects in the iNO and placebo groups, respectively. The most frequently reported AEs were similar in the 2 treatment groups. The 3 most frequently reported AEs in both groups were patent ductus arteriosus, 218 (55.2%) and 191 (48.1 %); anemia, 189 (47.8%) and 169 (42.6%); and hyperbilirubinemia, 122 (30.9%) and 121 (30.5%) in the iNO and placebo groups, respectively. Adverse events that occurred in 5% or more of subjects in either treatment group are shown in the table 10 below.

With regard to outcomes common in premature infants, a higher incidence of subjects in the iNO group (45 [11.4%]) experienced severe IVH compared with subjects in the placebo group (36 [9.1 %]). A total of 26 (6.6%) subjects in the iNO group and 27 (6.8%) subjects in the placebo group experienced IVH considered to be moderate in severity. A total of 43 (10.9%) subjects in the iNO group and 28 (7.1 %) subjects in the placebo group experienced IVH considered to be mild in severity. When the incidence of ductus arteriosus during the treatment period was examined, the incidence was higher in subjects in the iNO group: 218 (55.2%) subjects vs. 191 (48.1%) subjects in the placebo group.

The incidence of sepsis occurring at any time during the study was 24.6% in the iNO group compared with 22.4% of subjects in the placebo group. The percentage of subjects with the first occurrence of any sepsis earlier than or at 72 hours of life was slightly higher in the iNO group (26.1 %) compared with the placebo group (24.2%). There was no incidence of sepsis in subjects older than 72 hours of life. The incidence of necrotizing enterocolitis was 3.8% vs. 3% for iNO versus placebo, respectively. The incidences of retinopathy of prematurity were similar in the treatment groups: with 0.3% and 0.0% of subjects in the iNO and the placebo groups, respectively, reporting an incidence of retinopathy of prematurity at any time during the study.
[INOT27 Clinical Study Report, section 12.2.1, 12.2.2, 12.3.1.3, Tables 17, 18, 23]

Reviewer's Comment:
There is a numerical difference in the SAE during treatment of 8 patients (2.1% difference between groups) with iNO demonstrating more intracranial bleeding. However, this neonatal population inherently has a high rate of identified AE/SAE, there is no consistent pattern of SAE or AEs seen across studies INOT27, INOT25 and BALLR1, and none of these differences achieves the level of significance.

Table 10: INOT27 Common Adverse Events (5% or more)

System Organ Class	Adverse Event[a]	iNO (N =395) n(%)	Placebo (N =397) n(%)
Congenital, Familial and Genetic Disorders	Patent Ductus Arteriosus[b]	218 (55.2)	191 (48.1)
Blood and Lymphatic System Disorders	Anemia	189 (47.8)	169 (42.6)
Hepatobiliary Disorders	Hyperbilirubinaemia	122 (30.9)	121 (30.5)
Nervous System Disorders	Hemorrhage Intracranial[c]	114 (28.9)	91 (22.9)
Hepatobiliary Disorders	Jaundice	98 (24.8)	90 (22.7)
Infections and Infestations	Sepsis[d]	97 (24.6)	89 (22.4)
Metabolism and Nutrition Disorders	Hyperglycemia	90 (22.8)	69(17.4)
Respiratory, Thoracic, and Mediastinal Disorders	Apnea	73 (18.5)	78 (19.6)
Vascular Disorders	Hypotension	58 (14.7)	51 (12.8)
Metabolism and Nutrition Disorders	Metabolic Acidosis	49 (12.4)	52 (13.1)
Blood and Lymphatic System Disorders	Thrombocytopenia	30 (7.6)	29 (7.3)
Metabolism and Nutrition· Disorders	Hyponatremia	21 (5.3)	20 (5.0)
Respiratory, Thoracic, and Mediastinal Disorders	Pulmonary Hemorrhage	19 (4.8)	23 (5.8)

a Subjects with multiple occurrences of an AE are counted only once: Adverse events are organized by highest percentage of occurrence in the iNO group. b Patent ductus arteriosus, patent ductus arteriosus repair C Cerebellar hemorrhage, cerebral hemorrhage, cerebral hemorrhage neonatal, hemorrhage intracranial, intraventricular hemorrhage neonatal d Candida sepsis, enterobacter sepsis, enterococcal sepsis, Escherichia sepsis, sepsis, sepsis neonatal, septic shock, . serratia sepsis, staphylococcal sepsis

[INOT27 Clinical Study Report, section 12.2.2, Table 18]

Reviewer's Comment:
The AEs listed are expected events for a percentage of premature infants; no unexpected or novel AEs were identified.

Laboratory Findings

Methemoglobin and NO_2 levels were measured at baseline, 24 hours, Days 2 through 6, Day 7, Day 14, and Day 21 (or at the end of ventilatory support). Methemoglobinemia was defined in this study as a subject with a MetHb level exceeding 5% on 2 samples 6 hours apart or 7.5% on a single reading. There were no incidences of methemoglobinemia of 5% or greater on 2 samples or 7.5% or greater on a single reading observed during treatment. Study drug was discontinued if NO_2 levels exceeded 3 ppm on 2 readings 6 hours apart or 5 ppm on a single reading. One subject (0.3%) in the iNO group and 1 subject (0.3%) in the placebo group (0.3%) experienced elevated NO_2 levels. [INOT27 Clinical Study Report, section 12.4]

Vital Signs

There were no apparent differences in vital signs (HR, BP, etc) between iNO and placebo groups.

Summary and Conclusions

The 2 treatment groups were well matched at baseline and the extent of exposure was comparable between the treatment groups. Overall, INOT27 failed to meet its primary efficacy endpoint of survival without BPD (as measured by use of supplemental oxygen at 36 weeks PMA) in premature infants, with no statistical difference (P=0.734) between iNO treatment and placebo groups.

Adverse events were very common in both groups of these critically ill premature infants. Overall, there were more AEs in the iNO-treated group than in the placebo group (96.2% vs. 91.9%). This difference was most noticeable in the cohort of subjects 26 weeks or older GA, with an AE incidence of 95.3% of subjects in the iNO group compared with 88.6% of subjects in the placebo group.
The 3 most frequently reported AEs in both treatment groups were patent ductus arteriosus, anemia, and hyperbilirubinemia. There were no statistically-significant differences observed in the incidence of AEs between the treatment groups, including the incidence of IVH. Adverse events with a higher incidence rate in the iNO group compared with the placebo group (greater than 5%) included patent ductus arteriosus, intracranial hemorrhage, anemia, and hyperglycemia. The relevance of this difference is uncertain in this population of premature infants in which these types of AEs are common. The incidences of all other AEs were similar between the treatment groups. There were no incidences of methemoglobinemia reported during the treatment period. Two subjects, 1 in each treatment group, experienced elevated NO_2 levels. Serious adverse events reported by greater than or equal to 3% of subjects in either treatment group were patent ductus arteriosus, intestinal perforation, sepsis, intracranial hemorrhage, pneumothorax, and pulmonary hemorrhage. The incidences of these and all other SAEs were similar between the treatment groups. As noted earlier, perinatal care was very good, and the overall death rate was relatively low in both groups, compared to recent studies in a similar population. The number of withdrawals from treatment in each group were similar. The overall death rate in the Safety Population throughout the study was 53 to 46 in the iNO and placebo groups, respectively, for all treated subjects. The AEs of subjects who died were similar in both groups, with IVH or IVH-related causes being the most frequently reported cause of death in both treatment groups. Although there were slightly more IVH reported at baseline in iNO-treated infants, this difference does not account for the slight increase in fatal IVH seen in the active treatment group. No significant safety issues were raised in this study regarding the administration of iNO at a dose of 5 ppm within the first 24 hours after birth to preterm infants. However, given the absence of benefit of this treatment as studied, any imbalance in the rate of AEs must be assessed closely.

3. Review of Efficacy Study BALLR1

Title

Low dose inhaled nitric oxide (iNO) for prevention and treatment of chronic lung disease (CLD) in the preterm infant. [The NO CLD Study]

Protocol

Administrative

Study Initiated: 04 April 2000
Study Completed: 05 July 2005
Clinical Centers: Multicenter; NIH-funded trial with 16 lead investigators, performed at 21 study sites throughout the USA.
Study Report Dated: 03 June 2009

BALLR1 was conducted by NIH/NHLBI-funded investigators, and the Sponsor received the data sets after unblinding of data had occurred, and after publication of the results by the original investigators (BALLR1 and INOT25). Of note, statistical Analysis Plans by the Sponsor were written after data had been published.

The original protocol was issued in 2000. The protocol was amended once on 2 June 2004; this amendment included the sample size reduction from 726 subjects to 585 subjects.

Objectives

The primary objective for BALLR1 was to assess the beneficial effect of iNO on the occurrence and severity of chronic lung disease (CLD) in infants of 500 to 1,250 g birth weight who continued to require mechanical ventilation or nasal CPAP between 7 and 21 days of age.

The secondary objectives were: to assess airway resistance, length of ventilation and hospitalization, and duration of oxygen requirement; and to examine the safety of iNO in preterm infants at risk of CLD. Specifically, occurrence of intraventricular hemorrhage (IVH), pulmonary hemorrhage, or other clinically important bleeding; evidence of toxicity as indicated by the occurrence and severity of other common morbidities of the preterm infant, including necrotizing enterocolitis (NEC), PDA retinopathy of prematurity, and infection; plasma markers of oxidant stress (protein 3-nitrotyrosine [3-NT] and carbonyls); markers of inflammation (interleukin [IL]-8 and IL-IB, N-acetylglucosaminidase and hyaluronan), fibrosis (transforming growth factor *B,TGFB)* and oxidant injury (8-epi-prostaglandin F2a) in tracheal aspirate (TA) fluid; and neurodevelopmental outcome through at least 2 years of age.

Reviewer's Comment:
Note that the definition of BPD as evidenced by oxygen requirement at 36 weeks' PCA is not specifically stated, nor is the protocol for weaning oxygen to meet the criterion clearly delineated. The publication of these results by Ballard, et al, reports using the specific criteria for challenge set by Walsh and colleagues.

Study Design

The study was designed as a multicenter, randomized, placebo-controlled, and blinded trial of iNO therapy (20 ppm and less) in preterm infants at high risk for developing BPD at 36 weeks' PMA. Included were subjects aged 7 to 21 days with a PMA ≤ 32 weeks and birth weight 500 to 1,250 g. Subjects were randomized to receive wither nitric oxide (iNO) or placebo (nitrogen [N2] gas), administered for a minimum of 24 days. All infants received blinded drug and were stratified by 2 levels of birth weight: 500 to 799 g and 800 to 1,250 g. Co-gestational siblings received the same treatment assignment as the first eligible infant randomized, even if enrolled on different dates.

All sites collected clinical data on pulmonary course and outcome. The incidence of other common morbidities of the preterm infant, including infection, intracranial hemorrhage, NEC, and ROP was also accessed. Selected sites evaluated exploratory endpoints, such as plasma markers of antioxidant stress, NO response, markers of inflammation, markers of oxidant injury, and airway resistance. The results of these ancillary investigations have been published and were not presented in this study report. The study protocol involved follow-up evaluation of long-term neurodevelopmental outcome through the age of 2 years.

Reviewer's Comment:
The study report only includes data through the first hospital discharge following birth, allowing for assessment of the primary endpoint).

Duration

Subjects were treated with study drug or placebo for a minimum of 24 days. Study treatment with iNO was initially administered at 20 ppm for 48 to 96 hours, and subsequently decreased weekly to 10 ppm, 5 ppm, 2 ppm, and 0 ppm.

Study Population

There were 585 planned subjects, with 587 enrolled and 5 withdrawn. The intent-to-treat (ITT) population included 587 infants, and 584 subjects comprised the safety population, being preterm infants of 500 to 1,250 g birth weight who continued to require mechanical ventilation or nasal CPAP (nCPAP) between 7 and 21 days of age.

Reviewer's Comment:
This is a different neonatal study population than for INOT 27. These infants are stratified by weight (not GA), and are 7 to 21 days old at study start, who presumably have received surfactant at birth, and are still requiring ventilatory support despite standard-of-care management. Although the weight and GA is the same, those infants in INOT25 were randomized at less than 48 hours of life. The age of the Ballard neonates may account for the potential difference in results from the two other trials, as could the total higher iNO dose used for this trial over that in INOT25 and INOT27.

Inclusion Criteria
For inclusion into the trial, subjects were required to fulfill all of the following criteria:

- 500 to 1,250 g at birth
- ≤ 32 weeks gestation
- 500 to 799 g on ventilator or nCPAP or nasal ventilation

- ≥ 800 to 1,250 g on ventilator with severity score ≥ 2.0 (fraction of inspired oxygen [$Fi0_2$] x mean airway pressure [MAP])
- Aged 7 to 21 days at the time of study entry

Pertinent Exclusion Criteria

Any of the following was regarded as a criterion for exclusion from the trial:

1. Infants with anomalies (e.g., cardiac, thoracic, lethal chromosomal, human immunodeficiency virus [HIV] exposure).
2. Bilateral Grade 4 IVH
3. Receipt of prior inhaled NO therapy
4. Unstable condition - defined as the presence of any of the following:
    - NEC, steroids started for BPD, or methemoglobin level >3% within 72 hours prior to randomization
    - Sepsis with positive blood culture, hypotension unresponsive to dopamine > 20 mcg/kg/min, or thrombocytopenia (< 50,000 platelets) or clinical evidence of bleeding within 24 hours prior to randomization
5. Unstable clinical respiratory condition:
    - Ventilator mode change (e.g., controlled mechanical ventilation [CMV] to high-frequency ventilation [HFV] within the previous 24 hours prior to randomization; or
    - Sustained increase in FiO_2.≥ 0.50 or MAP ≥ 5 cm H_2O within the previous 6 hours prior to randomization.

Study Interruption/ Withdrawal Criteria

A subject's participation in the study was interrupted or discontinued if any of the following applied:

- Methemoglobin levels increased to ≥ 5%
- Significant respiratory decompensation upon initiation of study drug defined as: increased FiO_2 > 0.3 or increase in MAP ≥ 3 cm H_2O above baseline at study entry in an infant without other cause of deterioration. Once the infant stabilized, the study drug could be restarted if the infant was still aged 21 days or less.
- Platelet count of< 25,000 with no response to transfusions and/or clinically significant bleeding
- Hypotension unresponsive to volume or vasopressors, and hydrocortisone
- Investigator or subject's family considered discontinuation of the study to be in the subject's best interest

All discontinuations were documented on the Discontinuation of Treatment Gas Form.

Reviewer's Comment:

By disallowing severe intracranial hemorrhage, and by being 7-21 days old when randomized to the trial (the period after which IVH typically occurs), this trial has selected out for adverse intracranial events, and proves to have lower SAE/AE rates than seen in INOT27 or INOT25.

Disallowed Therapies and "rescue iNO"

Receipt of iNO therapy prior to enrollment was disallowed.

However, infants were eligible to receive "rescue" open-label iNO therapy if they met all of the following criteria:

- Aged ≥ 28 days
- 0.70 FiO_2
- 12 cm H_2O MAP

The decision to administer iNO in this open-label fashion must have been approved by the site Principal Investigator at each study site and reported to the OC within 72 hours. Study drug for open-label use was provided by INO Therapeutics LLC. Study INOvent® (masked system) and study drug tanks were not to be used to administer open-label, nitric oxide. The site was to use a hospital owned INOvent® for the administration of the open-label NO. If an infant remained on open-label iNO, the masked system was to be removed from the bedside. The masked system was to remain blinded. Sites were instructed to substitute study drug with an unblinded INOvent® and open-label NO, and begin treatment with 20 ppm. If the infant had a decrease in FiO_2 or remained unchanged for 72 hours, study drug administration was continued. At 72 hours, the infant was then weaned to 10 ppm and study personnel were to follow the weaning protocol for study drug. Data collection and AE reporting for infants treated with open-label NO was to continue per the study protocol.

Reviewer's Comment:
The use of "rescue" iNO confounds the outcomes somewhat, but would not skew the data in favor of the treatment; if placebo subjects received rescue, this would improve placebo outcomes and make the treatment effect more difficult to achieve.

Concomitant Treatments
Concomitant medications were recorded on the case report form (CRF).

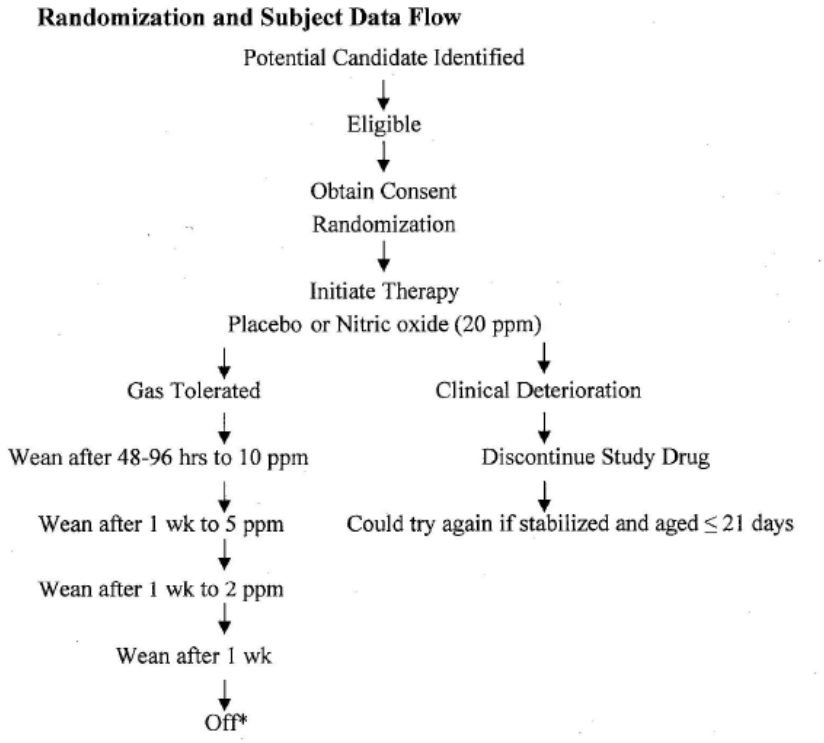
Infants were managed according to clinical management guidelines which addressed surfactant administration, administration of Vitamin A, general ventilatory parameters including partial pressure of carbon dioxide (PCO_2) targets, treatment of the patent ductus arteriosus, criteria for transfusion, and criteria for administration of postnatal steroids for BPD.

Study Treatments/ Blinding
The study drug was administered using the blinded version of the INOvent® continuous flow delivery system, using modified blinded software and a blinding shield, which provided for masked delivery of the study treatment. Personnel involved in the general care of the subject remained masked to treatment allocation during the course of treatment and until completion of the study. The study treatment remained blinded to parents, investigators, and staff involved in subject follow-up assessments pending completion of all follow-up procedures. Personnel who were trained to do routine maintenance and un-blind the INOvent® system using the security code were not involved in the general care of the specific infant beyond delivery of the study drug.
Random block size was employed to help maintain blinding of investigators. In the event that the treatment assignment of a specific subject became known, it would be impossible for an investigator to guess the next treatment assignment because of the random block size.

Schematic

The following schematic describes the randomization and subsequent weaning process used for infants in this trial. The protocol contains a controlled wean over the treatment period, and there is also a weaning protocol for infants who clinically deteriorate, which necessitated withdrawal from the trial.

Figure 1: Schematic of BALLR01



[BALLR1 Clinical Study Report, section 9.1, Figure1]

Efficacy Evaluations

Primary Endpoint

The primary efficacy variable for this study was survival without BPD at 36 weeks' PMA. BPD was defined by the physiologic definition by Walsh [Pediatrics 2004, 114 (5): 1305-1311]. An infant who was alive without BPD at 36 weeks' PMA was counted as "success." Any infant who died, or had BPD at 36 weeks' PMA was counted as "failure."

Reviewer's Comment:

This study report was prepared in 2009, although the original clinical study took place April 2000 through May 2005. While the study report notes using the criteria by Walsh, the Walsh article was not published until 2004, and previous report with pilot data was published in 2003. So presumably, some of these infants had oxygen challenge performed without the benefit of the Walsh "physiologic definition." However, no other information is provided in this study report submitted by the Sponsor.

Secondary Endpoints

The secondary objectives were to assess the following:

- Changes in airway resistance
- length of ventilation
- length of hospitalization
- duration of oxygen requirement

Reviewer's Comment:

Although listed as a secondary efficacy endpoint, the Sponsor does not include any of the data regarding airway resistance in this study report. Rather, they comment that this was evaluated in a publication. Please see "Analysis" section below for more information.

Safety Evaluations

The safety of iNO in premature infants was assessed by:

- Incidence of Adverse Events, including IVH, pulmonary hemorrhage, other bleeding
- Toxicity as indicated by occurrence of other morbidities including PDA, NEC, PVL, ROP and infections
- Incidence of elevated methemoglobin

Schedule of Evaluations

Study assessments were performed at frequent time points throughout the study period (baseline, daily for the first 28 days, weekly, at 36-wk PMA, at D/C), and also at 12 and 24 months for the longer neurodevelopmental and pulmonary outcomes.
Assessments at baseline include maternal history, delivery history, crib score, surfactant use, and Vitamin A administration. Ongoing assessments through the study period include daily ventilator parameters, FiO2, O2 saturation, mean BP, vasopressor use, medication use, post-natal steroid administration, I/O's, nutritional evaluations, drug flow (ppm), hearing screens, and BPD outcome at 36-weeks PMA. Other periodic evaluations include need for transfusions, volume administration, development of PDA, ROP, sepsis, as well as status of neurologic, GI, renal, hematologic and growth systems. Adverse events, protocol violations, and deaths were tracked throughout.

Data Analysis

Two interim analyses were performed based on an O'Brien-Fleming stopping rule. The exact boundaries and nominal p values for stopping were based on applying the Lan-DeMets use function. Because these boundaries were both for rejection and acceptance of the null hypothesis, the final analyses, considering that the trial was not stopped at either interim analysis, they used a $p = 0.05$ level of significance.

Following publication of the trial results by the investigators, the electronic database and electronic images of individual CRFs were retrieved by the commercial sponsor, INO Therapeutics LLC. Tabulations and analyses for this report were performed by INO Therapeutics LLC from the data provided by the data coordinating center (DCC), and in accordance with the statistical analysis plan, which was created by INO Therapeutics LLC after publication (SAP dated 2008). All data analysis and processing, as well as all tabulation of descriptive statistics, were performed at INO Therapeutics LLC primarily using SAS software (version 8.2 and 9.1.3) for Windows.

INO Therapeutics LLC performed data audits of both the clinical study report and the SAE narratives. Fully populated SAS data sets, described as "cleaned" were received from the Principal Investigator. INO Therapeutics Data Management department applied in-house designed edit checks and generated data queries. Queries were submitted to the representative of the Principal Investigator and/or the individual sites for resolution. Responses, as well as "notes to file", were considered in the cleaning process and the data were appropriately modified and documented. New SAS data sets were then created from the re-cleaned data based on the Study Data tabulation model (SDTM) standards. These new SAS data sets were used to create all the tables, listings, and figures for this report.

The primary statistical analysis was planned as an ITT approach using all randomized infants regardless of the amount of treatment received. A second analysis was planned based on the general estimating equation (GEE) with clustered twins and triplets from among all infants enrolled. An efficacy analysis using the safety population was originally planned in the protocol to ensure consistency and robustness of the results; an efficacy analysis using all subjects enrolled was performed. The safety population was defined as all subjects who received study drug treatment and also had safety information available after the beginning of treatment categorized by actual treatment received. Safety analyses were planned using the safety population.
[BALLR1 Clinical Study Report, section 9.7.1. 9.7.1.1]

Reviewer's Comments:
The statistical analyses should have adjusted the alpha, based on the interim evaluations.

There are questions regarding whether or not the initial statistical analysis (Multiple Outputation Technique) performed by the Investigator, which were all performed post-hoc, was correct, and then whether subsequent "cleaning" and analysis of data by the Sponsor is appropriate and whether and how those issues would effect the marginally positive results for the primary efficacy endpoint. The number and scope of concerning issues regarding the interpretation and handling of data and analyses which include data quality, application of several different statistical tests, and lack of adjustments to the level of significance based on 2 interim analyses are significant issues that may affect an efficacy determination.

The published paper handled the analysis of multiple gestation data in a different manner than the above described post-hoc method of analysis performed by the Sponsor. In addition, the method to account for siblings detailed in the SAP submitted by the Sponsor to the FDA in 2008 was not what was ultimately utilized in this submission. As a result, the level of significance of the primary efficacy endpoint varies with each analysis (but most retain significance).
For further details, please see Dr. Buenconsejo's statistical review.

Conduct

This study was an investigator initiated application to NHLBI conducted as a cooperative effort by the National Heart, Lung, and Blood Institute (NHLBI) extramural network. The study was designed and initiated via a research grant from NHLBI. The Principal Investigator and the study Steering Committee designed the protocol, data collection forms, the study manual of procedures, and conducted site monitoring, serious adverse event (SAE) reporting, and other regulatory activities during the conduct of the trial. Randomization of study drug was performed centrally for all participating institutions by the Data Coordinating Center (DCC) at the Division of Biostatistics at The Children's Hospital of Philadelphia. INO Therapeutics LLC provided study supplies and blinded INOvent® delivery devices to each institution. Following completion of the study and data analysis, the results were published in *The New England Journal of Medicine,* July 27, 2006, Volume 355, pages 343-353 (a correction was published on October 4,2007, Volume 357, page 14). Following publication, INO Therapeutics LLC, received permission from the Steering Committee and NHLBI to use all data and related documents for the preparation of this report.

The protocol was amended once on 2 June 2004; this amendment included the sample size reduction from 726 subjects to 585 subjects. Due to concern regarding the rate of enrollment and study completion, the Principal Investigator and primary study statistician initiated sample size discussions with the DSMB. Although these discussions occurred at the time of the second

interim analyses, the decision to reduce the sample size was based on the actual rates of data accrual and multiple gestation infants enrolled; outcome data were not considered in the determination of the reduced sample size. Three reasons are documented as the rationale for sample size reduction: an actual lower rate (7%) of multiple births than the estimated 17% rate; improvement of statistical software allowing for sample size calculations based on spacing of two interim looks at 20% and 50% rather than 33.3% and 66.7%; and a request to decrease the power to 80% from the 85% power suggested by the DSMB prior to opening the majority of the sites (DSMB minutes March 2004). Co-gestational siblings (infants of a multiple birth) which were assigned treatment as a group instead of randomized as individuals, did not conform with the ICH definition of randomized. Therefore, only the randomized sibling was considered part of the ITT population in the evaluations contained in this report, i.e. the ITT population is the group of individually randomized subjects, including the first enrolled of the co-gestational siblings. However, certain evaluations were performed on the entire group of subjects including all co-gestational siblings; this group is referred to as "all enrolled."
[BALLR1 Clinical Study Report, section 9.8.1, 9.8.2]

Results

Study Population

Disposition

A total of 587 subjects were enrolled and randomized to treatment. The first subject was enrolled 04 April 2000 and the last subject completed study treatment on 05 July 2005. Of the 587 subjects enrolled, 548 (93.4%) subjects completed the study according to the protocol: 278 (93.9%) of those in the iNO group and 270 (92.8%) of those in the placebo group. A total of 39 (6.6%) of 587 subjects discontinued study treatment while receiving treatment or within 72 hours of completing treatment: 18 (6.1 %) of 296 in the iNO group and 21 (7.2%) of 291 in the placebo group. Sixteen (5.4%) of 296 iNO subjects and 18 (6.2%) of 291 placebo subjects died while receiving study drug or within 72 hours after treatment with study drug. Forty-five subjects, the co-gestational siblings, were not included in the ITT population, since they did not meet the definition of “randomized.” They were included in the safety population.

Table 11: BALLR1 Subject Disposition

All Subjects enrolled	**Inhaled NO (N =296) n(%)**	**Placebo (N =291) n (%)**	**Total (N =587) n(%)**
Completed study according to protocol	278 (93.9)	270 (92.8)	548 (93.4)
Did not complete study	18 (6.1)	21 (7.2)	39 (6.6)
Death	16 (5.4)	18(6.2)	34 (5.8)
Consent Withdrawn	1 (0.3)	2 (0.7)	3 (0.5)
Delivery Device Failure	0(0.0)	1 (0.3)	1 (0.2)
Other	1 (0.3)	0(0.0)	1 (0.2)

[BALLR1 Clinical Study Report, section 10.1, Figure 1, Table 3]

Protocol Violations

Four subjects were noted to have violated inclusion/exclusion criteria: 3 subjects in the iNO group and 1 subject in the placebo group. Four subjects were randomized to one study drug, but initially received the opposite treatment, known only to the DRT, thereby receiving both treatments: 3 subjects in the iNO group and 1 subject in the placebo group. These subjects were included in the randomized groups for analyses. Other deviations from the study guidelines included inhaled steroids given, vitamin A not given per protocol as part of hospital policy, severity score too low, dosing schedule not followed as per protocol, and study drug dosage error.
[BALLR1 Clinical Study Report, section 10.2]

Demographics

In general, the baseline characteristics for subjects and mothers were similar between the inhaled NO and placebo treatment groups for the 542 subjects in the ITT Population. The treatment groups were comparable in PMA, sex, age, and race of mother, birth weight, head circumference, delivery method, RSS, Apgar scores at 1, 5, and 10 minutes, and baseline ventilation. With the exception of triplets, the percentages of multiple births were similar between the 2 treatment groups in the ITT Population; the incidence of triplets was higher in the iNO group than in the placebo group, 8 (3%) vs. 3 (1 %).

Table 12: BALLR1 Maternal Race Demographics (safety population)

Variable	Parameter	iNO (N=295)	Placebo (N=289)	Total (N=584)
RACE				
	White	161 (55)	142 (49)	303 (52)
	Black	74 (25)	90 (31)	164 (28)
	Other	47 (16)	52 (18)	99 (17)
	Unknown	13 (4)	5 (2)	18 (3)

The ratio of white mothers to black mothers of ITT randomized infants was slightly higher in the iNO group (2.1 %:1 %) than in the placebo group (1.5%:1 %).
[BALLR1 Clinical Study Report, section 11.2.1, Table 7, 8, 9, 10]

Efficacy Results

Analysis of Primary Endpoint

All primary efficacy analyses were evaluated at a level of significance of 0.05. Subjects who received drug but efficacy data at end point were missing were not included in any efficacy analyses.

Table 13: BALLR1 Primary Efficacy Endpoint

Outcome	iNO N=269 n(%)	Placebo N=268 n(%)	Total N=537 n(%)	Odds ratio	p Value
Success: Alive no BPD	121 (45.0)	95 (35.4)	216 (40.2)	1.489	0.028
Failure: Death or BPD	148 (55.0)	173 (64.6)	321 (59.8)		

[BALLR1 Clinical Study Report, section 11.4.1.1., Table 12]

Based on the Sponsor's statistical analysis for this study, treatment with iNO 20 ppm and less for 24 days significantly (p =0.028) increased survival without BPD at 36 weeks' PMA in preterm neonates with respiratory failure. There were 121 (45.0%) successes in the iNO group and 95 (35.4%) in the placebo group. The odds ratio of iNO to placebo was 1.489 (see table 13 above). [BALLR1 Clinical Study Report, section 11.4.1.1, Tables 12-20]

Reviewer's Comment:
The originally published paper had a primary efficacy p value of 0.043, which while still significant, is different from the 0.028 reported by the Sponsor. It is not clear from looking at the tables if this is purely due to the correction for multiple gestation infants, or due to the re-"cleaning" of the data that were performed by the Sponsor as noted in the "Data Quality" section above. Dr. Buenconsejo's biostatistical review addresses these concerns in detail, and raises additional questions regarding the statistical analysis of this data.

*The original Ballard data was published in the New England Journal of Medicine in 2006, and an erroratum was published in 2007, with a change in p value. The erroratum states, "*we reanalyzed our data by means of the multiple outputation approach used in the original analysis and obtained a P value of 0.03 (relative benefit of nitric oxide in the overall population, 1.26; 95% confidence interval [CI], 1.02 to 1.55) rather than a P value of 0.04 (relative benefit, 1.23; 95% CI, 1.01 to 1.51), as reported in our article (in the Abstract, Table 2, and the third paragraph of the Results section). Analysis by means of the generalized- estimating-equations method,2 which is also used for analysis of clustered data, gives a P value of 0.03 (relative benefit, 1.45; 95% CI, 1.03 to 2.04)."

As stated previously, while there is evidence of a treatment effect, the multiple analyses and re-adjudication of the data make it difficult to definitively state the statistical significance of such an effect.

Analysis of Secondary Endpoints
The Sponsor notes that secondary efficacy variables of time-to-end of ventilatory support, time-to-end of supplemental oxygen, and time-to-end of hospitalization were shorter for those infants receiving iNO as compared to those receiving placebo (see table 14 below).

Across all 3 time-to-event variables (ventilatory support, supplemental oxygen, and hospitalization), the median duration of care was approximately 1 week shorter for iNO-treated subjects compared with placebo-treated subjects: 10.9, 10.7, and 12.0 weeks for subjects treated with iNO, respectively vs. 12.1, 11.9, and 12.9 weeks for subjects treated with placebo, respectively.

Table 14: BALLR1Secondary Efficacy Endpoints

Outcome	Category	iNO (N = 271)	Placebo (N = 271)
Time of known survival (weeks)	Mean (SD)	13.0 (7.8)	14.0 (7.7)
	Median	12.0	13.0
	Range	(0.6, 106)	(0.0, 72.9)
Time on Mechanical Ventilation (weeks)	Mean (SD)	11.9 (8.0)	13.1 (7.8)
	Median	10.9	12.1
	Range	(0.6, 106)	(0.0, 72.9)
Time to End of Hospitalization (weeks)	Mean (SD)	12.7 (7.6)	13.4 (6.1)
	Median	12.0	12.9
	Range	(0.6, 106)	(0.0, 37.7)
Time to end of Oxygenation (weeks)	Mean (SD)	11.6 (8.1)	12.6 (7.1)
	Median	10.7	11.9
	Range	(0.6, 106)	(0.59.7)

[BALLR1 Clinical Study Report, section 11.4.1.2, Table 21, sections 11.4.1.1.1, 11.4.1.1.2, 11.4.1.1.3]

Reviewer's Comment:
*The originally stated secondary efficacy aim was as follows: "To assess airway resistance, length of ventilation and hospitalization, and decreased duration of oxygen requirement." The Sponsor does not address the airway resistance issue, other than to say it was evaluated, and is part of another publication. There is no data provided anywhere in this submission to assess whether this fourth point was met in terms of efficacy. Literature review identified the publication; (*Journal of Perinatology 2007; 27: 766–771*). A subset of 71 infants less than 32 weeks gestational age, at 7-21 days of life (34 received iNO, 37 received placebo) was assessed, with the following results:*

> *"At baseline expiratory resistance (231±71 versus 215±76 cm $H2O^{-1}$ s^{-1}) and compliance (0.49±0.14 versus 0.53±0.13 ml cm $H2O^{-1}$ kg^{-1}) were comparable between placebo and NO groups, respectively. There was no effect of NO on expiratory resistance or compliance at 1 h, 1 week or 2 weeks of study gas administration."*

With regard to the other three secondary efficacy variables, numeric differences between groups were noted to favor iNO, but there is considerable overlap in both groups, looking at both the standard deviation and the range.

Other Endpoints
The outcome stratified by various demographic characteristics showed the iNO treatment being favored over placebo, although not significantly (with the exception of race of mother equal to "black"). The subgroup analyses censoring at date of event showed positive results in PMA, with the ≥ 26 weeks group experiencing significantly shorter duration of mechanical ventilation than the < 26 weeks group (p = 0.0011, 57.6% more likely to be removed from support sooner). Similarly, non-whites were 61.4% more likely to be removed from ventilatory support sooner than whites (p =0.0001). The results of the subgroup analyses censoring at date of event showed significant differences in PMA (≥ 26 weeks vs. < 26 weeks, p =0.0033) and race (non-

white vs. white, p =0.0021). The subgroup analyses censoring at date of event showed positive results in PMA with the ≥ 26 weeks group being different from < 26 weeks (p = 0.0002, 47.8% more likely to be discharged sooner).

Table 15: BALLR1 Primary Outcome by Race

Outcome	iNO n (%)	Placebo n (%)	Odds Ratio	p value	
				Treatment	Breslow
Race of Mother White					
	(N = 148)	**(N = 131)**			
Success: Alive without BPD	52 (35.1)	44 (33.6)	1.071	0.8019	0.0892
Failure: Death or BPD	96 (64.9)	87 (66.4)			
Race of Mother Black					
	(N = 71)	**(N = 86)**			
Success: Alive without BPD	40 (56.3)	28 (32.6)	2.673	0.0036	
Failure: Death or BPD	31 (43.7)	58 (67.4)			
Race of Mother Other					
	(N = 46)	**(N = 50)**			
Success: Alive without BPD	26 (56.5)	23 (46.0)	1.526	0.3161	
Failure: Death or BPD	20 (43.5)	27 (54.0)			

Odds ratio is ratio of iNO over placebo; p value is based on Fisher's exact test.
Source: Section 14.2.1, Table 10E

[BALLR1 Clinical Study Report, section 11.4.1, Table 19, and section 11.4.7]

Reviewer's Comment:
It is not clear the importance, if any, one can give this data analysis, as the trial was not powered for these subset analyses and given that the same data quality and statistical analysis issues as mentioned above come into play. It is also difficult to separate treatment effect of iNO from the other possible confounders, such as the effect of being one of multiple gestation on lung maturation, how birth weight might have impacted lung development in subgroups, etc. While the Sponsor notes that the differences in groups stratified by demographics may be important clinically, they do not provide insight over what would already be expected clinically. For example, at 1-3 weeks of age, one would expect that a greater than 26 week gestation infant would be extubated sooner than a more immature neonate, and other studies in the literature have shown that in general, non-whites are removed from ventilation sooner than whites.

Safety Results

Exposure

The mean number of days of exposure to treatment was similar between treatment groups, 23.3 days for subjects in the iNO group and 23.1 days for subjects in the placebo group. The median number of days' exposure was the same for both groups (24 days). The maximum duration of exposure was 35 days in the iNO group and 46 days in the placebo group.

Subjects were eligible to receive "rescue" open-label iNO therapy who met the protocol specified criteria. A total of 33 (5.7%) of 584 subjects received open-label iNO, 15 (5.1 %) of 295 iNO subjects and 18 (6.2%) of 289 placebo subjects.
[BALLR1 Clinical Study Report, section 12.1, Table 22]

Reviewer's Comment:
Numbers of subjects who received "rescue" iNO were similar for both placebo and treatment.

Overview of Adverse Events

Adverse Events
An overview of Adverse Events for BALLR1 is presented in table 16 below:

Table 16: BALLR1 AE in >5% (safety)

System Organ Class	AE[a] (Preferred Term)	iNO (N =295) n (%)	Placebo (N =289) n (%)
Any AE		230 (78.0)	217 (75.1)
Infections and infestations	Bacterial sepsis	66 (22.4)	72 (24.9)
Congenital, familial and genetic disorders	Patent ductus arteriosus	52 (17.6)	48 (16.6)
Surgical and medical procedures	Therapy cessation[b]	32 (10.8)	24 (8.3)
Blood and lymphatic system disorders	Thrombocytopenia	22 (7.5)	15 (5.2)
Investigations	Platelet count decreased	21 (7.1)	18 (6.2)
Infections and infestations	Tracheitis	19 (6.4)	14 (4.8)
Gastrointestinal disorders	Gastroesophageal reflux disease	16 (5.4)	8 (2.8)
Nervous system disorders	Intraventricular haemorrhage neonatal	15 (5.1)	11 (3.8)
Gastrointestinal disorders	Necrotising enterocolitis neonatal	15 (5.1)	13 (4.5)
Investigations	Blood creatinine [Increased]	15 (5.1)	20 (6.9)
Nervous system disorder	Hydrocephalus	14 (4.7)	23 (8.0)
Eye disorders	Retinopathy of prematurity	13 (4.4)	16 (5.5)

[a] Adverse events are organized by highest percentage of occurrence in iNO treatment group
[b] Includes but is not limited to occurrences of equipment failure, ventilator changed to model incompatible with INOvent®, parent request, or subject met open-label criteria per protocol
[BALLR1 Clinical Study Report, section 12.2.2, Table 24]

Reviewer's Comment:
For this study report, the Sponsor only provides data in tabular form for AEs that occur in greater than 5% of the safety population.

Deaths

As previously stated, death was considered a Serious Adverse Event, and not part of the primary efficacy endpoint.

The rate of death over time from admission showed no difference between treatment groups (log rank test, p = 0.7000). The number and percentage of subject deaths is presented in the table below. The observed percentage of subjects who died while receiving therapy or within 72 hours of therapy completion was 5.4% in the iNO group vs. 6.2% in the placebo group. The primary cause of death for 5 subjects in the iNO group and 4 subjects in the placebo group was from a respiratory diagnosis. One infant in the iNO group died due to BPD, and this was considered not related to study drug. The number of subjects whose primary cause of death was from sepsis while receiving therapy or within the 72 hours following treatment was 7 subjects in the iNO group and 2 subjects in the placebo group. A total of 2 subjects in the iNO group and 5 subjects in the placebo group had a primary cause of death of NEC neonatal.

Table 17: BALLR1 Deaths-(safety population)

	Treatment	
Category	**iNO**	**Placebo**
Number of subjects treated	295	289
Number (%) of subjects who died	16 (5.4%)	18(6.2%)

BALLR1 Clinical Study Report, section 12.3.1.1, Table 26, Table 27]

Nonfatal Serious Adverse Events

The percentage of subjects that experienced an AE with a severity of life threatening was similar in the 2 treatment groups: 26 (9%) subject in the iNO group and 23 (8%) subjects in the placebo group.
Numbers were similar for NEC [6 (2%) vs. 5(2%)], bacterial sepsis [4 (1%) vs. 3 (1%)], fungal sepsis [2 (1%) vs. 2 (1%)], and therapy cessation [2 (1%) vs. 2 (1%)] for iNO versus placebo groups, respectively. Staphlococcal sepsis [2 (1%) vs. 0], Pulmonary pneumatocele [2 (1%) vs. 0], and hypotension [2 (1%) vs. 0] were seen more in the iNO group, and resuscitation [4 (1%) vs. 7 (2%)] and pneumothorax [0 vs. 2 (1%)] were seen more often in the placebo group.
[BALLR1 Clinical Study Report, section 12.3.1.2, Table 28]

Reviewer's Comment:
Differences are very small, and do not appear clinically meaningful, given the background rate of these occurrences in preterm infants outside of the clinical trials setting.

SAE and AE leading to discontinuation/ Dropout

The study drug was stopped then restarted in 12 (4%) and 8 (3%) subjects in the iNO and placebo groups, respectively. The study drug was discontinued in 18 (6%) and 13 (5%)

subjects in the iNO and placebo groups, respectively. The action taken data for 5 (2%) subjects in the iNO group and 8 (3%) subjects in the placebo group were unavailable or unknown.
[BALLR1 Clinical Study Report, section 12.3.1.3, Table 30]

Common Adverse Events

A total of 447 (76.5%) subjects experienced an AE during the study. Two hundred thirty (78.0%) subjects in the iNO group and 217 (75.1%) subjects in the placebo group experienced at least one AE. The most frequently reported AEs were similar in the two treatment groups. The most frequently reported AEs (2: 5%) in the iNO group were bacterial sepsis (22.4%), patent ductus arteriosus (17.6%), therapy cessation (10.8%), thrombocytopenia (7.5%), and platelet count decreased (7.1 %). The most frequently reported AEs in the placebo group were bacterial sepsis (24.9%), patent ductus arteriosus (16.6%), therapy cessation (8.3%), hydrocephalus (8.0%), and blood creatinine [increased] (6.9%).
[BALLR1 Clinical Study Report, section 12.2.2, 12.2.3]

Reviewer's Comment:
Adverse Events appear to be fairly well-distributed between groups, and no AE are noted that would not otherwise be expected in the premature infant population.

Outcomes commonly seen in premature infants and classified by Investigators as Special interest AEs included incidences of ventriculomegaly, IVH, PVL, NEC, patent ductus arteriosus, pulmonary hemorrhage, ROP and sepsis, as shown in the table below. In general, the incidences of special interest AEs were similar between the iNO and placebo treatment groups. The incidences of sepsis, ventriculomegaly, and ROP were slightly decreased in the iNO group compared with the placebo group (28.1% vs. 29.8%, 4.7% vs. 8.3%, and 4.4% vs. 5.5%, respectively). The incidences of PDA, IVH, and NEC were slightly increased in the iNO group compared with the placebo group (17.6% vs. 16.6%, 6.1% vs. 4.5%, and 5.1% vs. 4.5%, respectively).

Table 18: BALLR1 Special Interest AE

Category	Treatment	
	iNO (N = 295) n (%)	Placebo (N =289) n (%)
Number of Subjects Who had Study Drug Discontinued due to an AE	18 (6.1)	13 (4.5)
Number of subjects without IVH	277 (93.9)	276 (95.5)
Special interest adverse events [a,b]		
Sepsis[c]	83 (28.1)	86 (29.8)
Patent ductus arteriosus	52 (17.6)	48 (16.6)
IVH[d]	18 (6.1)	13 (4.5)
IVH Grades 3 and 4	4 (1.4)	3 (1.0)
Ventriculomegaly	14 (4.7)	24 (8.3)
Neonatal NEC	15 (5.1)	13 (4.5)
ROP	13 (4.4)	16 (5.5)
PVL	8 (2.7)	3 (1.0)
Pulmonary hemorrhage	1 (0.3)	2 (0.7)

IVH = intraventricular hemorrhage, PVL = periventricular leukomalacia, NEC = necrotizing enterocolitis, ROP = retinopathy of prematurity

[a] Subject that experienced more than 1 event in a category are each counted only once.

[b] Subjects may appear in more than 1 category and subjects whose cause of death was an AE of special interest may not be included in the above n (%) due to CRF design.

[c] Sepsis includes: bacterial, candida, enterobacter, fungal, sepsis, and staphyloccal sepsis (Section 14.3.3, Table S10)

[d] IVH includes: IVH neonatal, Grades 1, 2, 3, 4, and intracranial haemorrhage

[BALLR1 Clinical Study Report, section 12.3.1.3, Table 29]

Reviewer's Comment:

Although the differences in iNO versus placebo treated subjects are small, PDA, IVH and NEC are slightly higher in the iNO-treated group, similar to that of study INOT27. This is despite the fact that all subjects were at least 7 days old, all had received standard of care and continued to require ventilatory support, and most had received intratracheal surfactant shortly after birth. The percentage differences overall for special interest events in BALLR1 versus INOT27 are reflective of this age and stability difference; for example, IVH in INOT27 was 28.9% vs. 22.9% for iNO vs. placebo, whereas BALLR1 is 6.1% vs. 4.5% (Severe IVH and evidence of active bleeding within 24 hours of randomization were exclusion criteria for infants in BALLR1, so it makes sense that the overall incidence of IVH was low, since those babies were selected out, and the study period began after the natural time frame for IVH to occur. INOT27 randomized subjects before most of the children would present with an IVH, so the numbers should be higher in both treatment and placebo groups.) Again, there is no consistent trend in the studies; in comparison, INOT25 had decreased rates of overall AEs, deaths, and IVH events for iNO treatment as compared to placebo.

Laboratory Findings

Methemoglobin levels were obtained at baseline, between 0 to 72 hours, and between 24 and 48 hours after initiation of study treatment at 20 ppm. Baseline values ranged from 0 to 2.8 for both groups. Methemoglobinemia was defined in this study as a subject with a methemoglobin level obtained after the initiation of treatment of 5% or more. One iNO recipient experienced an elevated methemoglobin level of 4%, post-study treatment initiation; the repeat level on the following day was 2.0%. No methemoglobin level more than 5 % was reported for any subject in either treatment group and no case of overdose was reported. Most of the remainder of subjects had methemoglobin levels of 2% or less.
[BALLR1 Clinical Study Report, section 12.4.1,]

Vital Signs

Vital signs were recorded at all designated time points. Further evaluations were not performed. There were no apparent differences in vital signs (HR, BP, etc) between iNO and placebo groups.

Summary and Conclusions

The two treatment groups were well matched at baseline, and the extent of exposure was comparable between the two treatment groups. Overall, BALLR1 appears to have met its primary efficacy endpoint, but there are a number of data quality and statistical issues regarding data analysis that make a favorable P value difficult to interpret. There was no pre-specified statistical analysis plan for the original NIH Study, and all analyses were determined after the data had been unblinded. The primary endpoint was analyzed in several ways by Dr. Ballard and by the Sponsor, and all were performed in a post-hoc manner, after data had been unblinded. Therefore, after the original data was published, Dr. Ballard and the Sponsor also used two additional methods to analyze the primary endpoint, multiple outputation (MO) and generalized estimating equation (GEE).

From a safety perspective, the frequency and type of AEs were those expected in a population of premature infants (<36 weeks' PMA). The frequency, type, severity, relationship to study treatment, and outcomes of AEs were similar between the two treatment groups. The incidence of IVH and PVL was slightly increased in the iNO group compared with the placebo group. A total of 16 (5.4%) subjects in the iNO group and 18 (6.2%) subjects in the placebo group died during treatment or within 72 hours of stopping treatment. The rate of death from time of admission showed no statistical difference between treatments (log rank test, p = 0.7000). Although there is limited information on SAEs, there appeared to be a similar frequency of SAEs between the two treatment groups. The frequency of discontinuations due to AEs and the incidence of special interest AEs were also similar between the two treatment groups. The most common AE percentages between treatment groups were fairly comparable. In AE of special significance (including PDA, IVH, PVL, NEC, ROP, and sepsis), there were small numeric differences between groups, favoring placebo for IVH, NEC and PDA. The small difference and background level of these events in the premature population make a clinical relevance difficult to discern, however. This study was not specifically designed to examine the effect of iNO on brain injury, and infants were not enrolled until at least day 7 of life, after the peak risk period for IVH. Despite an initial concentration of NO at 20 ppm and a duration of therapy of 24 days, there was only 1 iNO subject with any methemoglobin level greater than 4%. No appreciable

elevations in methemoglobin were noted. No unexpected safety issues were raised in this study regarding the administration of iNO to preterm infants at risk for developing BPD.

Overall, there is evidence of a treatment effect for iNO in preterm infants ≤ 34 weeks' PMA who require assisted ventilation, but, because of issues raised above (data quality, different post hoc statistical analyses, and lack of p value adjustments for interim analyses, the statistical determination is difficult to discern.

4. Review of Efficacy Study INOT 25

Title

Inhaled nitric oxide for the prevention of chronic lung disease in premature newborns

Protocol

Administrative

Study Initiated: April 5, 2001
Study Completed: July 6, 2005
Clinical Centers: This was an NIH-funded trial with 16 Principal Investigators from 16 US Academic Institutions.
Study Report Dated: June 17, 2008
Study Sponsor: INO Therapeutics, LLC

INOT25 was carried out at multiple US centers, and was conducted by NIH/NHLBI-funded investigators. The Sponsor received the data sets after unblinding of data had occurred, and after publication of the results by the original investigators. Statistical Analysis Plan by the Sponsor were written after data had been published, but before the Sponsor began their own data analyses.

The original protocol was issued on May 1, 2000, and the study protocol was not amended at any time.

Objectives

The primary objective for this trial was to assess treatment with a low dose of iNO in reducing the incidence of BPD in premature infants with chronic lung disease, specifically to determine if inhaled nitric oxide reduces the combined endpoint of BPD/mortality in premature newborns (500 to 1,250g birth weight) with respiratory failure who require mechanical ventilation within the first 48 hours of life.

The secondary objectives are described in the following 3 aims:
Specific Aim #2: to determine if early serum and lung (tracheal aspirate) markers of inflammation are predictive of NO efficacy and the development of BPD, and to measure the effects of NO on these markers
Specific Aim #3: to assess the safety of NO in premature neonates
Specific Aim #4: to assess economic and long-term outcomes of premature neonates with respiratory failure treated with inhaled NO and with placebo

Reviewer's Comment:
Specific aims #2 and 4 are considered exploratory and are not addressed in this study report submitted by the Sponsor.

BPD was defined as "the need for supplemental oxygen or mechanical ventilation at 36 weeks PMA, plus abnormal findings on chest radiography, assessed by a centrally-read standardized scoring system," published by Toce and colleagues. Note this is different than that of INOT27 and BALLR1; (the Walsh "physiologic" criteria for determination of oxygen need in relation to defining BPD were not published until 2004, when this trial was already underway).

Study Design

The study was designed as a multicenter, randomized, placebo-controlled, and blinded trial of low-dose NO therapy (5 ppm) in premature newborns with respiratory failure requiring mechanical ventilation. Included were subjects <48 hours old with a gestational age <34 weeks and birth weight 500 to 1,250 g. Nitric oxide 5 ppm or placebo was administered for a 21-day period or until extubation. All subjects who required mechanical ventilation were randomized, regardless of the level of inspired oxygen. The randomization was stratified at each center by 3 levels of birth weight: 500 to 749 g, 750 to 999 g, and 1,000 to 1,250 g.

Randomization codes were generated at the Data coordinating center and sent directly to INO Therapeutics LLC in Port Allen, LA (the manufacturing facility) for label placement on the study cylinders. The cylinders were then sent directly to the study site. The respiratory therapist set up the cylinders per protocol. No randomization was done at the clinic sites.

This study was conducted as a cooperative effort between INO Therapeutics and the NHLBI extramural network. The study was designed and initiated by Dr. John Kinsella under a research grant from NHLBI. The study protocol and data collection forms were designed by Dr. Kinsella and the study Steering Committee, and data management was performed by Dr. Gary Cutter at the DCC, located at the University of Alabama, Birmingham. INO Therapeutics provided study supplies and blinded INOvent® delivery devices, as well as site monitoring and regulatory support; INO Therapeutics was responsible for serious adverse event (SAE) reporting. Following completion of the study and data analysis, the results were published by the Steering Committee in the *New England Journal of Medicine* in July 2006 (N Engl J Med 2006;355;4:354-64). Following publication, INO Therapeutics received permission from the Steering Committee and NHLBI to use all data and related documents for the preparation of this report.

Duration

Subjects received iNO 5 ppm or placebo for a 21-day period or until extubation.

Study Population

800 subjects (400 subjects per group) were planned. The intent-to-treat (ITT) population consisted of 395 subjects randomized to treatment with placebo and 398 subjects randomized to treatment with NO 5 parts per million (ppm). Of the 793 subjects who were randomized, 296 (77.7%) in the placebo group and 306 (78.3%) in the inhaled NO group either completed 21 days of the study or were successfully extubated.

Inclusion Criteria

For inclusion into the trial, subjects were required to fulfill all of the following criteria:

1. Birth weight 500 to 1,250 grams and gestational age <34 weeks
2. Age at enrollment <48 hours
3. Respiratory failure on mechanical ventilation
4. Absence of structural heart disease (except patent ductus arteriosus, atrial septal defect <1 cm, or muscular VSD <2mm if known prior to randomization)
5. Absence of lethal congenital anomaly

Pertinent Exclusion Criteria

Any of the following were regarded as criterion for exclusion:

1. Participation in another concurrent experimental study (observational studies were allowed with prior approval by the steering committee and DSMB)
2. Active pulmonary hemorrhage
3. Unevacuated pneumothorax
4. High-frequency jet ventilation
5. Expected short duration of ventilation (<48 hours from birth)

Withdrawal criteria

A subject's participation in the study was discontinued if any of the following applied:

- Successful extubation of > 48 hours
- Day 21
- Delivery and/or monitoring device malfunction that could not be rectified within 24 hours
- Investigator or subject's family considered discontinuation of the study to be in the subject's best interest
- Best medical care
- Methemoglobin levels increased to > 5%
- Nitrogen dioxide levels increased to > 3 ppm
- An SAE thought by the investigator to be probable or highly probable related to treatment gas
- Subject died

Disallowed Therapies

Corticosteroid use was prohibited in the first week of life. The only exception was the use of hydrocortisone replacement therapy in infants with systemic hypotension and low serum cortisol levels. Between the first and fourth weeks after birth, corticosteroid treatment was allowed using dexamethasone at 0.1 to 0.2 mg/kg/day for 3 days in ventilated subjects on FiO2 $\geq$ 0.4. If the inspired oxygen requirement subsequently increased, a second short course of therapy was permitted.

Concomitant Treatments

Exogenous surfactant was administered prophylactically in the delivery room to all infants with birth weight <800 g. Treatment of established respiratory distress syndrome with surfactant was performed in mechanically ventilated infants with radiographic signs of surfactant deficiency. Subsequent doses of surfactant were given to infants who required a mean airway pressure $\geq$ 7 cm H20 and fraction of inspired oxygen (FiO2) $\geq$0.4.

Study Treatments/Blinding

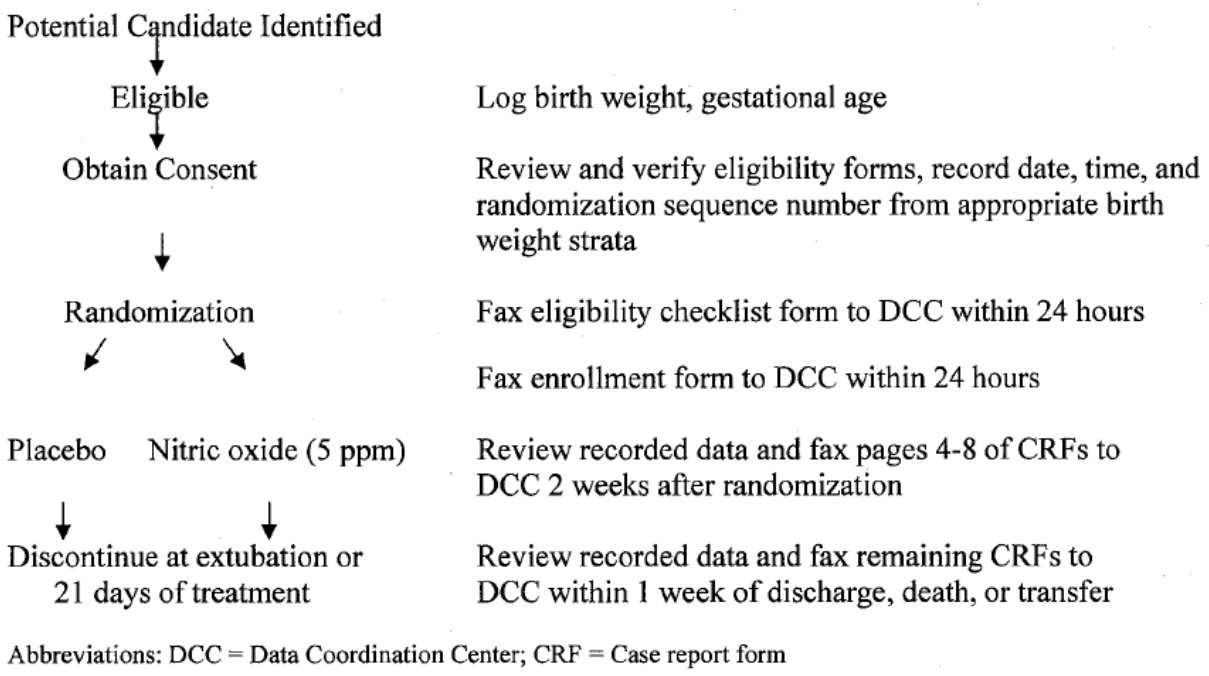
Nitric oxide for inhalation or placebo (100% Grade 5 N2 gas) was administered using the INOvent® delivery system per protocol and treatment was initiated within 48 hours of birth. The delivery system provides for masked delivery of the treatment gas. Nitric oxide was administered at a dose of 5 ppm for a 21-day period or until extubation. Personnel involved in the general care of the subject, including bedside nurses and attending physicians, remained masked to treatment allocation during the course of treatment and until completion of the study.

All other management decisions were at the discretion of the attending neonatologist. If a subject was re-intubated within 48 hours, the study gas could be restarted. Endotracheal intubation was used for all infants with birth weight <800 g, and intubation was determined by clinical manifestations of respiratory failure for all other infants.

Schematic

The following is the randomization and subject data flow schematic for the trial.

Figure 2: INOT25 randomization and subject flow



Abbreviations: DCC = Data Coordination Center; CRF = Case report form

[INOT25 Clinical Study Report, section 9.1, Figure 1]

Efficacy Evaluations

Primary Endpoint

The primary efficacy variable for this study was a binary endpoint determined by the assessment at 36 weeks' PMA. An infant who was alive without BPD at 36 weeks' PMA was counted as a "success". An infant who died or who had BPD at 36 weeks' PMA was counted as a "failure".

Subjects were considered to have BPD if they still required mechanical ventilation or supplemental oxygen and had accompanying radiographic changes.

Secondary Endpoints

The secondary endpoints for this study were:

- to determine if early serum and lung (tracheal aspirate) markers of inflammation are predictive of NO efficacy and the development of BPD, and to measure the effects of NO on these markers
- to assess the safety of NO in premature neonates
- to assess economic and long-term outcomes of premature neonates with respiratory failure treated with inhaled NO and with placebo

The first and third of these were not addressed in the study report, but some of the specific endpoints regarding safety were assessed, as listed below.

Safety Evaluations

Secondary safety endpoints that were evaluated include the following:

- Days requiring assisted ventilation
- Length of hospitalization
- Survival without significant brain injury (grade 3 or 4 IVH)

Schedule of Evaluations

Vital signs, ventilator settings, tracheal lavage fluid, serum cytokine levels, arterial blood gases (ABG), methemoglobin levels, and cranial ultrasound examinations were obtained at regular intervals for 21 days or until extubation. All adverse events (AEs) observed by study site personnel from the start of study gas until completion of a subject's participation in the study, defined as off study gas, were recorded. However, for subjects who prematurely discontinued treatment with study gas but remained in the study for follow-up, only AEs classified as serious were recorded. The SAEs were collected and recorded during study gas administration and throughout the entire hospitalization. They were collected and recorded only until 30 days after discharge home. Other events were collected at 12- and 24-month follow-up. Cranial ultrasound was performed at baseline, 7-14 days of life, and greater than 30 days of life.

Subjects were evaluated when they were 36 weeks' corrected gestational age. At that time, a chest x-ray was obtained. Subjects were considered to have BPD if they still required mechanical ventilation or supplemental oxygen and had accompanying radiographic changes.

After obtaining parental consent, all enrolled subjects could participate in the follow-up program, which occurs when the child is 12, 24, 36, and 54 months old. The follow-up includes a health questionnaire, a physical examination, and developmental testing, including the Bayley Scale of Infant Development. An audiology examination was performed at Month 12. Resource utilization data and quality-of-life related data were collected by structured telephone interviews at 3, 6, 9, 12, 18, and 24 months of age.
[INOT25 Clinical Study Report, section 9.1, Table 2]

Data Analysis

Data quality assurance was monitored throughout the clinical trial in a number of ways. Periodic reports detailing quality of information received, timeliness of communication and response time for error corrections were sent to clinical centers, NHLBI, the monitor group, and the DSMB. There were once-a-year summary reports completed as well. A GMP-quality audit was performed in February 2005, and an institutional audit was conducted for the clinical data

management for the University of Alabama Biostatistics, performed in 2005. Five clinical site investigator audits were also completed, with a random sample of 10% of total subjects enrolled at each site evaluation.

The primary efficacy variable for this study was a binary endpoint determined by the assessment at 36 weeks' PMA. An infant who was alive without BPD at 36 weeks' PMA was counted as a "success". An infant who had died or who had BPD at 36 weeks' PMA was counted as a "failure". The rate of success was analyzed using a generalized linear model with logarithmic link to obtain relative risks and odds ratios along with the raw probabilities of success and the corresponding 95% CIs. Adjusted RRs also were calculated, which adjusted for gestational age, baseline Oxygenation Index, and type of ventilation. Interactions between treatment group and these potentially important covariates were examined. All p values were 2-sided and evaluated at the 0.048 significance level. This is equivalent to 1-sided tests at the 0.024 significance level. These criteria are consistent with an overall type 1 error rate no larger than 0.05 while incorporating appropriate adjustments for the interim analyses performed. While the protocol did not state explicitly which alpha spending method was to be used, it was safe to assume that with the significance for the 3 interim analyses set at 0.001, the final alpha level should have been no more conservative than using an Obrien-Harrington-Fleming boundary of 0.048, very near the most conservative ,Bonferroni correction, which would be 0.047.

Three interim analyses were conducted during this study. Consistent with the prospectively determined interim analysis strategy defined in the protocol of using significance levels of 0.001 for each of the three interim analyses, this fourth and final analysis was conducted using a significance level of 0.048.
[INOT25 Clinical Study Report, section 9.5.3, 9.6, 9.7.1.7.1]

Reviewer's Comment:
Similar to BALLR1, the INOT25 trial was published before the Sponsor received the data, and their statistical analysis plan was written after data was unblinded/ published.

Results

Study Population

Disposition
800 subjects were planned. A total of 793 subjects were enrolled and randomly assigned to study treatment. The first subject was enrolled 5 April, 2001 and the end of treatment for the last subject was on 6 July, 2005. Of the 793 subjects enrolled, 793 subjects were included in the ITT Population and 763 subjects were included in the Safety Population. Of the 763 subjects in the Safety Population, 602 (78%) subjects completed 21 days of the study or were successfully extubated, 306 (78.3%) of those in the iNO group and 296 (77.7 %) of those in the placebo group. There were no listings of subjects who did not complete study treatment, other than those who died. A total of 69 (17.3%) iNO subjects and 83 (21.0%) placebo subjects did not complete the study due to death.
[INOT25 Clinical Study Report, section 10.1, Fig 2]

Protocol Violations
Protocol deviations were similar for each treatment group, and occurred in small numbers. 9 iNO and 13 placebo patients were placed on open-label iNOmax, and 2 iNO subjects remained on study gas longer than 21 days (0 in placebo group). 3 subjects were enrolled at >48 hours of life in each treatment group, and treatment was restarted at >48 hours in 4 iNO and 3 placebo

patients. The following other deviations occurred in the trial, comparing iNO vs. placebo, as follows: informed consent issue, 2 vs. 3; enrolled into wrong birth weight strata, 3 vs. 1; wrong gas cylinder used, 2 vs. 2; enrollment issue, 2 vs. 0; and other issue 4 vs. 3.
[INOT25 Clinical Study Report, section 10.2, Table 3]

Demographics

In general, the demographic and baseline characteristics for subjects and mothers were similar between the iNO and placebo treatment groups for the 793 subjects in the ITT Population. The treatment groups were comparable in GA, sex, race, age of mother, birth weight, and ventilation modality. There were some small differences between groups with regard to grade of scoring for cranial ultrasound, but treatment groups were similar in normal ultrasounds US (74.6% iNO vs. 71.1% placebo), periventricular cysts (3.5% vs. 2.8%), and ventriculomegaly (3.5% vs. 4.3%). Degree of respiratory disease as determined by oxygenation index (OI), OI strata, and respiratory severity score were similar between groups. The study report notes that groups were also well-matched for baseline medications that have effect on incidence of BPD, such as Vitamin A, caffeine, surfactants, and steroids, but data was not included in the study report. Maternal race/ethnicity information is listed in table 19 below.

Table 19: INOT25 Maternal Race Demographics

Variable	Parameter	iNO (N=398)	Placebo (N=395)	Total (N=793)
RACE				
	White/non-Hispanic	249 (62.6)	234 (59.2)	483 (60.9)
	Hispanic	41 (10.3)	48 (12.2)	89 (11.2)
	Black/non-Hispanic	94 (23.6)	98 (24.8)	192 (24.2)
	Asian/Pacific Islander	2 (0.5)	5 (1.3)	7 (0.9)
	American Indian	6 (1.5)	3 (0.8)	9 (1.1)
	Alaskan Native	2 (0.5)	2 (0.5)	4 (0.5)
	Other	4 (1.0)	5 (1.3)	9 (1.1)

[INOT25 Clinical Study Report, section 11.2.1, Table 6]

Efficacy Results

Analysis of Primary Endpoint

The primary outcome of this trial, change in the rate of survival without BPD, was analyzed using gestational age and birth weight as covariates. As shown in the table below, the success rate was 21 % for the placebo group and 23.6 % for the inhaled NO group. The p value obtained for the Wald test along with the odds ratio and corresponding confidence interval indicate that with gestational age as a covariate, the treatment success rate is not statistically significantly different in the two groups. Similar results were obtained with the birth weight as a covariate.

Table 20: INOT25 Primary Outcome Analysis

Outcome	Placebo (N = 395) n (%)	Inhaled NO (N = 398) n (%)	Total (N = 793) n (%)
Failure: Death or CLD	304 (79.0%)	292 (76.4%)	596 (77.7%)
Success: Alive without CLD	81 (21.0%)	90 (23.6%)	171 (22.3%)
Total	385 (100.00%)	382 (100.00%)	767 (100.00)%
Fisher's test to evaluate incidence of death by treatment, p value	0.2068		
Total[a]	395 (100.0%)	398 (100.0%)	

Model variable	p Value	Odds Ratio	95% CI
Gestational age as covariate			
Treatment (inhaled NO vs. placebo)	0.4041	0.85	(0.58, 1.24)
Gestational age	< 0.0001	0.74	(0.66, 0.83)
Birth weight as covariate			
Treatment (inhaled NO vs. placebo)	0.4102	0.853	(0.583, 1.246)
Birth weight	< 0.0001	0.997	(0.996, 0.998)

[a] Total percentage is based on the total number of subjects with non-missing CLD or death status at 36 weeks gestational age visit.
CI = confidence interval, NO = nitric oxide

[INOT25 Clinical Study Report, section 11.4.1, Tables 8-11]

When primary outcome was stratified by birth weight cohort, a p value <0.0001 was obtained for comparison of subjects with birth weight of 500-749 gram vs. 1,000 to 1,250 grams. There was a similar p value of <0.0001 when comparing gestational age strata of <26 weeks to ≥ 26 weeks. Infants weighing ≥ 1 kg treated with iNO had an increased likelihood of survival without BPD as compared to placebo infants (38.6% vs. 25.8%). For infants less than 1 kg, there was no difference between treatment groups. Older premature infants greater than or equal to 26 weeks GA had a numeric advantage over those less than 26 weeks (30.8% vs. 27.1%). The death rate in iNO-treated infants (17.3%) vs. placebo (21%) is numerically different, but does not reach statistical significance.

Reviewer's Comments:
P-values do not reach significance, and confidence intervals are large for most of the stratified analyses, except the two related groups listed above. This data should be viewed as hypothesis generating.

Analysis of Secondary Endpoints
The Sponsor has performed analyses on secondary endpoints. No treatment differences were observed.

Descriptive analyses noted that number of days of assisted ventilation was slightly more in iNO vs. placebo group, mean 42.5 versus 40.5 days, respectively. Data for length of hospitalization shows a slightly longer length for iNO, 39.52 days versus 98.3 days for placebo; however, data

is missing for 201 infants. Survival without significant brain injury data notes 62.5% of placebo versus 71.4 % of those on iNO met criteria (54 subjects had missing data, 27 in each group).
[INOT25 Clinical Study Report, section 11.4.1, Tables 12-16]

Safety Results

Exposure

The mean number of days of exposure to treatment was similar between the two groups; 12.1 days for subjects in the placebo group and 12.7 days for subjects in the inhaled NO group.
[INOT25 Clinical Study Report, section 12.1, Table 19]

Overview of Adverse Events

Adverse Events

The AEs were defined as any signs (including the clinical manifestations of abnormal laboratory results) or medical diagnoses noted by medical personnel, regardless of relationship to study gas, that:

- Had their onset any time after the start of study gas (signs, symptoms, and diagnoses occurring prior to the first dose of study gas were not considered to be AEs if they did not increase in severity) and
- Had worsened since the event was previously reported (this included worsening of signs, symptoms, or diagnoses that were present prior to dosing).

All AEs observed by study site personnel from the start of study gas until completion of a subject's participation in the study, defined as off study gas, were recorded in the subject's CRF. However, for subjects who prematurely discontinued study gas but remained in the study for follow-up, only AEs classified as serious were recorded. The AEs were recorded regardless of relationship to study treatment.

The Sponsor notes in this submission that a portion of AE data are missing.
Three hundred sixty-three (95.0%) subjects in the placebo group and 374 (95.7%) in the inhaled NO group experienced an AE. There were 92 (24.0%) SAEs reported in subjects receiving placebo and 100 (26.0%) SAEs in subjects receiving inhaled NO. There were 14 (3.7%) withdrawals from treatment reported in subjects receiving placebo and 6 (1.5%) withdrawals in subjects receiving inhaled NO. There were 86 (22.5%) deaths reported in subjects receiving placebo and 77 (19.7%) deaths in subjects receiving inhaled NO.
[INOT25 Clinical Study Report, section 9.5.1.3, section 12.2.2]

Deaths

There were 86 (22.5%) deaths in the placebo group and 77 (19.7%) deaths in the inhaled NO group.
[INOT25 Clinical Study Report, section 12.3.1.1, Table 22]

Nonfatal Serious Adverse Events

The percentage of subjects that experienced nonfatal SAEs was similar across the 2 treatment groups: 24.0% (92 of 382) in the placebo group and 26.0% (100 of 391) in the inhaled NO group.

A total of 192 subjects experienced nonfatal SAEs during this study; of these, 138 subjects (32.5%) were of gestational age < 26 weeks, and 54 (15.5%) subjects were of gestational age ≥ 26 weeks. Of the subjects who were of gestational age ≥ 26 weeks, fewer subjects in the inhaled NO group (14.1% in nitric oxide vs. 17.0% in placebo) experienced any nonfatal SAEs. The most common nonfatal SAEs were neonatal IVH (31 [8.0%] in placebo and 27 [7.4%] in nitric oxide); sepsis (14 [4.5%] in placebo and 21 [6.3%] in nitric oxide); pneumothorax (14 [3.8%] in placebo and 10 [2.6%] in nitric oxide); neonatal necrotizing enterocolitis (9 [2.4%] in placebo and 10 [2.3%] in nitric oxide); and pulmonary hemorrhage (7 [1.8%] in placebo and 8 [2.0%] in nitric oxide).

Twenty-one (5.5%) subjects from the placebo group and 23 (5.9%) subjects from the inhaled NO group, who experienced SAEs, died.
[INOT25 Clinical Study Report, section 12.3.1.2, Table 23]

SAEs and AEs Leading to Discontinuation/Dropout

There were a total of eight withdrawals due to an AE or SAE; 5 (1.3%) for placebo and three (0.8%) for iNO. There were a total of 24 deaths in the placebo group and 29 deaths in the iNO group due to an adverse event.
The number of subjects withdrawn from treatment because of AEs, and AEs of special interest are presented in the table below. Special interest AEs included incidences of ventriculomegaly, intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, patent ductus arteriosus, pulmonary hemorrhage, retinopathy of prematurity and sepsis.

Table 21: INOT25 Withdrawals due to AEs of Interest

Category	Treatment	
	Placebo	Inhaled NO
Number of subjects treated	382	391
Special interest adverse events		
Ventriculomegaly	2 (0.5%)	0 (0.0%)
IVH (all) [a]	55 (14.4%)	50 (12.8%)
IVH Grade 3 and 4 Only	32 (8.4%)	25 (6.4%)
PVL	7 (1.8%)	1 (0.3%)
Necrotizing enterocolitis	13 (3.4%)	13 (3.3%)
Patent ductus arteriosus	61 (16.0%)	46 (11.8%)
Pulmonary hemorrhage	9 (2.4%)	11 (2.8%)
Retinopathy	1 (0.3%)	2 (0.5%)
Sepsis	48 (12.6%)	65 (16.6%)

NO = nitric oxide, SD = standard deviation, IVH = intraventricular hemorrhage, PVL = periventricular leukomalacia
[a] Patients that experienced more than 1 event are each counted only once.

[INOT25 Clinical Study Report, section 12.3.1.3, Tables 24, 25]

Reviewer's Comment:

With regard to outcomes common in premature infants, the Sponsor did not present data with regard to all subjects with a Special interest AE. The patients who withdrew from the study based on those outcomes are in the withdrawal section above

Common Adverse Events

Three hundred sixty-three (95.0%) subjects in the placebo group and 374 (95.7%) in the inhaled NO group experienced an AE. The most frequently reported AEs in the placebo group were anemia (56.8%), decreased oxygen saturation (53.4%), respiratory acidosis (46.6%), hypoxemia (44.8%), bradycardia (41.1%), hyperglycemia (40.6%), hypercalcemia (33.8%), and respiratory gas exchange disorder (33.2%). The most frequently reported AEs in the inhaled NO group were anemia (60.9%), decreased oxygen saturation (56.5%), bradycardia (48.1%), hypoxia (46.5%), respiratory acidosis (40.9%), hyperglycemia (38.1 %), hypercalcemia (33.8%), and respiratory gas exchange disorder (31.7%). The outcome data for 357 (93.5%) subjects in the placebo group and 370 (94.6%) subjects in the inhaled NO group were described as 'missing'. The table below lists the most common AEs that occurred in greater than 30% of the population.

Table 22: INOT25 AE in Greater than 30% of Safety Population

Adverse Event	Inhaled NO N=391	Placebo N=382
Anemia	238 (60.9%)	217 (56.8%)
Bradycardia	188 (48.1)	157 (41.1)
Decreased oxygen sat.	221 (56.5)	204 (53.4)
Hypercalcemia	132 (33.8)	129 (33.8)
Hyperglycemia	149 (38.1)	155 (40.6)
Hypoxia	182 (46.5)	171 (44.8)
Resp. gas exchange d/o	124 (31.7)	127 (33.2)
Respiratory acidosis	160 (40.9)	178 (46.6)

[INOT25 Clinical Study Report, section 12.2.2, Table 21]

Reviewer's Comment:
The study report does not include any data regarding Adverse Events at a rate that occurred less than 30%. It is not clear how the 30% cutoff was decided.

Laboratory Findings

Methemoglobin levels were obtained at baseline and at 60 minutes. Baseline values were 0 (0%) for both groups. At 60 minutes, 1 (0.3%) subject in the placebo and 1 (0.3%) subject in the inhaled NO group had a > 5% threshold value of methemoglobin. No elevation of NO2 was reported in any subject in either group.
[INOT25 Clinical Study Report, section 12.4]

Vital Signs

There were no apparent differences in vital signs (HR, BP, etc) between iNO and placebo groups.

INOT25 Summary and Conclusions

The 2 treatment groups were well matched at baseline, and the duration of exposure to study treatment was comparable between the two treatment arms. Overall, the primary outcome of this trial, change in the rate of survival without BPD, was not different between placebo and iNO treatment groups. The data was not significant when analyzed using gestational age and birth weight as covariates; the success rate was 21 % for the placebo group and 23.6 % for the inhaled NO group.

The AE profile in the two groups was similar and comparable to what would be expected in a population of preterm infants of this size and weight. The most commonly recorded AEs recurring in at least 30% in both groups were not unexpected. The AEs of special interest were those that are known to occur commonly in preterm infants, and that have significant associated morbidity; including ventriculomegaly, IVH, PVL, necrotizing enterocolitis, patent ductus arteriosus, pulmonary hemorrhage, retinopathy of prematurity, and sepsis. Except for patent ductus arteriosus, which occurred more frequently in the placebo group, and sepsis, which occurred more frequently in the inhaled NO group, other special interest AEs occurred to a similar extent in both of the groups. The most common SAEs recorded during this study were neonatal IVH, sepsis, pneumothorax, neonatal necrotizing enterocolitis, and pulmonary hemorrhage. Neonatal IVH and pneumothorax appeared to occur more frequently in the placebo group, whereas sepsis occurred more frequently in the inhaled NO group. Occurrence of neonatal necrotizing enterocolitis and pulmonary hemorrhage was similar in both groups. There were 86 (22.5%) deaths in the placebo group and 77 (19.7%) deaths in the nitric oxide group.

5. Overall Summary and Conclusion

Clinical Studies INOT27, BALLR1, and INOT25

Two of these studies, INOT25 and INOT27, are very similar in design, drug dosing, and population. Neither of these studies met their primary efficacy endpoint, which leads one to conclude that a dose of iNOmax initiated at less than 48 hours of life, at 5ppm for 7-21 days in a 24 to 34 week PMA infant does not provide significant benefit in the reduction of oxygen requirement at 36 weeks' PMA, the surrogate marker used for the development of BPD/chronic lung disease of prematurity.

In contrast to the INOT studies, depending on the statistical analysis conducted, iNO at a higher dose (20 ppm) in premature infants with respiratory insufficiency at 7-21 days of life for a period of 28 days (b) (4) . As such, the trial design and data collection are not considered up to the regulatory standards generally required for a study used for regulatory purposes. Significant issues regarding the study design, conduct and statistical aspects include data quality, the application of several different statistical tests to the primary endpoint, and the lack of adjustment to the level of significance based on 2 interim analyses.

Thus, on the basis of the 3 studies submitted the use of nitric oxide in premature infants for the prevention of BPD is not supported and is reflected in the FDA labeling changes outlined below.

With regard to safety, in each individual study there were here were nominal differences in deaths or adverse events common to premature infants between the iNO and placebo groups, however, when taken as a whole, there were no meaningful differences between treatment groups with regard to deaths, methemoglobin levels, or adverse events commonly observed in premature infants, including intraventricular hemorrhage, patent ductus arteriosus, pulmonary hemorrhage, and retinopathy of prematurity.

Written Request

Based upon the requirements in the Written Request Letter dated 30 April, 2010, the Sponsor has fairly responded to each item in the WR. The Pediatric Exclusivity Board met on November 2, 2010 and concurred that the WR requirements had been met.

6. Appendices

Labeling Recommendations

Current Text

Nitric oxide for inhalation has been studied in a neonatal population (up to 14 days of age). No information about its effectiveness in other age populations is available.

Sponsor Proposed Text

(b) (4)

FDA Proposed Text

The safety and efficacy of INOmax for the prevention of chronic lung disease [bronchopulmonary dysplasia, (BPD)] in neonates ≤ 34 weeks gestational age requiring respiratory support has been studied in three large multi-center double-blind, placebo-controlled

clinical trials in a total of [redacted] preterm infants. Of these, [redacted] received placebo, and [redacted] received inhaled nitric oxide at doses ranging from 5-20 ppm, for treatment periods of 7-24 days duration. The primary endpoint for these studies was alive and without BPD at 36 weeks postmenstrual age. The need for supplemental oxygen at 36 weeks PMA served as a surrogate endpoint for the presence of BPD. Overall, efficacy for the prevention of bronchopulmonary dysplasia in preterm infants was not established. There were no meaningful differences between treatment groups with regard to deaths, methemoglobin levels, or adverse events commonly observed in premature infants, including intraventricular hemorrhage, patent ductus arteriosus, pulmonary hemorrhage, and retinopathy of prematurity.

The use of INOmax for prevention of BPD in preterm neonates ≤ 34 weeks gestational age is not indicated.

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/s/

KIMBERLY A WITZMANN
11/19/2010

ANTHONY G DURMOWICZ
11/19/2010