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APPLICATION NUMBER: NDA 20845

MEDICAL REVIEW(S)

McDonald

OCT 29 1999

Primary Medical Review of Inhaled Nitric Oxide (I-NO)

NDA 20-845

Food and Drug Administration
Division of Cardio-Renal Drug Products (HFD-110)

October 29, 1999

By
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NDA: 20-845

NAME OF DRUG: Inhaled Nitric Oxide (I-NO)
TRADE NAME: INOmax
FORMULATION: Gas for inhalation
RELATED APPLICATION: None

DATE OF SUBMISSION: 5.25.99
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PROPOSED INDICATION: Treatment of hypoxic respiratory failure in newborns

SPONSOR/MONITORS: INO Therapeutics, Inc.

/S/ h.o.

Douglas C. Throckmorton, M.D.
Primary Medical Reviewer

0.0 Overall Summary of Efficacy and Safety for I-NO

Inhaled Nitric Oxide (I-NO) has been proposed as a treatment for hypoxic respiratory failure in neonates. Clinical support for this indication comes from four clinical trials conducted in this population and submitted as part of this NDA, as well as an extensive published literature on the use of I-NO in this setting. Three of these trials were previously reviewed as part of an earlier NDA submission by the sponsor. The fourth trial was completed more recently and is reviewed as part of the present document.

The data from three trials (NINOS, INO-01/ -02 and CINRGI) demonstrate that I-NO administration is associated with a significant decrease in the use of extra-corporeal membrane oxygenation (ECMO), an invasive method of oxygenating the blood. This effect of I-NO to decrease ECMO use may well be due to the acute effect of I-NO to improve oxygenation, rather than due to any other beneficial effect on the course of the disease causing the hypoxic respiratory failure. In support of this contention, no beneficial effect of I-NO on mortality or any other clinical endpoint was demonstrated by the available data. No effect of I-NO on mortality (beneficial or adverse) has been demonstrated by the data. The effect of I-NO to improve oxygenation is significant, however, and avoidance of ECMO is a clinically-desirable outcome. In the absence of hard clinical benefit (e.g., decreased mortality, fewer days of hospitalization) the safety of I-NO needs to be firmly established prior to allowing its non-investigational use.

The following safety issues have been raised during one or both of my reviews:

1) The safety database included small number of subjects, and for most adverse events, the INO-01/ -02 was the primary source of information. Given the baseline differences between the subjects in the INO-01/ -02 and the other trials, extrapolating between the two populations is also difficult, and open to serious errors of omission due to inadequate data. These difficulties have been alleviated to some extent by the addition of 97 additional patients who were exposed to I-NO, bringing the total number of children exposed to I-NO in the NDA database to 375. The difficulties with differences in baseline characteristics are again present in the CINRGI trial, complicating its interpretation. Another potentially confounding variable between the CINRGI trial and the previous trials is the lower dose of I-NO administered in CINRGI (20 ppm reduced to 5 ppm if possible), compared with the NINOS and INO-01/ -02 trials (20-80 ppm).

2) The available safety database in the original NDA raised several potential safety issues. The most troubling of the adverse events, raised in the original medical review, was the possible association of I-NO with acute and chronic pulmonary toxicities. This association, like all of the safety data, relied on small numbers of subjects, although the association was plausible, given the available data. The addition of the CINRGI trial data, along with additional long-term follow-up data from NINOS and INO-01/ -02 has allayed some of the concerns, especially regarding the occurrence of chronic injury. The existing database is inadequate, however, to exclude the occurrence of pulmonary toxicity in association with the use of I-NO.

3) There was a definite association of I-NO with the development of methemoglobinemia and elevated NO_2 concentrations, identified in the NINOS and INO-01/ -02 trials (especially at the 80 ppm dose). This concern is minimized with the use of the lower doses of I-NO in the CINRGI trial (and the proposed dose for the label).

4) Several other adverse events were also possibly linked to the administration of I-NO based on the data available in 1997, although the data were insufficient to determine the seriousness of these potential adverse events, or to determine their duration or dose-response. The addition of the CINRGI data has resolved some of these safety concerns, and no new safety concerns have arisen as a result of the CINRGI trial review. The available data does suggest that rapid discontinuation of I-NO is associated with rebound hypoxia in some patients.

5) For some adverse events of interest, no data were obtained at all. Most critical of these was the effect of I-NO on coagulation parameters. Other clinical events for which we have either scarce or no clinical data include: musculoskeletal injury; non-glomerular renal injury; effects on the cardiac conduction system, and effects on serum electrolytes.

6) The number of patients exposed to I-NO is too small to adequately assess the potential interactions of I-NO with disease states, patient demographics and concomitant medications. The potential interaction of I-NO with other drugs is of particular importance for drugs commonly used to treat this condition, such as steroids and vasodilators (with the exception of tolazoline).

7) Finally, an issue that cannot be resolved from the database is the potential genotoxicity and carcinogenicity of I-NO. The available data on the genotoxicity of I-NO are mixed (see section 4.1 in my 1997 review for details). It is true that the duration of exposure to I-NO is limited in these studies, and that I-NO is produced (at many-fold lower concentrations) intracellularly. However, the cumulative years of risk for a newborn who receives I-NO is appreciably longer than an adult.

0.0 Overall Summary of Efficacy and Safety for I-NO (cont)

The data suggest that I-NO has a dose-dependent, acute effect on oxygenation in newborns with hypoxic respiratory failure. This improvement in the physiology translates into a reduced use of ECMO, an invasive procedure with significant potential morbidity and mortality. There are no data demonstrating a clear beneficial effect of I-NO on hard-endpoints (death, days of hospitalization, days of ventilation, incidence of chronic lung disease or neurological sequelae). Given that there are other effective therapies and a falling mortality rate from this disease, the safety of I-NO becomes more critical to assess.

Regarding safety, in distinction to the situation in 1997, the available database is more reassuring regarding the safety I-NO administration, especially the potential for neurologic injury. Additional data are needed to resolve the issue of pulmonary toxicity definitively. No new safety concerns have been identified from the CINRGI review. The safety database is inadequate as regards to certain key adverse events, and insufficient data exist on the interaction of I-NO with other medications used in the treatment of hypoxic respiratory failure.

In conclusion, a clear clinical benefit of I-NO in this population has not been demonstrated. A beneficial effect of I-NO on the physiology of these severely ill patients (improvement in oxygenation) has been demonstrated. This effect allows for a decreased use of an invasive and potentially dangerous procedure (ECMO). While the database does not address some safety concerns for I-NO, the available data suggest that the short-term use of I-NO is not associated with severe adverse events. Approval of I-NO to improve oxygenation in neonates with hypoxic respiratory failure is therefore recommended.

1.0 Materials Utilized in the Review

1) NDA 20-845
2) Medical/Statistical Review of I-NO by D.C. Throckmorton and Walid Nuri, completed 11.19.97.
3) Published literature pertaining to I-NO.
4) Draft Statistical review by Lu Cui, Ph.D., obtained 10.27.99.

2.0 Background

The initial submission of NDA 20-845, for inhaled nitric oxide (I-NO), took place in 1997. In the package were data from three substantive trials: NINOS, INOSG and INO-01/ -02. In addition, a fourth trial (INO-03) enrolled 14 patients before being halted. Two of these trials (NINOS and INO-01/ -02) were ultimately considered to be pivotal as regards to safety and efficacy. The NINOS trial was conducted by the NIH, while the INO-01/ -02 was a combined trial initiated and supported by the sponsor. All of the trials examined the effects of I-NO in newborn infants with hypoxic respiratory failure along with evidence (either clinical or by echocardiogram) of pulmonary hypertension. All of the trials were randomized, placebo-controlled, designed to be double-blind. Children with congenital diaphragmatic hernia/lung hypoplasia were excluded from these trials.

NINOS: With regard to efficacy, the NINOS trial was stopped early for perceived overwhelming clinical efficacy with regard to its primary endpoint (the incidence of ECMO or death). In this trial, I-NO (20-80 ppm) reduced the use of ECMO in patients with hypoxic respiratory failure and had an acute effect to improve oxygenation.

INC-01/ -02: After NINOS stopped early, the INO-01/ -02 trial had a severe decline in enrollment, and ultimately stopped early as well. While this trial did not show a significant effect of I-NO (5-80 ppm) on its primary endpoint (death, receipt of ECMO, evidence of neurologic or pulmonary sequelae at 28 days), there was a trend towards a reduction in the use of ECMO in the I-NO group as a whole. There was also a dose-dependent effect of I-NO to improve oxygenation. INO-01/ -02 enrolled patients with less severe hypoxia than the other trials in the NDA, and was the only trial in the original NDA submission to collect all reported adverse events and measure routine laboratories other than methemoglobin and NO_2 levels.

INOSG: The INOSG trial suffered from methodologic flaws that called into question the interpretation of some of its findings. It did demonstrate an acute effect of I-NO (80 ppm) to improve oxygenation, which was its pre-specified primary endpoint.

INO-03: A small trial, INO-03 enrolled 14 patients before similarly being halted. Its contributes to the overall safety database.

With regard to safety, concerns were raised regarding the long-term safety of I-NO. Most significant, in the final Medical/Statistical review document, dated 11.19.97, the possibility of acute and chronic lung injury following I-NO was raised by this reviewer.

2.0 Background (cont)

The sponsor ultimately chose to withdraw the NDA application prior to a decision about approvability. At the time of the review, and in subsequent meetings, the weaknesses of the database were discussed with the sponsor, and they were encouraged to collect long-term follow-up regarding the clinical course of the infants in the available studies (for the NINOS and INO-01/ -02 trials). The results of this follow-up have been summarized by the sponsor, and are presented below.

In addition, the sponsor obtained the data from a third randomized, placebo-controlled study of I-NO in hypoxic respiratory failure (the CINRGI trial). The results of this trial are presented by the sponsor in the present submission, and are reviewed below.

2.1 Organization of Medical Review

Because of the nature of the NDA submission, with a large portion of the data previously submitted and reviewed, the current document will differ somewhat from the usual format for an NDA review. In particular, those trials I have reviewed previously are summarized in the present review, and the reader is referred to the review dated 11.19.97 for further details. Tables that originally appeared in the previous review will be identified by having two numbers in their title: the numbering system used for the present review as well as the number corresponding to their location in the 1997 review (in parentheses).

This current review document will initially focus on the CINRGI trial data, followed by a review of the long-term data from the NINOS and INO-01/ -02 trials. The company has also performed additional analyses from the NINOS data addressing some of the concerns expressed by the Agency, and these will be presented in this section as well. The third section will focus on integrating the results from the three main clinical trials to assess the overall risk/benefit ratio of the use of I-NO in this neonatal population with respiratory failure. This section will include the summary materials for the efficacy and safety data of the NINOS, INOSG and INO-01/ -02, drawn largely from my original consult, incorporating the relevant data from the CINRGI trial. A review of the relevant published literature regarding the safety and efficacy of I-NO will also be included at this point.

The conclusions of the Medical Reviewer, including the recommendation regarding the approvability of I-NO, will follow the integrated efficacy and safety summaries. References are to be found at the end of the document, followed by the Appendices, which will include the following:

1) a list of abbreviations used,

2) narratives for the deaths in the CINRGI trial, and

3) the sponsor's proposed label, including comments from the medical, chemistry and pharmacology reviewers where appropriate.

The reader is referred to the original review of NDA 20-845, dated 11.19.97, for some of the normal aspects of an NDA review, including pharmacology, toxicology, carcinogenicity. The reviews of the NINOS, INOSG and INO-01/ -02 trials are also to be found there. Finally, the previous review contains an extensive review of the available animal and human literature published on nitric oxide.

The reader is also referred to the Statistical Review of NDA 20-845 by Dr. Cui, submitted along with the current Medical Review.

3.1 Title of Study

A comparison of conventional therapy and inhaled nitric oxide in the management of persistent pulmonary hypertension of the newborn (Clinical Investigation of Nitric Oxide Research Group Initiative; CINRGI).

3.2 Sites of Investigation and Investigators

The CINRGI investigators and their sites of investigation, along with the number of infants enrolled at each center, are summarized below. No financial disclosure information was available at the time of this review, so no statement is possible at this time regarding potential conflicts of interest on the part of the investigators.

Site Name	# of Enrolled Subjects
All Children's Hospital	1
Arnold Palmer Hospital	34
Carolina Medical Center	24
Children's Medical Center of Akron	4
Christ Hospital	3
Duke University Hospital	13
Egleston Children's Hospital	43
Georgetown University Medical Center	9
Grady Memorial Hospital	13
Greenville Memorial Hospital	5
Medical University of South Carolina	28
Ochsner Clinic	12
Richland Medical Center	3
St. Joseph's Hospital and Medical Center	10
University of South Dakota	1
University of Texas	1
Vanderbilt University	6
Wilford Hall Medical Center	2
Total Enrollment[a]	212

a. Of these, 186 enrolled patients met the entry criteria and were ultimately randomized.

3.3 Background

Initial protocol submitted: 12.95

This submission was submitted as an amendment to the pilot study protocol (unblinded) which was finalized 4.94. At that time, 36 patients had been enrolled. The protocol was changed to a randomized, double-blind study at this time.

First protocol amendment submitted: 12.97

1. Provided further details regarding the blinding of the trial, including the use of shrouds for the gas tanks.
2. The primary endpoint was changed from the 'number of infants who needed ECMO' to the 'number of infants who received ECMO.'

Second protocol amendment submitted: 6.98

1. A steering committee was established.
2. Monitoring of NO and NO_2 levels were added.

Study Initiation: 4.21.94

Enrollment Termination: 12.8.98

3.4 Study Design

CINRGI was a multicenter, double-blind, randomized, placebo-controlled trial in infants with evidence of persistent pulmonary hypertension of the newborn (PPHN), but without evidence of structural heart disease. An important feature of this trial, compared with the NINOS and INO-01/ -02 trials, is that the use of high-frequency oscillatory ventilation was encouraged prior to study enrollment for patients with significant parenchymal lung disease.

Eligible patients were first categorized according to disease type (e.g., respiratory distress syndrome, meconium aspiration syndrome), and then randomized to receive either placebo (N_2) or I-NO.

I-NO was started at 20 ppm and continued for at least 4 hours. At that point, if the PaO_2 was >60 mm Hg with a pH ≤7.55, the dose was decreased to 5 ppm (otherwise the I-NO was continued at 20 ppm for a maximum of 24 hours). Infants could be continued on I-NO 5 ppm for up to 96 hours of gas administration or the patient was 7 days of age.

Treatment gas was continued until FiO_2 was <0.7, the patient had received 96 hours of therapy, or the patient was 7 days old, whichever came first. Once the FiO_2 ≤0.7, weaning attempts were made. Treatment gas could be restarted if the patient required an FiO_2≥0.80 to support a PaO_2≥60 mm Hg. During the first 24 hours, the gas was re-started at 20 ppm. After 24 hours, the gas could only be restarted at 5 ppm. If the patient failed to respond to the reinitiation of study gas, they were deemed a treatment failure and the gas was discontinued.

Treatment Failure/ Patient Discontinuation

After initiation of treatment, patients were assessed for the occurrence of treatment failure, defined as:

1. Sustained hypoxemia:
    a. OI >40 cm H_2O on 3 of 5 ABGs drawn 30 minutes apart.
    b PaO_2<40 mm Hg for ≥2 hours. or
    c. PaO_2 <35 for >1 hour.
2. Systemic hypotension (mean arterial systemic BP <35 mm Hg) unresponsive to medical management.
3. Inadequate response to treatment gas:
    a. PaO_2<60 mm Hg after 24 hours. or
    b. deterioration in oxygenation status on the initiation of study gas, as evidenced by:
        i. a drop of >10 mm Hg PaO_2 or to a value <40 mm Hg,
        ii. a drop in oxygen saturation of >5%, or
        iii. a drop in SaO_2 to <88%.
4. Failure to tolerate weaning from gas
    a. Failure to tolerate a decrease in the study gas to 5 ppm after 24 hours at 20 ppm.
    b. Failure to tolerate discontinuation of study gas at the end of 96 hours of treatment.
    c. Oxygen saturation falls by >5% or to <88%.
5. Elevated methemoglobin levels (>4%).
6. Elevated NO_2 levels (>5 ppm).
7. Parents withdraw informed consent.

Blinding

An unblinded investigator monitored the patients for NO_2 and methemoglobin levels at all sites, necessitating a second, blinded, team who were responsible for all activities and data collection, excluding those data which could indicate the therapy (methemoglobin levels, NO_2 levels, settings and calibration of the study gas delivery device). Every three hours the unblinded respiratory therapist recorded the NO_2 and NO levels, regardless of the treatment gas administered to the patients.

Concomitant Medications

It has been suggested that the effectiveness of I-NO may vary depending on the type of ventilatory support given to the patients. To minimize this, the investigators attempted to standardize the delivery of high frequency oscillatory ventilation (HFOV) and surfactant. Infants with evidence of parenchymal lung disease on CXR and/or poor lung inflation were started on HFOV prior to entry into the study, and patients with RDS were treated with at least one dose of surfactant.

3.4 Study Design

Concomitant Medications (cont)

All other therapies were allowed by the protocol, although two disease-specific guidelines were given to the investigators(NDA vol. 9.6, section 9.4.6):

1. *Pulmonary hypertension with associated parenchymal lung disease.* The goal in these patients is to optimize lung inflation. In some patients, the optimization of lung inflation was expected to decrease pulmonary artery pressure. In patients with radiographic signs of low lung volume, end-expiratory or mean airway pressure was increased in an attempt to recruit collapsed alveoli.

2. *Pulmonary hypertension without significant parenchymal lung disease.* In these patients, the primary goal was selective pulmonary vasodilatation. Every attempt was made to avoid lung over-inflation and pressure-induced lung injury.

3.5 Study Objective/ Primary and Secondary Endpoints

The objective of the CINRGI trial were to assess the safety and efficacy of I-NO added to conventional therapy for PPHN, compared with conventional therapy alone. Patients with congenital diaphragmatic hernia (CDH)/ lung hypoplasia were eligible for enrollment in the trial, but were excluded from the primary analyses.

Primary Endpoint

1. The number of patients in each treatment group that received ECMO.

Secondary Endpoints

1. Improvement in arterial oxygenation, measured by arterial-alveolar oxygen ratio (a-A ratio), the alveolar-arterial oxygen gradient ($A\text{-}aDO_2$), the arterial partial pressure of oxygen (PaO_2), and the oxygenation index (OI) in the treatment groups.

2. Incidence of the following in the two treatment groups:
   - a. Physiologic measures
     - *i.* blood pressure,
     - *ii.* gas exchange,
     - *iii.* methemoglobin levels.
   - b. Safety measures
     - *i.* discharge home on O_2 and/or pulmonary medications,
     - *ii.* neurologic abnormalities
     - *iii.* survival to discharge.

3.6 Number of Subjects/ Randomization

Central randomization codes were created using blocks of ten patients in each disease stratum (e.g., RDS, MAS). Enrollment books were created for each center including sealed envelopes containing the randomization packet. Each envelope was labeled with the site designation, the underlying disease stratum, and a sequential patient number. The blinded investigators were to open the envelopes sequentially within each stratum.

3.7 Inclusion/ Exclusion Criteria

Inclusion Criteria (all must be present)

1. Estimated gestational age ≥34 weeks.
2. Age <96 hours at time of entry.
3. Severe respiratory failure defined as OI >25 on optimized ventilator settings (including HFOV in all patients with significant parenchymal disease).
4. Postductal arterial line for sampling blood gases.

At least one of the following must also be present:

5. Echocardiographic evidence of pulmonary hypertension without structural heart disease.

and/or 6. Clinical evidence of pulmonary hypertension with at least one of the following:
   - a. differential oxygenation in preductal and postductal areas.
   - b. marked clinical lability in oxygenation despite optimized treatment of the lung disease.
     - 1) >2 desaturation events within 12 hours due to PPHN and not vent setting.
     - 2) need for extreme alkalosis to maintain adequate oxygenation (pH >7.60).

3.7 Inclusion/ Exclusion Criteria (cont)

Exclusion Criteria (none can be present)

1. Urgent need for ECMO:
   a. Refractory hypotension (< 35 mm Hg) despite vasopressors and volume support.
   b. Refractory hypoxemia (PaO_2 <30 mm Hg) despite maximum ventilatory support.
2. Lethal congenital anomaly.
3. Significant bleeding diathesis.
4. Active seizures on anticonvulsants, or a history of prolonged severe asphyxia:
   a. PaO_2<10 mm Hg for 1 hour or PaO_2<20 mm Hg for 2 hours.
   b. pH <7.0 for >1 hour despite resuscitative efforts.
5. Cyanotic congenital heart disease.
6. Any other reason that would exclude the use of ECMO.

3.8 Dosage/ Administration

Treatment gas was administered using a delivery device that diluted the gas (100% N_2 or NO 800 ppm) 1:20 delivered to the endotracheal tube. Adjustments to the study gas rates were similar whether the patient received placebo or I-NO.

3.9 Duration/ Adjustment of Therapy

Treatment gas (I-NO or N_2) was started at 20 ppm and continued for at least 4 hours. At that point, if the PaO_2 was >60 mm Hg with a pH ≤7.55, the dose was decreased to 5 ppm (otherwise the I-NO was continued at 20 ppm for a maximum of 24 hours). Infants could be continued on I-NO 5 ppm for up to 96 hours of gas administration or the patient was 7 days of age.

Adjustments made to study gas are discussed in section 3.4 above.

3.10 Safety and Efficacy Endpoints Measured

The timing of the various tests performed as part of CINRGI is presented in tabular form below.

Table 3.10.1 Timetable for clinical observations and lab measurements in CINRGI[a].

Test	Study Day			
	0	1-30	Discharge	Day 30/ Completion
Inclusion/Exclusion	X			
Labs	X			
Hemodynamics	X	X		X
Oxygenation parameters	X	X	X	X
Ventilation parameters	X	X		X
NO_2/MetHbg levels	X	X		
Head U/S	X		X	
Echocardiogram	X			
Neurological exam			X	
Outcome				X
Adverse Events		X		X

a. Data from NDA vol. 9.6, Table 2.

3.11 Statistical Considerations

Power

The sample size was based on an expected rate of ECMO use in the placebo group of 50% compared with a 30% ECMO use in the I-NO group. Using an overall alpha of 0.05, with 80% power, along with a one-to-one randomization of placebo/I-NO group it was determined that 103 patients in each arm would be needed.

Multiplicity

No adjustments for multiplicity were performed.

Interim Analyses

There were no interim analyses.

3.11 Statistical Considerations (cont)

Statistical Analysis

The primary endpoint of the trial was the number of patients in each treatment group who received ECMO. The principal analysis was an intent-to-treat analysis stratified by disease using Cochran-Mantel-Haenszel (CMH) method.

The secondary endpoints were analyzed using CMH, Fisher's 2-tailed exact test, or Student's t test as appropriate.

Because infants with pulmonary hypoplasia were expected to have substantially higher morbidity/mortality as well as responses to I-NO, the safety outcomes were analyzed separately and then integrated with the other infants in the trial.

3.12 Efficacy Outcomes for the CINRGI Trial

3.12.1 Subject Demographics & Baseline Characteristics

The demographic and clinical background data for the subjects enrolled in CINRGI are summarized below.

Table 3.12.1.1 Demographics of CINRGI[a].

Demographic	Placebo N=89	I-NO N=97	p Value[b]
Gender			
Male	52 (58.4%)	44 (45.4%)	0.08
Race (n (%))			
Caucasian	44 (49.4%)	40 (41.2%)	0.30
Black	33 (37.1%)	43 (44.3%)	
Hispanic	10 (11.2%)	8 (8.2%)	
Other	2 (2.2%)	6 (6.2%)	
Mean Age Since Birth (hrs ±SD)	29.9±16.5	30.0±20.2	0.95
Mean Age (±SD)[c]	38.8±2.1	39.2±1.7	0.20
Mean Weight, kg (±SD)	3.3±0.6	3.3±0.6	0.81
Apgar Scores			
1 Minute	5.4±2.8	5.2±2.5	0.69
5 Minute	7.3±2.2	7.4±1.8	0.72

a. Data from CINRGI study report, table 7-8.
b. p Value per sponsor.
c. Mean age assessed by physical exam at birth.

Table 3.12.1.2 Birth demographics of CINRGI[a].

Peri-Natal Demographic	Placebo N=89	I-NO N=97	p Value[b]
Obstetrical complications	64/89 (71.9%)	54/97 (55.7%)	0.023
Fetal Distress	48/89 (53.9%)	46/96 (47.9%)	0.46
Cesarean Section	52/89 (58.4%)	42/97 (43.3%)	0.042
Resuscitation at birth	9/89 (10.1%)	8/96 (8.3%)	0.80
CPR at birth	1 (1.1%)	1 (1.0%)	1.00
Normal Head U/S	79/87 (90.8%)	88/95 (92.6%)	0.79

a. Data from CINRGI study report, table 7-8.
b. p Value per sponsor.
c. Mean age assessed by physical exam at birth.

The two treatment groups were well-balanced with regard to the cause of the hypoxic pulmonary failure.

Table 3.12.1.3 Underlying disease leading to hypoxic respiratory failure in CINRGI[a].

Underlying Disease	Placebo N=89	I-NO N=97
Meconium aspiration	35 (39%)	34 (35%)
Pneumonia/Sepsis	21 (24%)	24 (25%)
Respiratory Distress Syndrome	8 (9%)	8 (8%)
Persistent Pulmonary Hypertension	25 (28%)	31 (32%)

a. Data from CINRGI study report, table 32.

3.12.1 Subject Demographics & Baseline Characteristics (cont)

The investigators collected individual information on the severity of the pulmonary disease at entry as well. Note that the control group had significantly more airleak and more pulmonary hemorrhage compared with the I-NO group. The severity of the pulmonary injury on CXR was similar in the two groups.

Table 3.12.1.4 Pulmonary disease at birth in CINRGI[a].

Peri-Natal Demographic	Placebo N=89	I-NO N=97	p Value[b]
Airleak Syndrome	22 (24.7%)	11 (11.3%)	0.021
Pulmonary Hemorrhage	8 (9.0%)	4 (4.1%)	0.24
Lung Disease on CXR			
None	6 (6.7%)	4 (14.4%)	0.60
Mild	28 (31.5%)	26 (26.8%)	
Moderate	42 (47.2%)	41 (42.3%)	
Severe	13 (14.6%)	16 (16.5%)	

a. Data from CINRGI study report, table 7-8.
b. p Value per sponsor.

Vasoactive drugs used at time of entry were well-balanced as to class among the treatment groups, as summarized below. There was a trend towards more use of epinephrine in the I-NO group among a small number of infants. In data not shown, the average dose of epinephrine and tolazoline in the small number of infants receiving those therapies was higher numerically in the I-NO group.

Table 3.12.1.5 Baseline medications for patients in the CINRGI study[a].

Baseline Medications (%)	Placebo N=89	I-NO N=97	p Value
Dopamine	78 (87.6%)	85 (87.6%)	1.00
Dobutamine	33 (37.1%)	34 (35.1%)	0.88
PGE	3 (3.4%)	3 (3.1%)	1.00
Epinephrine	5 (5.6%)	13 (13.4%)	0.086
Tolazoline	1 (1.1%)	4 (4.1%)	0.37
Surfactant	42 (47.2%)	34 (35.7%)	0.10
Sodium Bicarbonate	64 (71.9%)	75 (77.3%)	0.41
Steroids	9 (10.1%)	7 (7.2%)	0.60

a. Data from CINRGI study report, tables 13 and 14.

As part of the entry criteria, all infants were to have evidence of pulmonary hypertension, and almost all infants had cardiac ECHOs. Beyond the presence of pulmonary hypertension, the treatment groups were balanced with regard to the presence or absence of the following (see CINRGI study report tables 15, 16 for details):

1) Patent ductus arteriosus (including right-to-left and left-to-right shunts).
2) Atrial shunts (including right-to-left and left-to-right shunts).
3) Mean ejection fraction.
4) Tricuspid regurgitation.

The hemodynamics at baseline are summarized below. Note that the I-NO group had a lower mean arterial pressure. Data are shown only for those infants with available data (approximately 90% of enrolled subjects). Recall also that many of the infants were on vasoactive medications that might affect blood pressure.

Table 3.12.1.6 Baseline hemodynamics for patients in the CINRGI study[a].

	Placebo	I-NO	p Value
Arterial Pressure (mm Hg)	55.8±12.3	51.6±11.0	0.019
Heart Rate	157±24	152±25	0.21

a. Data from CINRGI study report, tables 18.

3.12.1 Subject Demographics & Baseline Characteristics (cont)

The next table summarizes the available data on the gas exchange at baseline in the two groups. Note the significant differences in the oxygenation and % saturation between the two groups.

Table 3.12.1.7 Baseline oxygenation status for patients in the CINRGI study[a].

	Placebo	I-NO	p Value
pH	7.44±0.13	7.46±0.13	0.25
PaO_2 (mm Hg)	54.3±36.1	77.6±68.3	0.007
$PaCO_2$ (mm Hg)	35.6±12.4	34.2±13.2	0.49
SaO_2 (%)	84.1±16.6	89.6±12.6	0.018
OI (cm H_2O/ mm Hg)	43.9±22.7	35.0±20.9	0.011

a. Data from CINRGI study report, tables 18-19.

One possible explanation offered by the sponsor for this difference was that some infants had their final PaO_2 done shortly after starting the treatment gas. This occurred because, per protocol, there were two teams working on the infants, and due to the acute severity of their illness the specific timing of samples was inadvertently missed. One test of this possibility is to look at the PaO_2 and OI measured (per protocol) at 2 and 4 hours before baseline (thus well-prior to the institution of study gas). These data are summarized in the table and graph below. The pulmonary airway pressure (PAW) is also summarized as it is incorporated in the formula for determining OI (see Appendix One). The $\%O_2$ inspired (FiO_2) did not change between time periods. Note that at all time points measured the oxygenation was better for the I-NO group, relative to the control group. Note also that the rates of change for PaO_2 and OI appear to be higher in the control group than in the I-NO group.

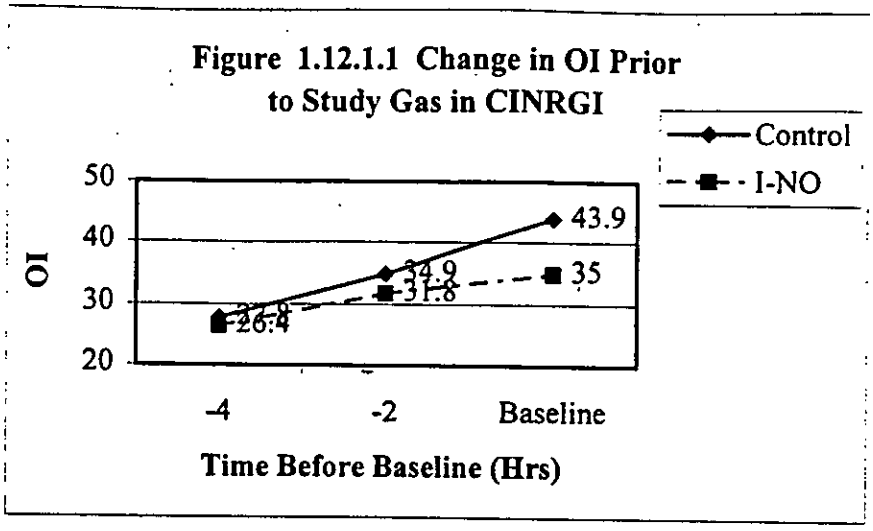
Table 3.12.1.8 Baseline oxygenation status for patients in the CINRGI study[a].

	PaO_2	Change in PaO_2[b]	OI	Change in OI[b]	P_{AW}
Control					
- 4 Hours	74.8	–	27.8	–	16.8
- 2 Hours	63.7	-11.1	34.9	+7.1	17.8
Baseline	54.3	-9.4	43.9	+9.0	19.0
I-NO					
- 4 Hours	81.8	–	26.4	–	15.5
- 2 Hours	77.6	-4.2	31.8	+5.4	16.3
Baseline	77.6	0.0	35.0	+3.2	17.6

a. Data from CINRGI study report, tables 18-19.
b. Change in PaO_2 (in mm Hg/ 2 hours) calculated as difference between timepoints and previous average value.

The change in OI with time is shown below. There appears to be a consistent trend towards a higher OI in the control group, relative to the I-NO group, evident from at least 2 hours prior to baseline. Even if one assumes that the recorded baseline values are 'contaminated' by individuals receiving study gas (I-NO or control), the values at – 2 hours still suggest some baseline differences between the two treatment groups with regard to oxygenation status. It is also worth noting that the average infant in the control group meets the 'OI' criterion for treatment failure at the time of randomization (OI >40).

3.12.1 Subject Demographics & Baseline Characteristics (cont)



Information on the method of ventilation was available for 85/89 patients in the control group (95.5%) and 93/97 of the I-NO subjects (95.9%). A higher percentage of the control patients were on high frequency ventilation at the time of entry (62.3% for control vs. 49.4% for I-NO).

3.12.2 Disposition of Subjects

There were 248 neonates enrolled in CINRGI, including 36 enrolled in the randomized but unblinded pilot study. These are not included in the following analyses, unless noted, leaving 212 patients. Of these, 26 had a diagnosis of lung hypoplasia, and were not included in the primary analysis per protocol. The efficacy population is thus 186 patients in the CINRGI trial.

Within the 186 patients, 14 patients were randomized by failed to meet entry criteria at time of starting treatment gas and were thus excluded from the primary efficacy analysis (8 I-NO, 6 control gas).

Appendix three is a diagram of the disposition of the subjects in CINRGI.

3.12.2a Subject Selection

No information is available about the subjects who were considered but not enrolled in CINRGI.

3.12.2b Protocol Violations & Deviations

As the CRF did not record whether the infants met all entry criteria, this analysis was performed retrospectively. There were 15 patients with 18 entry criteria violations. Note that there were 2 infants in the placebo group with immediate need for ECMO, as well as 7 infants in the I-NO group whose entry OI was too low (i.e., their pulmonary disease was not sufficiently severe).

Table 3.12.2b.1 Entry criteria violations in CINRGI[a].

Entry Criteria Violation	Placebo	I-NO
OI <25	1	7
Gestational age <34 weeks	1	0
No evidence of pulmonary HTN	1	2
Urgent need for ECMO	2	0
Bleeding diathesis	1	0
Cyanotic heart disease	0	1
No postductal arterial line	0	1
Prolonged birth asphyxia	0	1

a. Data from CINRGI study report, table 4.

In addition to these individuals, randomization envelopes were incorrectly opened on four occasions (e.g., I-NO delivery device not available, opened from wrong disease strata). In all cases, the envelopes were not re-used.

3.12.2c Patient Randomization and Completion

Table 3.12.2c.1 Summary of subjects entered into each dose group in CINRGI[a].

Demographic	MAS	Pneumonia/ Sepsis	RDS	PPHN	Lung Hypoplasia
Randomized[b]	69	45	16	56	26
Received I-NO	35	24	8	31	11
Received Control gas	34	24	8	31	11
ECMO					
I-NO Received ECMO	10 (29%)	8 (33%)	1 (13%)	9 (29%)	0 (0%)
Control Gas Received ECMO	21 (60%)	12 (57%)	7 (88%)	7 (28%)	0 (0%)
Death					
I-NO Died	0 (0%)	0 (0%)	2 (25%)	0 (0%)	0 (0%)
Control Gas Received ECMO	0 (0%)	0 (0%)	0 (0%)	1 (4%)	1 (7%)

a. Data from NDA vol. 9.6, CINRGI study report, fig. 1.
b. This table does not include those infants enrolled in the pilot, unblinded portion of the study (36 total). They are included in the publication resulting from these studies.

Because of the definitions used, patients were discontinued for 'treatment failure,' as defined above. This definition includes patients who received ECMO as well as those who were discontinued for failure to respond or due to worsening clinical condition. These events are summarized below, with all of the types of neonatal respiratory failure combined.

Table 3.12.2c.2 Summary of subjects entered into each dose group in CINRGI[a].

	Placebo	I-NO	p Value[b]
Patients treated	89	97	
Treatment successes[b]	32 (36.0%)	60 (61.8)	0.0001
Treatment failures	57 (54.0%)	37 (38.1%)	
Due to ECMO criteria	46 (51.7%)	25 (25.8%)	0.219
Due to toxicity	0 (0%)	2 (2.1%)	0.152
Due to failure to respond	31 (34.8%)	12 (12.4%)	0.056
Due to condition worsening	14 (15.7%)	6 (6.2%)	0.441

a. Data from NDA vol. 9.6, CINRGI study report, Table 3.
b. The definitions of success and failure are included above in section 1.4.

Appendix three is a diagram of the disposition of the subjects in CINRGI.

3.12.2d Study Drug and Concomitant Therapies Administered During Study

The first table below shows the amount of study drug administered to each of the treatment groups. The difference in the time of study gas administration is related to the increased number of drop-outs for treatment failure in the control group.

Table 3.12.2d.1 Dose and duration of infusion of study drug in CINRGI[a].

Study Group	Placebo (n=87)	I-NO (n=93)
Mean±SD time on study gas	27.1±30.6	40.1±32.3
Median time on gas (hours)	73.3	27.8

a. Data from CINRGI study report, table 41. Similar data, including the infants with CDH, is not shown (table 42).
b. Shown as mean±sd (range).

The sponsor summarized the information on the use of vasoactive drugs (i.e., dopamine, PGE, epinephrine) and other drugs commonly used in the treatment of pulmonary hypertension. This information has been included in the Demographics section above table, 3.12.1.5.

3.12.3 Primary Analyses of CINRGI Trial Results

The primary endpoint of the CINRGI trial was the number of infants receiving ECMO in each treatment group, with adjustment for the underlying disease using Cochran-Mantel-Haenszel (CMH). Per protocol, the primary analysis excluded those infants who had lung hypoplasia. The results are shown below for the intent-to-treat population.

Table 3.12.3.1 Primary efficacy outcome from the CINRGI trial[a].

	Placebo N=89	I-NO N=97	p Value[b]
Received ECMO	51 (57.3%)	30 (30.9%)	0.001
Did not receive ECMO	38 (42.7%)	67 (69.1%)	

a. Data from CINRGI study report table 23. Shown for the non-lung hypoplasia population.
b. p Value using CMH adjusting for underlying disease. p Value by Fisher's exact test =0.0004 per sponsor.

Similar results were obtained if the non-lung patients were analyzed according to the gas actually received. One infant received I-NO after being randomized to placebo.

Table 3.12.3.2 Primary efficacy outcome from the CINRGI trial[a].

	Placebo N=88	I-NO N=98	p Value[b]
Received ECMO	50 (56.8%)	31 (31.6%)	0.001
Did not receive ECMO	38 (43.2%)	67 (68.4%)	

a. Data from CINRGI study report table 31. Shown for the non-lung hypoplasia population.
b. p Value using CMH adjusting for underlying disease. p Value by Fisher's exact test =0.0004 per sponsor.

When the 15 infants listed in section 3.12.2b above as not meeting entry criteria were removed, I-NO use was still associated with a nominally significant reduction in the use of ECMO.

Table 3.12.3.3 Primary efficacy outcome from the CINRGI trial[a].

	Placebo N=85	I-NO N=86	p Value[b]
Received ECMO	49 (57.6%)	26 (30.2%)	0.001
Did not receive ECMO	36 (42.4%)	60 (69.8%)	

a. Data from CINRGI study report table 30. Shown for the non-lung hypoplasia population.
b. p Value using CMH adjusting for underlying disease.

The FDA statistician also examined if the baseline imbalances altered the results seen in CINRGI, and concluded that 'the reviewer and sponsor's analyses suggest a difference in the rate of use of ECMO between the two groups even adjusted for the imbalance of baseline OI.' The reader is referred to Dr. Cui's review for further details.

3.12.4 Secondary Analyses of CINRGI Trial Results

There were two pre-specified endpoints of the CINRGI trial: 1) improvement in arterial oxygenation, and 2) safety measures (e.g., chronic lung disease, neurologic abnormalities, survival to hospital discharge). The effects of I-NO on oxygenation will be considered after a summary of the safety endpoints measured during the trial.

Discharge Pulmonary Status

Fewer infants in the I-NO group were discharged with 'chronic lung disease', as defined by the individual investigators. This difference achieved nominal statistical significance, although the endpoint was not pre-specified. There was also a trend favoring I-NO with regard to use of O_2 or pulmonary medications at time of discharge.

Importantly, there was no significant relationship between the presence of lung injury at the time of entry into the trial (e.g., airleak syndrome) and the presence of chronic lung disease (analyzed by the FDA). This is important as there was a baseline imbalance in the incidence of both airleak syndrome and pulmonary hemorrhage at the time of entry into the trial (see table 3.12.1.4 above, higher incidence in the control group).

Table 3.12.4.1 Discharge status of infants in CINRGI[a].

	Placebo	I-NO	p Value
Chronic lung disease	11/82 (13.4%)	3/92 (3.3%)	0.023
Discharged on home O_2	7/81 (8.6%)	3/90 (3.3%)	0.195
Discharged on pulmonary medications	6/81 (7.4%)	4/90 (4.4%)	0.520
Discharged on O_2 or pulmonary medications	10/89 (11.2%)	6/97 (6.2%)	0.296

a. Data from CINRGI study report, table 28. Data shown only for those infants with available data.

3.12.4 Secondary Analyses of CINRGI Trial Results (cont)

Duration of Hospitalization

Duration of hospitalization was similar between the two treatment groups.

Table 3.12.4.2 Duration of hospitalization in CINRGI[a].

Hospital stay (days)	Placebo	I-NO	p Value
Mean±sd	25.3±17.3	22.5±10.3	0.198[b]
Median	21.0	20.0	
# of Patients with Data	79	90	

a. Data from CINRGI study report, table 29. Data shown only for those infants with available data.
b. p Value using Student's t test per sponsor.

Discharge Neurologic Status

The neurologic status at time of discharge is summarized below. Note that only information on these data are missing for approximately 50% or more of the infants in both treatment groups.

Table 3.12.4.3 Discharge neurologic status in the CINRGI trial[a].

	Placebo	I-NO	p Value
Abnormal Head U/S	12/52 (23.1%)	5/42 (11.9%)	0.188
Abnormal Head CT	8/34 (23.5)	12/25 (48.0%)	0.058
Abnormal Neurologic Exam	8/41 (19.5%)	7/48 (14.6%)	0.580
Abnormal CT, U/S or Neurologic Exam	19/89 (21.3%)	17/9 (17.5%)	0.579

a. Data from CINRGI study report, table 67. P Values per sponsor.

Vital Status at 28 Days

The mortality rate was similarly low in both treatment groups. There is no suggestion of increased mortality through 28 days following I-NO treatment.

Table 3.12.4.4 Mortality through 28 days in the CINRGI trial[a].

	Placebo	I-NO
Dead	5/88 (5.7%)	3/97 (3.1%)
Alive	83/88 (94.3%)	94/97 (96.9%)

a. Data from CINRGI study report, table 71.

Changes in Oxygenation

The sponsor performed several analyses comparing the effect of I-NO and control gas on oxygenation, as measured by the following parameters: arterial-alveolar oxygen ratio (a-A ratio), the alveolar-arterial oxygen gradient ($A\text{-}aDO_2$), the arterial partial pressure of oxygen (PaO_2), and the oxygenation index (OI). Because all of these show the same trends, only the changes in PaO_2 will be included here. The reader is referred to the CINRGI NDA study report for the other measurements as needed (Tables 34 to 37). All of these assessments are limited by the large percentage of children who were deemed 'treatment failures' (reflecting in many cases the initiation of ECMO) and thus dropped from the study.

The table on the next page summarizes the changes in the mean PaO_2 through 24 hours for the subjects with available data. At all time points measured, including baseline and the earliest time-point after initiation of study gas, the infants in the I-NO group had a higher mean PaO_2.

3.12.4 Secondary Analyses of CINRGI Trial Results (cont)

Table 3.12.4.5 Arterial PaO_2 over time (mm Hg) in the CINRGI trial[a].

Time		Placebo	I-NO
Baseline	Mean±sd	54.3±36.1	77.6±68.3
	Median	47	53.5
	Number of pts	81	90
30 Minutes	mean±sd	75.9±68.2	136.7±105.8
	median	54.5	92.5
	number of pts	82	86
1 Hour	mean±sd	100.9±102.8	141.4±104.7
	median	60.0	108
	number of pts	77	94
4 Hour	mean±sd	117.6±96.3	145.1±102
	median	74.5	101
	number of pts	62	88
12 Hours	mean±sd	130.9±93.2	147.2±79
	median	92	127
	number of pts	45	80
24 Hours	mean±sd	116.6±67	141.6±77.5
	median	90	115
	number of pts	44	73

a. Data from CINRGI study report, table 36.

The data from CINRGI were also analyzed by the FDA statistician, Dr. Cui. He concluded that the CINRGI ...'analyses fail to support the conclusion that there is a sustained treatment effect of NO on oxygenation.' He also concludes that ...'the NO treatment affected patients more rapidly as compared to the conventional therapy used (as the background therapy) in placebo.' An analysis of the CINRGI oxygenation data from Dr. Cui's draft review is found below. The reader is referred to his review for further details.

Table 3.12.4.6a (from Dr. Cui's review, table 2.4) Analysis of change in OI and PaO_2.

OI			
Time	Control (n, Δ)	NO (n, Δ)	Nominal p-value*
Baseline	(n=75) 43.9	(n=83) 35.0	0.0119
30 min	(n=65) –4.4	(n=71) –11.8	0.0230
1 hour	(n=69) –4.9	(n=81) –12.0	0.0679
4 hours	(n=71) –4.9	(n=82) –13.3	0.0374
12 hours	(n=71) –8.1	(n=82) –14.9	0.0943
24 hours	(n=71) –8.8	(n=82) –15.1	0.1198
PaO_2			
Time	Control (n, Δ)	NO (n, Δ)	Nominal p-value*
Baseline	(n=81) 54.3	(n=90) 77.6	0.0055
30 min	(n=75) 23.7	(n=80) 57.8	0.0093
1 hour	(n=78) 38.3	(n=89) 63.9	0.1023
4 hours	(n=79) 44.4	(n=89) 63.6	0.2422
12 hours	(n=79) 45.3	(n=89) 61.4	0.3005
24 hours	(n=79) 36.6	(n=89) 46.5	0.4874

* for group difference

3.12.5 Additional Analyses of CINRGI Trial Results

Sub-group analyses on the use of ECMO

Because of the presence of several baseline imbalances in the trial between treatment groups, the sponsor examined the interactions of several baseline variables with the incidence of death and/or ECMO. In this post-hoc analysis, the effect of I-NO to reduce the use of ECMO persisted when all of the baseline imbalances were included in the logistic regression model.

Table 3.12.5.1 Effect of baseline variables on I-NO effect on ECMO, using logistic regression analysis[a].

Parameters in the model	# of Patients	p Value for I-NO effect on ECMO
OI PaO_2	178	0.021
Underlying disease OI/PaO_2	178	0.021
Underlying disease OI/PaO_2 Airleak Syndrome on entry Delivery by C-section Obstetrical Complications No prenatal care prior to 3rd trimester	172	0.014
OI Delivery by C-section Obstetrical complications No prenatal care prior to the 3rd trimester	172	0.014
OI Airleak Syndrome at entry Delivery by C-section Obstetrical complications No prenatal care prior to the 3rd trimester	172	0.014

a. Data from CINRGI study report, table 26. Based on the non-lung hypoplasia population.

Because the I-NO group had a lower PaO_2 and lower OI at baseline, the sponsor also analyzed the results by stratifying the infants according to entry PaO_2 and OI. A significant fraction of the decrease in ECMO use was in 'moderately' ill infants.

Table 3.12.5.2 Rate of ECMO stratified by baseline PaO_2[a].

	Placebo	I-NO
≤30 mm Hg	11/11 (100%)	5/6 (83.3%)
30 to ≤50	20/40 (50.0%)	13/32 (40.6%)
50 to ≤70	12/19 (63.2%)	4/25 (16.0%)
70 to ≤100	2/6 (33.3%)	3/13 (23.1%)
>100	1/5 (20%)	2/14 (14.3%)

a. Data from CINRGI study report, table 24. Subject with unknown PaO_2 at baseline not shown.

The sponsor also used CMH to test the rate of ECMO controlling for baseline PaO_2, and reported a p Value= 0.007.

Table 3.12.5.3 Rate of ECMO stratified by baseline OI[a].

	Placebo	I-NO
≤30 cm H_2O/ mm Hg	7/20 (35.0%)	9/43 (20.9%)
30 to ≤40	9/20 (45.0%)	4/15 (26.7%)
40 to ≤50	7/11 (63.6%)	6/11 (54.5%)
>50	12/24 (50.0%)	8/14 (57.%)

a. Data from CINRGI study report, table 24.

The sponsor used CMH to test the rate of ECMO controlling for baseline OI, and reported a p Value = 0.007.

3.12.5 Additional Analyses of CINRGI Trial Results (cont)

Recall also that there was an imbalance with regard to the OI in the treatment groups, and that there appeared to be a difference in the rate at which the infants were getting sicker (table 3.12.1.8 and Figure 1.12.1.1 above). From these data, the I-NO patients appeared to be more stable with regard to their pulmonary status than the control group. To address the impact of this imbalance, the sponsor performed additional modeling, seeking to incorporate the change in the baseline. Per their analysis, incorporating this into the model does not eliminate the significant effect of I-NO to reduce the use of ECMO in CINRGI. The p-Values for this analysis are shown in the table below.

Table 3.12.5.4 Logistics model examining effect of varying baseline slope for oxygenation parameters on reduction in ECMO by I-NO[a].

Parameter	p-Value for I-NO effect on ECMO[b]	p-Value for I-NO effect on ECMO[c]
OI (n=101)	0.045	0.0025
PaO_2 (n=150)	0.0013	0.0006
FiO_2	0.0002	0.0002

a. Data from sponsor at request of Medical Reviewer. Not confirmed by FDA analysis.
b. For change in parameter prior to baseline.
c. For change in slope of change in parameter prior to baseline.

Finally, the sponsor also analyzed the results of the trial according to the underlying disease. ECMO use was reduced in each stratum in the I-NO group except the lung hypoplasia/CDH group. Note that relatively few patients with PPHN received ECMO in either treatment group.

Table 3.12.5.5 Infants receiving ECMO grouped by underlying disease, from the CINRGI trial[a].

	Placebo N=89	I-NO N=97
Meconium aspiration	21/35 (60.0%)	11/34 (32.4%)
Pneumonia/sepsis	14/21 (66.7%)	9/24 (37.5%)
Respiratory Distress Syndrome	7/8 (87.5%)	1/8 (12.5%)
Persistent Pulmonary HTN	9/25 (36.0%)	9/31 (29.0%)
Lung Hypoplasia/ CDH	13/15 (86.7%)	9/11 (81.8%)

a. Data from CINRGI study report table 32 and 33.

<u>Efficacy outcomes in children with congenital diaphragmatic hernia (CDH)/ lung hypoplasia</u>

It was anticipated that I-NO would have little clinical efficacy in the CDH population, leading to its exclusion from the population used for the primary efficacy analysis. The sponsor did collect information on this sub-group separately, which is summarized below. No beneficial effects of I-NO in this population were detected.

Table 3.12.5.6 Efficacy outcomes in infants with lung hypoplasia due to CDH[a].

Clinical Event	Placebo N=15	I-NO N=11	p-Value[b]
Receipt of ECMO	13 (87%)	9 (82%)	1.00
Death within 28 days	6 (40%)	4 (36%)	1.00
Hospital Stay (days)	70±41	53±37	0.40

a. Data from CINRGI study report, table 33.
b. p Value calculated using Student's t test.

3.13 Safety Outcomes from the CINRGI Trial

3.13.1 Comparisons of Defined Safety Endpoints

The protocol-specified safety outcomes, death, duration of hospitalization, discharge pulmonary status and discharge neurologic status, are included in section 1.13.2 above.

3.13.2 Comments on Specific Safety Parameters

Deaths

Mortality was assessed at the end of 28 days and again at 6 months (for 185 patients with available follow-up). Narratives of the deaths are to be found in appendix two.

Table 3.13.2.1 All-cause mortality through 28 days in CINRGI[a].

Treatment Group	Placebo N=89	I-NO N=97
Dead	5 (5.7%)	3 (3.1%)
Alive	83 (94.3%)	94 (96.9%)

a. Data from CINRGI study report, table 71 and from electronic datasets.

Table 3.13.2.2 All-cause mortality through 6 months in CINRGI[a].

Treatment Group	Placebo N=	I-NO N=
Dead	5 (5.7%)	4 (4.1%)
Alive	83 (94.3%)	93 (95.9%)

a. Data from CINRGI study report, table 72 and from electronic datasets.

As anticipated from the literature, the mortality rate in the patients with lung hypoplasia/congenital diaphragmatic hernia was substantially higher at 28 days.

Table 3.13.2.3 All-cause mortality for infants with lung hypoplasia through 28 days in CINRGI[a].

	Placebo N=15	I-NO N=11
Dead	6/15 (40.0%)	4/11 (36.4%)
Alive	9/15 (60.0%)	7/11 (63.6%)

a. Data from CINRGI study report table 33. Shown for the lung hypoplasia population.

3.13.2 Serious Adverse Events in CINRGI

The trial did not collect information on the incidence of adverse events that were classified as Serious Adverse Events.

3.13.3 Adverse Events in CINRGI

The sponsor collected adverse events as reported by the investigators through 30 days. The first table summarizes those reported events. For each system, any patient with more than one AE reported for any one body system is counted only once. Individual adverse events are included if they occurred in ≥2% of either treatment group, or if the difference between the two treatment groups was ≥2X (these are also shaded), or if the comparison was of special clinical interest (e.g., renal failure).

3.13.3 Adverse Events in CINRGI (cont)

Table 3.13.3.1 Adverse events reported for the non-lung hypoplasia group in the CINRGI study[a].

Adverse Event	Placebo N=89	I-NO N=97
Body as a Whole	32 (36.0%)	31 (32.0%)
Cellulitis	0 (0%)	5 (5.2%)
Infection	3 (3.4%)	6 (6.2%)
Sepsis	2 (2.2%)	7 (7.2%)
Generalized Edema	8 (9.0%)	3 (3.1%)
Cardiovascular System	42 (34.8%)	31 (32.0%)
Bradycardia	4 (4.5%)	1 (1.0%)
Hemorrhage	10 (11.2%)	6 (6.2%)
Hypotension	9 (10.1%)	13 (13.4%)
Pneumothorax	10 (11.2%)	8 (8.2%)
Nervous System	27 (30.3%)	20 (20.6%)
Cerebral hemorrhage	5 (5.6%)	2 (2.1%)
Convulsion	10 (11.2%	10 (10.3%)
Intracranial hemorrhage	0 (0%)	1 (1.0%)
Gastrointestinal System	21 (23.6%)	22 (22.7%)
LFTs Abnormal	2 (2.2%)	1 (1.0%)
Respiratory System:	34 (38.2%)	32 (33.0%)
Pneumothorax	10 (11.2%)	8 (8.2%)
Stridor	3 (3.4%)	5 (5.2%)
Bronchiolitis	0 (0%)	1 (1.0%)
Hemic & Lymphatic System	61 (68.5%)	50 (51.5%)
Anemia	12 (13.5%)	9 (9.3%)
Coagulation disorder	7 (7.9%)	3 (3.1%)
Leukopenia	15 (16.9%)	4 (4.1%)
Thrombocytopenia	38 (42.7%)	36 (37.1%)
Methemoglobinemia	0 (0%)	2 (2.1%)
Metabolic/Endocrine System	None	
Bilirubinemia	23 (25.8%)	24 (24.7%)
Hyperglycemia	6 (6.7%)	8 (8.2%)
Hypernatremia	5 (5.6%)	2 (2.1%)
Hypomagnesemia	7 (7.9%)	2 (2.1%)
Renal and GU System	16 (18.0%)	9 (9.3%)
Hematuria	5 (5.6%)	8 (8.2%)
Oliguria	7 (7.9%)	0 (0%)
Skin and Appendages System	3 (3.4%)	7 (7.2%)
Skin Ulcer	0 (0%)	3 (3.1%)
Special Senses	3 (3.4%)	6 (6.2%)

a. Data from CINRGI study report, section 12.2. Shown for the non-lung hypoplasia group.

Next, the reported AEs for the lung hypoplasia group is summarized. This sicker population had a higher incidence of adverse events overall than did the infants whose pulmonary failure was not due to lung hypoplasia.

Table 3.13.3.2 Adverse events reported for the lung hypoplasia group in the CINRGI study[a].

Adverse Event	Placebo N=15	I-NO N=11
Body as a Whole	10 (66.7%)	10 (90.9%)
Cardiovascular System	7 (46.7%)	3 (27.3%)
Gastrointestinal System	10 (66.7%)	6 (54.5%)
Respiratory System:	10 (66.7%)	8 (72.7%)
Hemic & Lymphatic System	13 (86.7%)	11 (100%)
Nervous System	2 (13.3%)	4 (36.4%)
Metabolic/Endocrine System	11 (73.3%)	8 (72.7%)
Renal and GU System	6 (40.0%)	7 (63.6%)
Skin and Appendages System	2 (13.3%)	0 (0%)
Special Senses	2 (13.3%)	1 (9.1%)

a. Data from CINRGI study report, section 12.2. Shown for the lung hypoplasia group.

3.13.4 Discontinuations in CINRGI

Adverse events leading to patient discontinuation were not systematically collected in the CINRGI trial.

3.13.5 Lab Adverse Events and Special Studies

Laboratory Measurements

The laboratory data submitted by the sponsor compared the mean changes over time. Initially, laboratory data were collected at baseline, at 24-48 hours after entry, at time treatment was stopped, and 24-48 hours after stopping treatment. Starting in 3.98, values were collected daily until the infant was discharged or through 28 days. Retrospective analysis was used to collect data for infants entered prior to that date.

Overall, the data summary reveals no significant changes in mean serum chemistries or hematology. The reader is referred to my original review of the changes in lab chemistries reported in the INO-01/-02 trial.

Two laboratory changes bear individual examination: elevated methemoglobin and NO_2 levels.

1. Elevated Methemoglobin levels

Two infants in the I-NO group had methemoglobinemia >4% during the treatment period (1.9% of the infants exposed to I-NO). No control infant had elevated methemoglobin. The I-NO group also had a higher mean methemoglobin level during the treatment period on average, when compared with the control group ($p = 0.001$ per sponsor). In data not summarized, there were no cases of elevated methemoglobin levels in the lung hypoplasia sub-group.

2. Elevated NO_2 Levels

No infant in either treatment group developed NO_2 levels >5 ppm during the study. Likewise, there was no significant difference between the two treatment groups with regard to the changes in mean NO_2 levels during the treatment period ($p=0.83$). In data not summarized, there were no cases of elevated NO_2 levels in the lung hypoplasia sub-group.

6-Month and 12-Month Follow-up Data

The sponsor collected long-term follow-up data on as many infants as possible at 6 and 12 months of age. The table below summarizes the available data with regard to some of the safety concerns raised previously for I-NO, and suggest that there were no large increase in the incidence of serious neurologic or pulmonary injury in the I-NO group. Note that the percentage of patients with available follow-up is <50% for all items.

Table 3.13.5.1 Six-month follow-up data from the CINRGI trial[a].

	Placebo	I-NO
Abnormal Neurologic Exam	7/38 (18.4%)	9/32 (28.1%)
Hospital Admissions		
Median	0	0
Mean±SD	0.26±0.58	0.13±0.41
On O_2	2/48 (4.2%)	0/42 (0%)
On pulmonary medications	7/48 (14.6%)	3/42 (7.1%)

a. Data from CINRGI study report, table 38.

Fewer patients have 12-month follow-up data available.

Table 3.13.5.2 Twelve-month follow-up data from the CINRGI trial[a].

	Placebo	I-NO
Abnormal Neurologic Exam	0/24 (0%)	3/20 (15.0%)
Hospital Admissions		
Median	0	0
Mean±SD	0.30±0.26	0.26±0.53
On O_2	0/36 (0%)	0/32 (0%)
On pulmonary medications	6/36 (16.7%)	4/32 (12.5%)

a. Data from CINRGI study report, table 39.

3.14 CINRGI Efficacy Summary

CINRGI was a multicenter, double-blind, randomized, placebo-controlled trial in infants with evidence of persistent pulmonary hypertension of the newborn (PPHN), but without evidence of structural heart disease. All infants were to be receiving high-frequency oscillatory ventilation prior to study enrollment.

Eligible patients were first categorized according to disease type (e.g., respiratory distress syndrome, meconium aspiration syndrome), and then randomized to receive either placebo (N_2) or I-NO.

I-NO was started at 20 ppm and continued for at least 4 hours. At that point, if the PaO_2 was >60 mm Hg with a pH ≤7.55, the dose was decreased to 5 ppm (otherwise the I-NO was continued at 20 ppm for a maximum of 24 hours). Infants could be continued on I-NO 5 ppm for up to 96 hours of gas administration or the patient was 7 days of age.

Treatment gas was continued until FiO_2 was <0.7, the patient had received 96 hours of therapy, or the patient was 7 days old, whichever came first. Once the FiO_2 ≤0.7, weaning attempts were made. Treatment gas could be restarted if the patient required an FiO_2≥0.80 to support a PaO_2≥60 mm Hg. During the first 24 hours, the gas was re-started at 20 ppm. After 24 hours, the gas could only be restarted at 5 ppm. If the patient failed to respond to the reinitiation of study gas, they were deemed a treatment failure and the gas was discontinued.

The primary endpoint was the number of patients in each treatment group that received ECMO.

1. Demographics: With the exceptions discussed below, there were no clinically-significant differences between the demographics of the two treatment groups at time of entry into the trial. These include the underlying disease state, racial and gender demographics, and baseline medications used.

1a. Birth Demographics

The control group had a higher incidence of birth by Cesarean section (table 3.12.1.2).

1b. Pulmonary Disease at Entry Into Trial

The control group had a higher incidence of Airleak Syndrome and Pulmonary Hemorrhage at the time of entry into the trial.

Table 3.14.1 Pulmonary disease at birth in CINRGI[a].

Peri-Natal Demographic	Placebo N=89	I-NO N=97	p Value[b]
Airleak Syndrome	22 (24.7%)	11 (11.3%)	0.021
Pulmonary Hemorrhage	8 (9.0%)	4 (4.1%)	0.24

a. Data from CINRGI study report, table 7-8.
b. p Value per sponsor.

1c. Baseline Hemodynamics

The I-NO group had a higher mean arterial pressure at baseline. Interpretation of this value is difficult, as many of the infants were receiving vasoactive and pressor medications (see table 3.12.1.6).

1d. Baseline Oxygenation

The control group had significantly worse oxygenation at baseline compared with the I-NO group.

Table 3.14.2 Baseline oxygenation status for patients in the CINRGI study[a].

	Placebo	I-NO	p Value[b]
pH	7.44±0.13	7.46±0.13	0.25
PaO_2 (mm Hg)	54.3±36.1	77.6±68.3	0.007
$PaCO_2$ (mm Hg)	35.6±12.4	34.2±13.2	0.49
SaO_2 (%)	84.1±16.6	89.6±12.6	0.018
OI (cm H_2O/ mm Hg)	43.9±22.7	35.0±20.9	0.011

a. Data from CINRGI study report, tables 18-19.
b. p Value per sponsor.

3.14 CINRGI Efficacy Summary (cont)

One possible explanation offered by the sponsor for this difference was that some infants had their final PaO_2 done shortly after starting the treatment gas. This occurred because, per protocol, there were two teams working on the infants, and due to the acute severity of their illness the specific timing of samples was inadvertently missed.

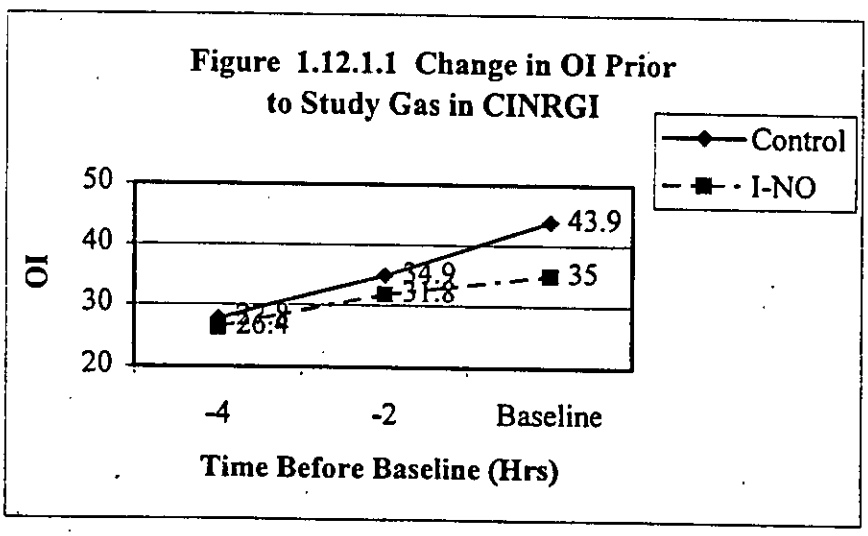
One test of this possibility is to look at the PaO_2 and OI measured (per protocol) at 2 and 4 hours before baseline (thus well-prior to the institution of study gas). These data are summarized in the table and graph below. The pulmonary airway pressure (PAW) is also summarized as it is incorporated in the formula for determining OI (see Appendix One). The $\%O_2$ inspired (FiO_2) did not change between time periods. Note that at all time points measured the oxygenation was better for the I-NO group, relative to the control group. Note also that the rates of change for PaO_2 and OI (shaded boxes) appear to be higher in the control group than in the I-NO group. If true, this suggests that the control group was 'getting sicker faster' than the I-NO group, a significant imbalance between the two treatment groups.

Table 3.14.3 Baseline oxygenation status for patients in the CINRGI study[a].

	PaO_2	Change in PaO_2[b]	OI	Change in OI[b]	PAW
Control					
- 4 Hours	74.8	–	27.8	–	16.8
- 2 Hours	63.7	-11.1	34.9	+7.1	17.8
Baseline	54.3	-9.4	43.9	+9.0	19.0
I-NO					
- 4 Hours	81.8	–	26.4	–	15.5
- 2 Hours	77.6	-4.2	31.8	+5.4	16.3
Baseline	77.6	0.0	35.0	+3.2	17.6

a. Data from CINRGI study report, tables 18-19.
b. Change in PaO_2 (in mm Hg/ 2 hours) calculated as difference between timepoints and previous average value.

The change in OI with time is shown below. There appears to be a consistent trend towards worsening OI in the control group, relative to the I-NO group that is evident from at least 2 hours prior to baseline. Even if one assumes that the recorded baseline values are 'contaminated' by individuals receiving study gas (I-NO or control), the values at 2 hours before baseline still suggest a clinically-significant difference between the two treatment groups with regard to oxygenation status (3 OI units, a difference of 14 mm Hg in PaO_2). It is also worth noting that the average infant in the control group meets the 'OI' criterion for treatment failure at the time of randomization (OI >40).



2. Clinical Efficacy: Primary Endpoint Analysis

The primary endpoint of the CINRGI trial was the number of infants receiving ECMO in each treatment group, with adjustment for the underlying disease using Cochran-Mantel-Haenszel (CMH). Per protocol, the primary analysis excluded those infants who had lung hypoplasia. The results are shown below for the intent-to-treat population, and demonstrate a significantly lower incidence of ECMO use in the I-NO group.

3.14 CINRGI Efficacy Summary (cont)

Table 3.14.5 Primary efficacy outcome from the CINRGI trial[a].

	Placebo N=89	I-NO N=97	p Value[b]
Received ECMO	51 (57.3%)	30 (30.9%)	0.001
Did not receive ECMO	38 (42.7%)	67 (69.1%)	

a. Data from CINRGI study report table 23. Shown for the non-lung hypoplasia population.
b. p Value using CMH adjusting for underlying disease. p Value by Fisher's exact test =0.0004 per sponsor.

Similar results were obtained if the non-lung patients were analyzed according to the gas actually received (table 3.12.1.2) and when the 15 infants who enrolled but did not meet entry criteria (table 3.12.3.3) were removed.

Because the I-NO group had a lower PaO_2 and lower OI at baseline, the sponsor also analyzed the results by stratifying the infants according to entry PaO_2 and OI. The majority of the differences in the treatment groups with regard to ECMO use occurred in the patients with PaO_2 between 50 and 100 mm Hg.

Table 3.14.6 Rate of ECMO stratified by baseline PaO_2[a].

	Placebo	I-NO
≤30 mm Hg	11/11 (100%)	5/6 (83.3%)
30 to ≤50	20/40 (50.0%)	13/32 (40.6%)
50 to ≤70	12/19 (63.2%)	4/25 (16.0%)
70 to ≤100	2/6 (33.3%)	3/13 (23.1%)
>100	1/5 (20%)	2/14 (14.3%)

a. Data from CINRGI study report, table 24. Subject with unknown PaO_2 at baseline not shown.

Because of the presence of several baseline imbalances in the trial between treatment groups, the sponsor examined the interactions of several baseline variables with the incidence of death and/or ECMO (table 3.12.5 above). Factoring in the baseline imbalances due to entry OI, PaO_2, presence of Airleak Syndrome, and birth by Cesarean section did not eliminate the nominal statistical significance of the difference in the use of ECMO between the two treatment groups. The FDA statistician agreed with 'the reviewer and sponsor's analyses (that) suggest a difference in the rate of use of ECMO between the two groups even adjusted for the imbalance of baseline OI.' The reader is referred to Dr. Cui's review for further details.

Table 3.14.7 Effect of baseline variables on I-NO effect on ECMO, using logistic regression analysis[a].

Parameters in the model	# of Patients	P Value for I-NO Effect on ECMO
OI PaO_2	178	0.021
Underlying disease OI/PaO_2	178	0.021
Underlying disease OI/PaO_2 Airleak Syndrome on entry Delivery by C-section Obstetrical Complications No prenatal care prior to 3rd trimester	172	0.014
OI Delivery by C-section Obstetrical complications No prenatal care prior to the 3rd trimester	172	0.014
OI Airleak Syndrome at entry Delivery by C-section Obstetrical complications No prenatal care prior to the 3rd trimester	172	0.014

a. Data from CINRGI study report, table 26. Based on the non-lung hypoplasia population. p Values per sponsor.

3.14 CINRGI Efficacy Summary (cont)

The sponsor also analyzed the results of the trial according to the underlying disease. ECMO use was reduced in each stratum in the I-NO group except the lung hypoplasia group. The use of ECMO in the PPHN patients was low in both treatment groups. This contrasts with the results from the NINOS trial, where 70% of the infants in the control group of the PPHN sub-set received ECMO.

Table 3.14.8 Infants receiving ECMO grouped by underlying disease, from the CINRGI trial[a].

	Placebo N=89	I-NO N=97
Meconium aspiration	21/35 (60.0%)	11/34 (32.4%)
Pneumonia/sepsis	14/21 (66.7%)	9/24 (37.5%)
Respiratory Distress Syndrome	7/8 (87.5%)	1/8 (12.5%)
Persistent Pulmonary HTN	9/25 (36.0%)	9/31 (29.0%)
Lung Hypoplasia/ CDH	13/15 (86.7%)	9/11 (81.8%)

a. Data from CINRGI study report table 32 and 33.

2. Clinical Effect (cont): Secondary Efficacy Analyses

Discharge Pulmonary Status

The sponsor collected data on the incidence of pulmonary injury at the time of discharge in the trial for the following endpoints: chronic lung disease, discharge home on O_2, and discharge home on pulmonary medicines. It should be noted that the incidence of 'chronic lung disease' was not a pre-specified endpoint of the trial, that the definition of 'chronic lung disease' was not specified, and its occurrence was left to the investigators to determine. For the two pre-specified endpoints (discharge to home on O_2 and pulmonary medication), there was a trend towards less pulmonary disease at the time of discharge in the I-NO group, as assessed by the incidence of 'chronic lung disease' and the use of home O_2 or pulmonary medications.

There was no significant relationship between the presence of lung injury at the time of entry into the trial (airleak syndrome) and the presence of chronic lung disease at the time of follow-up (analyzed by the FDA). This is important as there was a baseline imbalance in the incidence of both airleak syndrome and pulmonary hemorrhage at the time of entry into the trial (see table 3.12.1.4 above, showing the higher incidence of these abnormalities at baseline in the control group).

Table 3.14.9 Discharge status of infants in CINRGI[a].

	Placebo	I-NO	p Value
Chronic lung disease	11/82 (13.4%)	3/92 (3.3%)	0.023
Discharged on home O_2	7/81 (8.6%)	3/90 (3.3%)	0.195
Discharged on pulmonary medications	6/81 (7.4%)	4/90 (4.4%)	0.520
Discharged on O_2 or pulmonary medications	10/89 (11.2%)	6/97 (6.2%)	0.296

a. Data from CINRGI study report, table 28. Data shown only for those infants with available data.

Duration of Hospitalization

Duration of hospitalization was similar between the two treatment groups.

Table 3.14.10 Duration of hospitalization in CINRGI[a].

	Placebo	I-NO	p Value
Hospital stay (days)			0.198[b]
Mean±sd	25.3±17.3	22.5±10.3	
Median	21.0	20.0	
# of Patients with Data	79	90	

a. Data from CINRGI study report, table 29. Data shown only for those infants with available data.
b. p Value using Student's t test per sponsor.

3.14 CINRGI Efficacy Summary (cont)

Discharge Neurologic Status

No significant differences were detected in the abnormalities at discharge that were detected by radiology (CT, U/S) or neurologic examination. Note that information is missing for approximately 50% or more of the infants in both treatment groups.

Table 3.14.11 Discharge neurologic status in the CINRGI trial[a].

	Placebo	I-NO	p Value
Abnormal Head U/S	12/52 (23.1%)	5/42 (11.9%)	0.188
Abnormal Head CT	8/34 (23.5)	12/25 (48.0%)	0.058
Abnormal Neurologic Exam	8/41 (19.5%)	7/48 (14.6%)	0.580
Abnormal CT, U/S or Neurologic Exam	19/89 (21.3%)	17/9 (17.5%)	0.579

a. Data from CINRGI study report, table 67. P Values per sponsor.

3. Physiological Efficacy

Exposure to I-NO had an acute effect to improve the oxygenation of infants, which persisted for the duration of exposure to I-NO. Note that oxygenation improved in the placebo group from baseline to 1 hour, even though the number of infants remained more or less constant (that is, while there was not a significant drop-out due to failure).

Table 3.14.4 Arterial PaO_2 over time (in mm Hg) from the CINRGI trial[a].

Time		Placebo	I-NO
Baseline	mean±sd	54.3±36.1	77.6±68.3
	median	47	53.5
	number of pts	81	90
30 Minutes	mean±sd	75.9±68.2	136.7±105.8
	median	54.5	92.5
	number of pts	82	86
1 Hour	mean±sd	100.9±102.8	141.4±104.7
	median	60.0	108
	number of pts	77	94
4 Hour	mean±sd	117.6±96.3	145.1±102
	median	74.5	101
	number of pts	62	88
12 Hours	mean±sd	130.9±93.2	147.2±79
	median	92	127
	number of pts	45	80
24 Hours	mean±sd	116.6±67	141.6±77.5
	median	90	115
	number of pts	44	73

a. Data from CINRGI study report, table 36.

These data were also analyzed by the FDA statistician, Dr. Cui. He concluded that the CINRGI ...'analyses fail to support the conclusion that there is a sustained treatment effect of NO on oxygenation.' He also concludes that ...'the NO treatment affected patients more rapidly as compared to the conventional therapy used (as the background therapy) in placebo.' Further details of his analysis are to be found in section 5.5 below.

3.15 CINRGI Safety Summary

In addition to the efficacy variables measured above, which also yield information about the safety profile of I-NO, the following safety parameters were also examined.

Deaths

Mortality was assessed at the end of 28 days and again at 6 months (for 185 patients with available follow-up). Narratives of the deaths are to be found in appendix two.

Table 3.15.1 All-cause mortality through 28 days in CINRGI[a].

Treatment Group	Placebo N=89	I-NO N=97
Dead	5 (5.7%)	3 (3.1%)
Alive	83 (94.3%)	94 (96.9%)

a. Data from CINRGI study report, table 71 and from electronic datasets.

Table 3.15.2 All-cause mortality through 6 months in CINRGI[a].

Treatment Group	Placebo	I-NO
Dead	5 (5.7%)	4 (4.1%)
Alive	83 (94.3%)	93 (95.9%)

a. Data from CINRGI study report, table 72 and from electronic datasets.

Adverse Events

No clinically significant differences in the incidence of reported adverse events are clear from the CINRGI trial (see table 3.13.3.1). Individual adverse events will be discussed in the context of the overall trial database in the Integrated NDA Safety Summary below (section 6.0).

Elevated NO_2 and Methemoglobin Levels

Elevated Methemoglobin levels

Two infants in the I-NO group had methemoglobinemia >4% during the treatment period (1.9% of the infants exposed to I-NO). No control infant had elevated methemoglobin. The I-NO group also had a higher mean methemoglobin level during the treatment period on average, when compared with the control group ($p = 0.001$ per sponsor).

Elevated NO_2 Levels

No infant in either treatment group developed NO_2 levels >5 ppm during the study. Likewise, there was no significant difference between the two treatment groups with regard to the changes in mean NO_2 levels during the treatment period ($p=0.83$).

Six-Month and 12-Month Follow-up

The available data do not suggest a large increase in pulmonary or neurologic toxicity (see tables 3.13.5.1, 3.13.5.2). The strength of this conclusion is limited in that less than 50% of the subjects had follow-up for the evaluations through 6 months.

3.16 CINRGI Medical Reviewer's Conclusions

CINRGI started as an unblinded study to investigate the use of low-dose I-NO in infants with pulmonary hypertension and hypoxic respiratory failure (4.94), which had as its endpoint 'the number of infants who needed ECMO.' Approximately 18 months later, the trial was changed to a double-blind study (12.95), followed by a change in the primary endpoint to 'the number of infants who received ECMO' 2 years later (12.97). This chronology is relevant because it reflects the changing emphasis of the trial from a small, investigative trial looking at a numbers-oriented primary endpoint to a large, double-blind trial with an endpoint that relied on the individual judgement of the investigators.

This change in trial goals may, in part, account for the imbalances that are present in the patient populations at baseline in the trial. These imbalances are not trivial, and make it difficult to interpret the results of the trial clearly. The most crucial of these imbalances is the apparent difference in the degree of 'sickness' in the two treatment groups; the control group was getting sicker, faster, and had a higher entry OI than the I-NO group. While the sponsor has submitted modeling that concludes these imbalances do not invalidate the primary findings of the study, they lower the degree of confidence this reviewer has about the results somewhat.

That having been said, what did the trial show with regard to the efficacy of I-NO?

1. First, exposure to I-NO causes an acute increase in oxygenation in as the population of infants with hypoxic respiratory failure. While there is some question about the durability of the effect (see Dr. Cui's review), this effect was similar to what has been seen in all of the other available trials of I-NO (NINOS, INO-01/ -02, and INOSG).

2. Children randomized to the I-NO group receive less ECMO than do infants in the control/placebo group, even when the baseline imbalances were accounted for. There is little overall disagreement that avoiding ECMO is a good thing (e.g., avoids cannulation of large blood vessels, lower risk of infection) if the decision to use ECMO is a truly blinded one. Unfortunately, there is every indication that the decision to proceed to ECMO in the trials of I-NO is a surrogate for the failure of the control gas to improve oxygenation. Since I-NO improves oxygenation (makes babies 'pink'), receipt of ECMO can be seen as a reflection of the acute physiological response to I-NO, and not demonstration of a clinically-relevant effect of I-NO.

3. The other pre-specified endpoints measured in the trial largely focused on the safety of I-NO administration (i.e., duration of hospitalization, neurologic and pulmonary status). The only difference detected was a trend towards fewer infants with evidence of chronic pulmonary damage, as assessed by the investigators, and by the use of either O_2 or pulmonary medications. These data will be relevant in the overall discussion of I-NO toxicity, as there are data from other studies suggesting that I-NO use may be associated with chronic pulmonary damage.

What can be said about the contribution of the CINRGI trial to the overall safety assessment of I-NO?

1. There was no evidence of a large adverse effect on mortality, and the numerical trend favored I-NO.

2. Methemoglobinemia and elevated NO_2 levels are not significant concerns at the doses of I-NO used in CINRGI.

3. The long-term follow-up data are incomplete, but raise no new safety concerns, especially about the neurologic or pulmonary effects of I-NO. Similarly, no evidence of a long-term effect of I-NO on total mortality relative to control was detected.

Individual lab and clinical adverse events will be integrated into the overall safety discussion of I-NO where relevant.

In conclusion, the CINRGI trial, like NINOS and INO-01/ -02, demonstrates that the acute administration of I-NO in the 5-20 ppm range is associated with an improvement in oxygenation within 30 minutes of administration. The difference between the control and I-NO group was clinically-significant (37 mm Hg at 30 minutes). Associated with this improvement, fewer infants received ECMO in the I-NO group, a finding that was statistically significant even when corrected for the baseline imbalances in oxygenation, presence of lung injury, birth demographics, and the rate of clinical deterioration.

Beyond the improvement in oxygenation and decreased ECMO, no effect of I-NO on durable clinical efficacy (duration of hospitalization, neurologic status at discharge) was demonstrated. There was a trend towards less evidence of pulmonary injury at the time of discharge, which is complicated by the baseline imbalance in pulmonary status. Using incomplete follow-up data through one year, no adverse or beneficial effects of I-NO on mortality or neurologic/pulmonary statuses were identified, again relative to the control group.

4.0 Long-Term Follow-Up for INO-01/ -02 and NINOS

During my initial review of NDA 20-845, concerns were raised regarding the long-term safety of I-NO. In the final review document, dated 11.19.97, this reviewer raised the possibility of lung injury following I-NO, based on data in the initial NDA database (see section 8.2.7.2 of original review for details). First, data from INO-01/ -02 suggest that there may be an increase in reactive airways disease in the period during and after exposure to I-NO, in agreement with data in the literature about the acute effects of I-NO in adults. Second, there was an increased rate of pneumothoraces in infants exposed to I-NO in the INO-01/ -02 and NINOS trials. Finally, a higher percentage of the infants discharged following treatment with I-NO used O_2 for a longer period of time during the first year after discharge. At the time of the review, the inadequacies of the database were discussed with the sponsor, and they were encouraged to collect long-term follow-up regarding the clinical course of the infants in the available studies (NINOS and INO-01/ -02 trials). The results of this follow-up have been summarized by the sponsor, and are presented below by study (NINOS and INO-01/ -02).

In addition, the sponsor had proposed the collection of long-term neurodevelopmental data on all available infants. For the present NDA submission, they have summarized the neurodevelopmental data on all infants with follow-up, and these data are summarized below.

4.1 NINOS Follow-Up

NINOS Trial Design

The NINOS trial was a multi-center, multi-national, double-blind, placebo-controlled trial designed ...'to evaluate the efficacy of I-NO (inhaled nitric oxide) in the treatment of term and near-term infants with hypoxic respiratory failure.'

Subjects with hypoxic respiratory failure were randomized to one of two groups:

1) a control group who received either oxygen (or no flow of I-NO);

2) a treatment group who received I-NO for up to 336 hours (14 days). Initially, subjects received I-NO 20 ppm.

After 30 minutes on study gas ('low-flow'), the subject's response was measured:

Full response: > 20 mmHg increase in PaO_2.

Partial response: 10-20 mmHg increase in PaO_2.

No response: <10 mmHg increase in PaO_2.

Subjects who responded fully were continued on the 'low-flow' study gas (either placebo (O_2) or I-NO, 20 ppm).

Subjects who had no response, or responded partially, were entered into the 'high-flow gas' protocol. These subjects were administered either placebo gas (O_2) or to I-NO, 80 ppm, depending on their initial randomization. Their response was measured after another 30 minutes, and based on the above criteria for successful response, the high-flow gas was either continued or discontinued.

Non-responders to the high-flow gas were weaned off of the study gas. They were eligible for a repeat trial of the same study gas (either low- or high-flow) after 6 hours, so long as the infant was still otherwise eligible. This process could be repeated three times. If no positive response was observed after 3 repeat trials (a total of 4 trials), the subject was labeled a non-responder.

Responders to high-flow study gas (I-NO 80 ppm or O_2 placebo) underwent regularly scheduled weaning attempts every 2 hours for the first 12 hours, and then every 12±2 hours. Weaning attempts continued until either the gas was completely discontinued, the weaning attempt was unsuccessful or the maximal exposure to study gas was reached.

Responders to low-flow study gas (I-NO 20 ppm or O_2 placebo) also underwent regularly scheduled weaning attempts every 12±2 hours. Weaning attempts continued until either the gas was completely discontinued, the weaning attempt was unsuccessful, or the maximal exposure to study gas was reached.

After 240 hours of gas administration, the study gas concentration had to have been ≤5 ppm I-NO or equivalent O_2 flow. Maximum exposure to study gas administration was to 336 hours (14 days).

Both groups received all other conventional therapies for hypoxic respiratory failure, including surfactant and high frequency jet ventilation. Each center's guidelines determined the mode of ventilation, the use of tolazoline and therapies to maintain arterial pressure, induce alkalosis, and provide sedation/ analgesia.

The primary endpoint of the NINOS trial was the receipt of ECMO and/or death prior to 120 days, to be compared between the two study populations. The sponsor also collected data on a variety of secondary endpoints: duration of hospitalization, need for O_2 at discharge, and the incidence of Air-Leak Syndrome and chronic lung disease.

4.1 NINOS Follow-Up (cont)

Receipt of ECMO was to be guided by a set of criteria (although the final decision to proceed to ECMO was to be made by each investigator individually). The criteria for ECMO were:

1. OI>40 on two ABGs separated by at least 30 minutes, or OI>35 for 4 hours.
2. A-aDO_2 >630 for 4 continuous hours, or A-aDO_2 >620 for 12 continuous hours.
3. Acute deterioration/unresponsiveness to medical therapy (any 2 of 4):
    a. PaO_2 <55 mmHg for at least 2 hours.
    b. pH <7.15, or <7.4 if alkalosis attempted for at least 2 hours.
    c. Mean blood pressure <40 mmHg for at least 2 hours.
    d. Severe barotrauma (at least 4 of the following 7 criteria):
        1. Pulmonary interstitial emphysema/pseudocyst
        2. Pneumothorax/pneumomediastinum
        3. Pneumoperitoneum
        4. Pneumopericardium
        5. Subcutaneous emphysema
        6. Persistent air leak >24 hours
        7. Mean Airway Pressure (MAP) >15 cm H_2O

Infants, including those who received to ECMO, were followed for adverse events until death, discharge to home, or 120 days. Survivors were also followed after discharge, for a planned analysis of neuro-developmental outcomes to be performed †8-24 months after initial study gas administration. In addition, information regarding other medical conditions (e.g., seizures, use of home O_2) was collected by the sponsor.

4.1.1 Additional NINOS Analyses: Receipt of ECMO by the two study populations

Since the original submission, the sponsor has performed additional analyses from the NINOS study data. While the pre-specified endpoint in NINOS relied on the ITT population, there were a number of protocol violations, and the sponsor has performed analyses looking at the clinical endpoints in the population based on actual receipt of study gas.

In addition, there was concern about the difference in the results between those infants who met the criteria for needing ECMO, and those who actually received it. Those analyses are also presented below.

Intent to Treat Population (original study population)

The primary endpoint of the NINOS trial was the receipt of ECMO and/or death prior to 120 days, to be compared between the two study populations. In the original trial results, there was a significant reduction in the incidence of this endpoint, along with a significant reduction in the receipt of ECMO in the I-NO group, compared with the control group. There was, however, no significant difference between the two groups with respect to the number of infants who met the criteria for receipt of ECMO (see table below).

Table 4.1.1.1 (from original NDA review, table 6.0.1.12.2d.2) Incidence of Primary and secondary endpoints from NINOS[b].

	Control n=121	I-NO Therapy n=114	p Value[a]
Met combined primary endpoint[a]	77 (63.6%)	52 (45.6%)	0.0061
Death	20 (16.5%)	16 (14%)	0.596
Met criteria for transfer to ECMO	83 (69%)	67 (59%)	0.12
Received ECMO	66 (54.5%)	44 (38.5%)	0.014
Changes in oxygenation parameters			
Change in PaO_2 (mmHg)[d]	9.7±51.7	58.2±85.2	<0.001
Change in OI[d]	0.8±21.1	-14.1±21.0	<0.001
Change in A-a DO_2 (mmHg)[d]	-6.7±57.5	-60.0±85.1	<0.001
Among survivors:			
Length of hospital stay (days)	29.5±23	36.4±45	0.17
Duration of assisted ventilation (days)	11.1±13	12.3±14	0.47
Chronic lung disease	14 (14%)	16 (16%)	0.71
Air-leak	15 (14%)	17 (16%)	0.62
Supplemental O_2 at time of discharge	15 (15%)	14 (14%)	0.89

a. Primary endpoint: death before discharge or 120 days (whichever comes first) and/or the initiation of ECMO, analyzed by chi-square test.

b. Data from original NDA submission, volume 2.14 and SAS primary datasets based on ITT population.

c. See above for ECMO criteria.

d. Change in this parameter measured from baseline to 30 minutes.

4.1.1 Additional NINOS Analyses: Receipt of ECMO by the two study populations (cont)

The sponsor also collected information on which of the ECMO criteria were met by each of the subjects. The majority of infants met the criteria for ECMO with an elevated OI, as shown below, supporting a link between the oxygenation of the infant the use of ECMO.

Table 4.1.1.2 (from initial NDA submission, table 6.0.1.12.2d.4) ECMO criteria met by subjects in NINOS trial.

	Control (n=121)	I-NO Therapy (n=114)	p Value
Met ECMO criteria	83/121 (69%)	67/114 (59%)	0.12
ECMO criteria met			
OI >40 twice in at least 30 minutes, or OI >35 for 4 hours	63/83 (76%)	53/67 (79%)	0.64
A-aDO_2 >630 for 4 hours or >620 for 12 hours	2/83 (2%)	3/67 (5%)	
Lack of response to treatment gas[a]	11/83 (13%)	5/67 (8%)	
Other criteria	7/83 (8%)	6/67 (9%)	
Transported for ECMO	12/121 (10%)	9/114 (8%)	0.59
Received ECMO	66/121 (55%)	44/114 (39%)	0.014

a. Lack of response defined as any two of the following: 1. PaO_2 <55 for >2 hours; 2. pH <7.15 or >7.40 if alkalosis was attempted for > 2 hours; 3. mean systemic BP <40 for > 2 hours; 4. severe barotrauma.

As shown in the table below, the most common reason for an infant meeting the criteria for, but not receiving, ECMO, was 'Improved', which occurred more frequently in the infants receiving I-NO.

Table 4.1.1.3 (from 6.0.1.12.2d.5) Reasons subjects met criteria for ECMO but did not receive it in NINOS trial[a].

	Control	I-NO Therapy)	p Value[b]
Number meeting ECMO criteria but not receiving ECMO	17/121 (14%)	23/114 (20%)	0.065
Reasons[a]			
Died prior to ECMO	2 (12%)	2 (9%)	0.46
Improved	8 (47%)	14 (61%)	
Support withdrawn	2 (12%)	1 (4%)	
Contraindications for ECMO	1 (6%)	4 (17%)	
Other reasons	4 (24%)	2 (9%)	

a. Data from NDA volume 9.1, table 3. Reasons were collected during trial, as per individual investigators.
b. p value calculated using unadjusted chi-square.

'Treatment Received' Population

The analysis above is based on the Intent-to-Treat (ITT) population: the subjects were analyzed according to the category they were randomized to (control gas or I-NO), regardless of whether they received the gas, or whether they received the correct type of gas. In the course of the trial, however, five individuals were randomized but did not receive gas. Another 6 infants received I-NO after being randomized to control, and one infant received control gas after being randomized to receive I-NO.

a. Individuals who did not receive study gas: (data from NDA volume 2.15, page 048408):

1. Center 56, Network 120, Subject # A11. Infant was randomized to control gas despite OI<25. Study gas never started.

2. Center 15, Network 27, Subject #A19. Infant with coarctation of the aorta (an exclusion criteria) picked up on ECHO after randomization to control gas. Study gas never started.

3. Center 51, Network 2, Subject # A13. After randomization to control gas, it was discovered that infant was being ventilated improperly. When ventilator was fixed, infant improved dramatically. Study gas never started.

4. Center 57, Network 101, Subject # A01. Infant randomized to control gas, but died before study gas was administered. Study gas never started.

5. Center 58, Network 110, Subject # A01. Infant randomized to I-NO, then ECHO revealed coarctation of the aorta (exclusion criteria). Study gas never started.

4.1.1 Additional NINOS Analyses: Receipt of ECMO by the two study populations (cont)

b. Individuals who received the 'incorrect' gas: (data from NDA volume 2.14, page 032908).

The first group of infants were randomized to control gas, and instead received I-NO. Six of these seven subjects who incorrectly received I-NO ultimately received ECMO and two of the seven died. The specified intent-to-treat analysis above attributes these events to the control group, when in fact the infants received I-NO.

1. Center 5, Network 9, Subject # A09. Infant with meconium aspiration randomized to control gas on day 11, but received I-NO. The infant met criteria for ECMO and ECMO was started on day 15, 94 hours after starting study gas. The infant was discharged on day 34.

2. Center 12, Network 3, Subject # A02. This infant with meconium aspiration was randomized to control gas but received I-NO (up to 80 ppm). She met the criteria for ECMO and received ECMO after 6 hours on study gas. She was discharged on day 22.

3. Center 14, Network 2, Subject # A02. This infant with pneumonia and sepsis was randomized to control gas, but received I-NO on day 2. The infant met the criteria and received ECMO the same day. He died on day 21.

4. Center 51, Network 3, Subject # A19. Infant with meconium aspiration was randomized to control gas, but received I-NO. She met the criteria for and received ECMO 2 hours and 26 minutes after study gas was initiated. The infant was discharged on day 54.

5. Center 54, Network 138, Subject # A08. Infant with meconium aspiration was randomized to control gas, but received I-NO. He met the criteria for, and received ECMO 35 hours and 54 minutes after study gas was started. He was discharged on day 38.

6. Center 54, Network 153, Subject # A14. Infant with pneumonia/sepsis and pulmonary hemorrhage was randomized to control gas, but received I-NO. She had a full response to I-NO, 80 ppm. After 70 hours on study gas, her condition deteriorated and she met criteria for ECMO (by OI criterion). She then improved, so that ECMO was not attempted, but then crashed acutely, became to unstable to transport for ECMO, and died.

7. Center 55, Network 69, Subject # A13. Infant with Respiratory Distress Syndrome, was randomized to control,gas, but received I-NO. The infant did not respond to I-NO at 20 or 80 ppm, met the criteria for and received ECMO, after 4 hours and 25 minutes on study gas. The infant was discharged on day 35.

One infant was randomized to receive control gas, after first receiving I-NO. Because this infant received I-NO initially, this infant is considered to have received I-NO for purposes of later statistical analysis.

8. Center 52, Network 56, Subject # A07. Infant with meconium aspiration was randomized to, and received I-NO. After a partial response to I-NO, 20 and 80 ppm, the infant met criteria for use of ECMO, and was transferred to another center for ECMO. There, another trial of study gas was made, and the infant incorrectly received control gas, rather than I-NO. The infant later received ECMO.

Analysis according to actual study gas received

An analysis of the data according the what gas the infants actually received is below. In this analysis, the infants who were randomized, but did not receive study gas, are excluded. The infants who received I-NO (correctly or incorrectly according to randomization) are grouped together, as are the infants who received control gas. The last individual, who received both control and I-NO gas, is attributed to the I-NO group. This analysis, grouping the subjects according to the gas actually received reduces, but does not eliminate, the statistical significance of the primary endpoint. Also note that there is still no difference in the incidence of death in the two populations.

Table 4.1.1.4 (from 6.0.1.12.3a.1) Analysis of primary endpoints study gas received in NINOS[b].

	Control	I-NO Therapy	p Value
Met combined primary endpoint[a]	71/112 (63%)	56/118 (47%)	0.015
Death	17/112 (15%)	17/118 (14%)	0.869
Received ECMO	62/112 (55%)	48/118 (41%)	0.026

a. Primary endpoint: death before discharge or 120 days (whichever comes first) and/or the initiation of ECMO.
b. Data from electronic datasets, analyzed using unadjusted chi-squared test.

Finally, if the Cochran-Mantel-Hanszell adjusted chi-squared test is used, to account for variability between centers, an analysis of the primary endpoint again showed a significant difference between the control and I-NO groups (p value = 0.022).

4.1.1 Additional NINOS Analyses: Receipt of ECMO by the two study populations (cont)

Subpopulation Analysis

At the time of the initial review, concern was raised that these data suggested that receipt of ECMO reflected the improvement in oxygenation that occurs following I-NO therapy (72% of infants given I-NO had a full or partial response, that is, increased their PaO_2). There were, in fact, no other clinical benefits of I-NO (e.g., reduction in the duration of hospitalization) were demonstrated in NINOS. In this interpretation, fewer infants in the I-NO group received ECMO because they were 'pink,' and the investigators delayed ECMO because of perceived clinical improvement. This delay allowed the many other therapeutic modalities also being used by the physicians to treat these desperately ill infants to have effect (e.g. high-frequency ventilation, vasodilators, alkalization).

To investigate this possibility, the sponsor has performed a series of additional data collections regarding the timing of when ECMO criteria were met compared with the initiation of study gas and the results of a treatment-received analysis of the primary outcome. These are summarized in this section.

The intent of the collection of data on the need for ECMO was to assess it after the initiation of study gas. Unfortunately, the timing of the assessment for ECMO criteria was not specified in the protocol, so that the majority of the infants were assessed for ECMO criteria before randomization, while others were only assessed while on study gas. Information on the timing of the assessment for ECMO was obtained by sending a supplemental case report form (CRF) to the investigative centers, requesting more information about the timing and reasons for deciding to place an infant on ECMO.

Supplemental CRF for timing of assessment for ECMO

Investigators were asked the following set of questions:

1) Did the patient meet the criteria for ECMO before randomization to study drug (Y/N)?
2) If NO, did the patient meet these criteria after randomization to ECMO (Y/N)?
3) Did the patient receive ECMO (Y/N)?

3a. If YES, why the did infant receive ECMO? (followed by list of criteria for ECMO, as above).

3b. If NO, why not?

1. Never met ECMO criteria.
2. Met above ECMO criteria by had contra-indication to ECMO.
3. Met above ECMO criteria but transport not available/ considered too dangerous.
4. Met above ECMO criteria but parental permission for ECMO not granted.
5. Met above ECMO criteria by patient improving (borderline or decreasing severity of illness from baseline).

This information was obtained on 235 patients (84.7% of the original enrollees). The sponsor analyzed three sets from these patients: those who met the criteria for ECMO prior to randomization, those who met ECMO criteria after randomization, and those who never met ECMO criteria. The first table shows the # of patients in each category. As expected, given the high mean OI at entry into the trial (46), the majority of the infants who were assessed before starting study gas met criteria for ECMO prior to randomization. Note the small number of patient in the I-NO group who met the criteria for ECMO after randomization with available data. However, in this group, fewer I-NO treated patients received ECMO. In addition, the number of infants who never met ECMO criteria were higher in the I-NO group.

Table 4.1.1.5 Time criteria for ECMO was met for sub-group with available data from NINOS[a].

	Control N=106	I-NO Therapy N=93
Met ECMO criteria prior to randomization[b]	62 (58.5%)	54 (58.1%)
Met ECMO criteria after randomization[b]	23 (21.7%)	12 (12.9%)
Never met ECMO criteria[b]	21 (19.8%)	27 (29.0%)
Total with information[c]	106 (87.6%)	93 (81.6%)

a. Data from NDA volume 9.1, table 4.
b. Calculated as a percentage of the infants with available information regarding timing of ECMO assessment.
c. Expressed as a percentage of the original randomized population.

4.1.1 Additional NINOS Analyses: Receipt of ECMO by the two study populations (cont)

1. Patients who met ECMO criteria prior to randomization

Fewer individuals who met the ECMO criteria prior to randomization into the I-NO group received ECMO compared with individuals randomized to control gas.

Table 4.1.1.6 Use of ECMO in patients meeting ECMO criteria prior to randomization from NINOS[a].

	Control N=62	I-NO Therapy N=54
Received ECMO	38 (61.3%)	26 (48.2%)
No ECMO	24 (38.7%)	28 (51.9%)

a. Data from NDA volume 9.1, table 5. Shown for individuals with available therapy.

Of the individuals who received ECMO, the most common cause cited was meeting oxygenation thresholds (OI, PaO_2, and/or A-a DO_2).

Table 4.1.1.7 Reasons for ECMO in patients meeting ECMO criteria prior to randomization in NINOS[a,b].

	Control N=38	I-NO Therapy N=26
OI threshold met	18 (47.4%)	9 (34.5%)
OI and PaO_2 thresholds met	4 (10.5%)	6 (23.1%)
OI and A-a DO_2 thresholds met	4 (10.5%)	6 (23.1%)
Acute deterioration, OI, pH and PaO_2 thresholds met	2 (5.3%)	3 (11.5%)

a. Data from NDA volume 9.1, table 6. Shown for individuals with available therapy.
b. Shown are the four most frequent cited reasons for ECMO, based on CRFs from individual investigators.

Of the individuals who did not receive ECMO, after meeting ECMO criteria, the reasons given by the investigators are listed below. Clinical improvement is the most commonly-cited reason given.

Table 4.1.1.8 Reasons for not receiving ECMO in patients meeting ECMO criteria prior to randomization from NINOS[a,b].

	Control N=24	I-NO Therapy N=28
Improved	14 (58.3%)	18 (64.3%)
Transport problems	0 (0%)	1 (3.6%)
Contraindications to ECMO	6 (25.0%)	6 (21.4%)
Failed to meet local ECMO criteria[b]	4 (16.7%)	3 (10.7%)

a. Data from NDA volume 9.1, table 6. Shown for individuals with available therapy.
b. Some examples of these local, stricter, criteria are longer periods of hypoxia, stricter definitions of contraindications to ECMO, and the clinical impression of lability.

2. Patient meeting ECMO criteria after randomization

The second sub-group was infants who met the ECMO criteria after randomization. Recall that fewer of the infants in the I-NO group fell into this category (21.7% in control vs. 12.9% in I-NO, see above). Once again, fewer infants in this I-NO sub-group received ECMO.

Table 4.1.1.9 Use of ECMO in patients meeting ECMO criteria after randomization from NINOS[a].

	Control N=23	I-NO Therapy N=12
Received ECMO	19 (82.6%)	7 (58.3%)
No ECMO	4 (17.4%)	5 (41.7%)

a. Data from NDA volume 9.1, table 8. Shown for individuals with available therapy.

4.1.1 Additional NINOS Analyses: Receipt of ECMO by the two study populations (cont)

Of the individuals who received ECMO, the most common cause cited was meeting oxygenation thresholds (OI, PaO_2, and/or A-a DO_2).

Table 4.1.1.10 Reasons for ECMO in patients meeting ECMO criteria after randomization from NINOS[a,b].

	Control N=19	I-NO Therapy N=7
OI threshold met	8 (42.1%)	4 (57.1%)
OI and PaO_2 thresholds met	3 (15.8%)	0 (0%)
Acute deterioration, OI and PaO_2 thresholds met	2 (10.5%)	1 (14.3%)
Acute deterioration, OI, pH and PaO_2 criteria met	1 (5.3%)	2 (28.6%)

a. Data from NDA volume 9.1, table 6. Shown for individuals with available therapy.
b. Shown are the four most frequent cited reasons for ECMO, based on CRFs from individual investigators.

Of the individuals who did not receive ECMO, after meeting ECMO criteria, the reasons given by the investigators are listed below. Clinical improvement is the most commonly-cited reason given.

Table 4.1.1.11 Reasons for not receiving ECMO in patients meeting ECMO criteria after randomization from NINOS[a,b].

	Control N=4	I-NO Therapy N=5
Improved	3 (75.0%)	5 (100%)
Contraindications to ECMO	1 (25.0%)	0 (0%)

a. Data from NDA volume 9.1, table 6. Shown for individuals with available therapy.

3. Patient never meeting ECMO criteria

Finally, there were 48 infants (24.1% of the total population with available data) who never met the criteria for ECMO. Given the small numbers, it is not clear if there was a reduction in the use of ECMO in this population. The most common cause for ECMO in this group, per the sponsor, was cardiac instability.

Table 4.1.1.12 Use of ECMO in patients who never met ECMO criteria NINOS[a].

	Control N=21	I-NO Therapy N=27
Received ECMO	7 (33.3%)	7 (25.9%)
No ECMO	14 (66.7%)	20 (74.1%)

a. Data from NDA volume 9.1, table 11. Shown for individuals with available therapy.

To conclude, the critical portion of the analyses presented above comes from table 4.1.1.5, which looked at those infants who were assessed for ECMO after starting study gas. In that population there were fewer infants in the I-NO group who got ECMO, and more infants who never met the criteria. This supports (in a small way with broad confidence intervals) that children who started I-NO were less likely to meet the criteria for ECMO. This is important because these criteria were less open to interpretation by the investigators.

Overall, the results of this post-hoc analysis suggest that regardless of when the infants met the criteria for ECMO, use of I-NO was associated with a lower use of ECMO. The primary reason for use of ECMO was persistent or progressive hypoxia.

4.1.2 NINOS Follow-Up

The outcomes for the 235 patients enrolled in NINOS are shown below. A total of 173 of the patients have available follow-up data at 18-24 months of age (73.6% of the original cohort). Statistics are based on the treatment group to which the patients were initially randomized, not the treatment they ultimately received. Recall that eight infants were randomized to control but instead were given I-NO (no infant was randomized to I-NO but received control gas).

Table 4.1.2.1 Follow-up in the NINOS trial[a].

Degree of Follow-Up	Control	I-NO
Enrolled: 235 Infants	121	114
Acute Hospitalization		
Survived	101	98
Died	20 (16.5% of initial cohort)	16 (14.0% of initial cohort)
Follow-up Period (18-24 months)		
With Follow-up Data Available	88 (72.7%)	85 (74.6%)
Died During Follow-up	0 (0%)	1 (<1%)
No Available Data	13 (10.7%)	
Refused Follow-up	5 (4.1%)	4 (3.3%)
Lost to Follow-up	7 (5.8%)	6 (5.3%)
Left State	1 (<1.0%)	2 (1.8%)

a. Data from NDA vol. 11.1, Figure 1.

1. Demographics of Long-term F/U Group

The first table summarizes the characteristics of the study subgroup. Other than the % that received ECMO, no significant differences exist.

Table 4.1.2.2 Demographics of the study group with long-term F/U in the NINOS trial[a].

	Control N=88	I-NO N=85
Birthweight (mean±sd)	3370±595	3448±582
Gender		
Female	33 (37.5%)	37 (43.5%)
Gestational Age (mean±sd weeks)	39±2	39±2
Received ECMO	66 (75.0%)	44 (51.8%)
Duration of Hospitalization	29±41	30±26
Age at time of evaluation (months)	20.8±2.7	20.4±2.5

a. Data from NDA vol. 11.1, Table 17.

In data not shown, the two study groups were well-balanced with regard to their socioeconomic indicators, including the identity of the primary caregiver (e.g., father, mother) and primary caregiver's demographics (education, employment, occupation, and income).

2. Need for Special Medical Programs

There were no significant differences in the use of special medical or social services either during the follow-up period (not shown) or at the time of evaluation (shown below).

Table 4.1.2.3 Use of special services in subjects with long-term F/U in the NINOS trial[a].

	Control N=88	I-NO N=85
Receiving visiting nursing services	4 (4.7%)	2 (2.4%)
Receiving occupational therapy	13 (15.1%)	14 (16.7%)
Receiving speech therapy	10 (11.6%)	5 (6.0%)
Receiving early intervention services	13 (15.1%)	12 (14.3%)
Receiving social work services	7 (8.1%)	7 (8.3%)
Receiving specialty medical services	34 (39.5%)	33 (39.3%)

a. Data from NDA vol. 11.1, Table 35.

4.1.2 NINOS Follow-Up (cont)

3. Need for Re-hospitalization

While a large number of the infants in both study groups were re-hospitalized during the follow-up period, the two groups were similar with regard to both need for, and duration, of re-hospitalization. There was a lower incidence of operations during the follow-up period reported for the I-NO group.

Table 4.1.2.4 Need for re-hospitalization in subjects with long-term F/U in the NINOS trial[a].

	Control N=88	I-NO N=85
Hospitalized since discharge	34 (39.1%)	28 (32.9%)
Mean number of re-hospitalizations[b]	2.1±1.8	3.0±4.2
Operations after discharge	48 (55.2%)	33 (38.8%)

a. Data from NDA vol. 11.1, Table 36.
b. For infants who were re-hospitalized.

4. Medications and other Therapies used During the Follow-up Period

Only a minority of subjects were taking medications regularly at the time of follow-up. There was no significant increase in the use of bronchodilators or anticonvulsants in the I-NO subjects with available follow-up.

Table 4.1.2.5 Medications used regularly by the subjects with long-term F/U in the NINOS trial[a].

	Control N=88	I-NO N=85
Taking medications regularly	23 (26.1%)	20 (23.5%)
Diuretics	0 (0%)	1 (1.2%)
Bronchodilators	7 (8.0%)	9 (10.6%)
Anticonvulsants	6 (6.9%)	3 (3.5%)

a. Data from NDA vol. 11.1, Table 37.

Table 4.1.2.6 Other medical therapies used by the subjects with long-term F/U in the NINOS trial[a].

	Control N=88	I-NO N=85
Currently using apnea monitor	0 (0%)	1 (1.2%)
Previously used apnea monitor[b]	8 (9.2%)	9 (10.6%)
Currently using O_2	0 (0%)	1 (1.2%)
Previously used O_2[b]	14 (16.1%)	13 (15.3%)
Currently on ventilator/CPAP	0 (0%)	1 (1.2%)
Currently on tube feedings or using a gastrostomy tube	4 (4.6%)	2 (2.4%)
Previously used tube feedings/gastrostomy tube	4 (4.6%)	5 (5.9%)
Require special equipment for ambulation[c]	5 (5.7%)	8 (9.4%)

a. Data from NDA vol. 11.1, Table 38, 39.
b. Refers to those patients who used a given therapy after discharge but before the long-term F/U visit.
c. Includes adapted stroller/wheelchair, braces/orthotics, and walkers.

5. Physical Examinations

The sponsor also summarized the available data on the physical examinations performed at the follow-up examination. In data not shown, there were no significant differences in the growth parameters of the infants (i.e., height, weight, head circumference) or in the incidence of physical abnormalities (e.g., visual or hearing deficiencies detected on physical examination among the treatment groups (see text tables 42-50 for details).

4.1.2 NINOS Follow-Up (cont)

6. Neurologic Examination

The overall diagnosis of the infants in the follow-up group is summarized below, followed by the incidence of cerebral palsy and seizures in the population. Note that there were fewer seizures reported in the I-NO group at follow-up

Table 4.1.2.7 Neurologic diagnoses for the subjects with long-term F/U in the NINOS trial[a].

	Control N=88	I-NO N=85
Normal	69 (79.3%)	66 (77.6%)
Global hypotonia	3 (3.4%)	0 (0%)
Monoplegia	2 (2.3%)	2 (2.4%)
Diplegia	3 (3.4%)	2 (2.4%)
Hemiplegia-right side	1 (1.1%)	2 (2.4%)
Quadraplegia	5 (5.7%)	4 (4.7%)
Truncal hypotonia	4 (4.6%)	4 (4.7%)

a. Data from NDA vol. 11.1, Table 52.

Table 4.1.2.8 Cerebral palsy in the subjects with long-term F/U in the NINOS trial[a].

	Control N=88	I-NO N=85
Cerebral palsy present	9 (10.3%)	10 (11.9%)
Mild or Moderate Cerebral Palsy	4 (4.6%)	5 (6.0%)
Severe Cerebral Palsy	5 (5.7%)	5 (6.0%)
Seizures present	13 (14.9%)	4 (4.7%)

a. Data from NDA vol. 11.1, Table 53.
b. p Value per the sponsor = 0.039 by Fischer's exact test.

7. Mental Development

Bayley's assessments were attempted for all children at the F/U visit, and were successful in 79 of the control patients (86.2%) and 79 of the I-NO patients (92.9%). In this group, there were no significant differences in the Mental Development Index (MDI) means.

Table 4.1.2.9 Bayley's Scale of infant development mean scores for subjects with long-term F/U in the NINOS trial[a].

	Control N=73/88	I-NO N=77/85
Mean MDI ±sd	87.0±18.7	85.0±21.7
MDI<50	5 (6.8%)	6 (7.8%)
MDI 50-69	9 (12.3%)	15 (19.5%)
MDI 70-84	12 (16.4%)	9 (11.7%)
MDI 85-99	29 (39.7%)	21 (27.3%)
MDI≥100	18 (24.7%)	26 (33.8%)

a. Data from NDA vol. 11.1, Table 62.
b. Subjects were tested using the Bayley Scales of Infant Development and the standardized Mental Development Index (MDI) was calculated.

No significant differences in psychomotor development were detected.

Table 4.1.2.10 Bayley's scale of psychomotor development mean scores for subjects with long-term F/U in the NINOS trial[a].

	Control N=73/88	I-NO N=77/85
Mean PDI±sd	93.6±17.5	85.9±21.2
PDI <50	3 (4.0%)	9 (11.5%)
PDI 50-69	6 (8.0%)	8 (10.3%)
PDI 70-84	4 (5.3%)	12 (15.4%)
PDI 85-99	27 (36.0%)	25 (32.1%)
PDI ≥100	35 (46.0%)	24 (30.8%)

a. Data from NDA vol. 11.1, Table 63.
b. Subjects were tested using the Bayley Scales of Psychomotor Development and the standardized Psychomotor Development Index (PDI) was calculated.

4.1.2 NINOS Follow-Up (cont)

Finally, a behavioral rating was determined for the follow-up group.

Table 4.1.2.11 Bayley's behavioral rating scale for subjects with long-term F/U in the NINOS trial[a].

	Control N=73/88	I-NO N=77/85
Mean orientation/engagement ±sd	58.4±29.4	49.2±28.6
Mean emotional regulation±sd	53.8±27.7	44.4±28.6
Mean motor quality±sd	58.5±33.2	44.0±31.8
Mean total score±sd	61.6±30.9	47.7±31.6

a. Data from NDA vol. 11.1, Table 64.
b. Subjects were tested using the Bayley Scales of Infant Development.

Summary of long-term safety results from the NINOS trial, conducted at 18-24 months of age

The results of the long-term follow-up will be summarized in the Integrated Efficacy and Safety Summary, sections 3.0 and 4.0 of this document.

4.2 INO-01/ -02 FOLLOW-UP

This was a multi-center, double-blind, randomized, placebo-controlled trial was designed to...'demonstrate a reduction in morbidity and mortality of PPHN with the use of inhaled nitric oxide' and ...'to study the effective and safe dose range and duration of therapy in the treatment of PPHN.' (initial NDA 20-845 submission, volume 2.17, page 077708).

Eligible subjects were randomized to one of four treatment groups: placebo (N_2); I-NO 5, 20, or 80 ppm. Randomized subjects received treatment gas for up to 14 days, or until one of the following sets of criteria were fulfilled:

1. The subject met 'treatment failure' criteria, defined by one of the following criteria:
   a. PaO_2 <40 mm Hg at the beginning and end of a 30-minute period not attributable to a mechanical problem.
   b. Mean systemic arterial pressure <35 mm Hg after volume or vasopressor therapy.
   c. Death.
   d. Methemoglobin >7% at two consecutive times at least 30 minutes apart.
   e. NO_2 level >3 ppm for 30 minutes.

or 2. The subject treatment is discontinued for one of the following reasons:
   a. Parental consent was withdrawn.
   b. Subject was found to have met an exclusion criteria.
   c. Delivery or monitoring device malfunction which could not be repaired.
   d. Principal investigator felt that withdrawal was in the best interests of the subject.

or 3. the subject received 14 days of therapy.

or 4. the subject improved clinically so that the criteria for decreasing treatment gas were met:
   a. FiO_2 <60%
   b. Mean Airway Pressure (P_{AW}) <10 cm H_2O
   c. post-ductal PaO_2 >60 mm Hg

If the subject was determined to be a treatment failure, or withdrawn for other reasons, the treatment gas was reduced by 20% repeatedly, at 0-1 hour intervals, until the subject was weaned over approximately 5 hours.

Treatment failures were maintained on conventional therapy after discontinuation of the study gas, at the discretion of the investigator. These might include ECMO or high-frequency jet ventilation (HFJV), as well as the use of surfactant and vasodilators. Subjects who were categorized as treatment failures were followed after withdrawal from study gas, and had the same data collected after discontinuation as subjects who continued in the trial.

The primary endpoint of the INO-01/ -02 trial was the incidence of one of the following: 1) death, 2) initiation of ECMO, 3) Evidence of abnormal neurological, and 4) Bronchopulmonary dysplasia.

Subjects who survived were examined during a 1 year follow-up examination for the following: a physical examination; medical and family history taken; a review of any hospitalizations; an audiology test; and a Bayley developmental test. In addition, the sponsor collected available clinical data, and these follow-up results are summarized below.

Long-term safety results from the INO-01/ -02 trial, conducted at 12 months of age

1. Demographics

The first table shows the degree of follow-up available for the infants in the study. It should be noted that the incomplete nature of the follow-up may introduce bias. Since infants with poor outcomes tend to be hospitalized more often, their records may be more complete.

Table 4.2.1 (from 6.0.1.13.3.1) Follow-up for infants in INO-01/ -02 at 1 month and 1 year of age[a].

	Placebo	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Initial # of subjects	41	41	36	37	114
# Died	2 (4.9%)	2 (4.9%)	5 (13.9%)	3 (8.1%)	10 (8.8%)
# Lost to Follow-up	3 (7.3%)	3 (7.3%)	2 (5.6%)	3 (8.1%)	8 (7.0%)
Telephone F/U only	1 (2.4%)	1 (2.4%)	1 (2.8%)	2 (5.4%)	4 (3.5%)
Seen in Clinic at 1 Yr	35 (85.4%)	35 (85.4%)	28 (77.8%)	29 (78.4%)	92 (80.7%)

a. Data from NDA vol. 9.3, Figure 1.

4.2 INO-01/-02 Follow-Up (cont)

The demographics of the individuals listed above are summarized below. Note that the mean age at follow-up was approximately 2 months younger in the placebo group than in any of the I-NO groups. In data not shown, the socioeconomic status of the primary caregivers in the treatment groups was similar, with the exception of marriage status, where a higher percentage of the placebo group were cared for by single parents (44.4% for placebo vs. 30.2% for the entire I-NO group). Family histories of mental or physical handicaps and other relevant medical illnesses (e.g., asthma, seizures) were also similar between the treatment groups.

Table 4.2.2 Demographics of infants followed in the INO-01/-02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Male	23 (63.9%)	16 (44.4%)	15 (51.7%)	18 (58.1%)	49 (51.0%)
Female	13 (36.1%)	20 (55.6%)	14 (48.3%)	13 (41.9%)	47 (49.0%)
Age (mean±SD in days)	409±80	462±182	463±195	461±187	462±186
Race					
White	17 (47.2%)	22 (61.1%)	15 (51.7%)	15 (48.4%)	52 (54.2%)
Non-White	19 (52.8%)	14 (38.9%)	14 (48.3%)	16 (51.6%)	44 (45.8%)

a. Data from NDA vol. 9.3, Tables 2,3.

2. Need for Special Medical Programs

The infants in the placebo group were less likely to be enrolled in special medical programs.

Table 4.2.3 Enrollment in special programs for infants followed in the INO-01/-02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Any program	6 (16.7%)	11 (30.5%)	8 (27.6%)	9 (29.0%)	28 (29.2%)
Infant stimulation	3 (8.3%)	5 (13.9%)	3 (10.3%)	6 (19.4%)	14 (14.6%)
Physical therapy	3 (8.3%)	5 (13.9%)	3 (10.3%)	6 (19.4%)	14 (14.6%)
Occupational therapy	1 (2.8%)	3 (8.3%)	5 (17.2%)	2 (6.5%)	10 (10.4%)
Speech therapy	0 (0%)	0 (0%)	1 (3.4%)	1 (3.2%)	2 (2.1%)
Feeding therapy	2 (5.6%)	0 (0%)	0 (0%)	3 (9.7%)	3 (3.1%)

a. Data from NDA vol. 9.3, Tables 7.

3. Rehospitalizations

There was no apparent difference between the treatment groups with regard to the incidence of re-hospitalization after discharge home.

Table 4.2.4 Rehospitalization after discharge for infants followed in the INO-01/-02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
# of infants re-hospitalized	6 (16.7%)	8 (22.2%)	10 (34.5%)	7 (22.6%)	25 (26.0%)
Mean # of hospitalization[b]	1.8	1.5	2.5	1.7	2.0

a. Data from NDA vol. 9.3, Tables 7.
b. For patients re-hospitalized.

4. Need for Long-Term Respiratory Medications

More infants in the I-NO treatment groups were taking respiratory medications at the time of their follow-up visit.

Table 4.2.5 Post-discharge medications at the one-year follow-up visit in the INO-01/-02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Taking medications	5 (13.9%)	12 (33.3%)	11 (37.9%)	9 (29.0%)	32 (33.3%)
Respiratory medications	2 (5.5%)	6 (16.7%)	6 (20.7%)	2 (6.5%)	14 (14.6%)
Anticonvulsants	0 (0%)	0 (0%)	2 (6.9%)	0 (0%)	2 (2.1%)
Diuretics	0 (0%)	0 (0%)	2 (6.9%)	0 (0%)	2 (2.1%)

a. Data from NDA vol. 9.3, Tables 9.

4.2 INO-01/ -02 Follow-Up (cont)

5. Review of Systems/Illnesses During Follow-up

A review of systems performed at the follow-up visit found relatively few problems. The respiratory ROS is summarized separately.

Table 4.2.6 Review of systems for the infants seen in follow-up from the INO-01/ -02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Cardiovascular problems	1 (2.8%)	3 (8.3%)	2 (6.9%)	3 (9.7%)	8 (8.3%)
GI problems	7 (19.4%)	1 (2.8%)	7 (24.1%)	5 (16.1%)	13 (13.5%)
Urogenital problems	0 (0%)	0 (0%)	2 (6.9%)	1 (3.2%)	3 (3.1%)
Hematological problems	1 (2.8%)	1 (2.8%)	2 (6.9%)	1 (3.2%)	4 (4.2%)
Musculoskeletal problems	6 (16.7%)	2 (5.6%)	5 (17.2%)	5 (16.1%)	12 (12.5%)
Sinusitis	2 (5.6%)	2 (5.6%)	3 (10.3%)	2 (6.5%)	7 (7.3%)
Strabismus	1 (2.8%)	1 (2.8%)	6 (20.7%)	4 (12.9%)	11 (11.5%)
Hearing problems	3 (8.3%)	5 (13.9%)	3 (10.3%)	1 (3.2%)	9 (9.4%)
Otitis media	18 (50.0%)	19 (52.8%)	18 (62.1%)	14 (45.2%)	51 (53.1%)
Speech problems	4 (11.1%)	5 (13.9%)	6 (20.7%)	4 (12.9%)	15 (15.6%)

a. Data from NDA vol. 9.3, Tables 10.

Next, the incidence of illnesses related to respiratory disease are summarized. Note that while the use of home O_2 occurred only in the I-NO groups, there was no detectable differences in the occurrence of other pulmonary disease. Fewer patients in the I-NO group were reported to have had severe URIs.

Table 4.2.7 Pulmonary review of systems for the infants seen in follow-up from the INO-01/ -02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Home Oxygen	0 (0%)	8 (22.2%)	1 (3.4%)	5 (16.1%)	14 (14.6%)
Mean age when O_2 was D/C'd (months)[b]	--	4.0±3.0	1.0±0.0	3.3±1.3	3.5±2.5
Asthma	5 (13.9%)	7 (19.4%)	3 (10.3%)	2 (6.5%)	12 (12.5%)
Bronchiolitis	4 (11.1%)	7 (19.4%)	4 (13.8%)	2 (6.5%)	13 (13.5%)
Bronchitis	2 (5.6%)	4 (11.1%)	3 (10.3%)	2 (6.5%)	9 (9.4%)
Pneumonia	3 (8.3%)	3 (8.3%)	3 (10.3%)	2 (6.5%)	8 (8.3%)
Severe URI	11 (30.6%)	8 (22.2%)	6 (20.7%)	6 (19.4%)	20 (20.8%)

a. Data from NDA vol. 9.3, Tables 11.

b. For infants who received O_2 at time of initial discharge.

The sponsor also collected data on the occurrence of seizures in the follow-up population. The only infants with seizures were in the 20 ppm and 80 ppm I-NO groups. There were, however, no differences noted in the incidence of abnormal neurologic examinations at 1 year.

Table 4.2.8 Incidence of seizures in the infants seen in follow-up from the INO-01/ -02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Seizures Present	0 (0%)	0 (0%)	4 (13.8%)	3 (10.3%)	7 (7.3%)
Cerebral Palsy Present	2 (5.6%)	0 (0%)	4 (13.8%)	3 (9.7%)	7 (7.3%)
Neurologic Abnormalities on Physical Exam					
None	28 (77.8%)	31 (86.1%)	20 (69.0%)	23 (74.2%)	74 (77.1%)
Mild	3 (8.3%)	1 (2.8%)	2 (6.9%)	1 (3.2%)	4 (4.2%)
Moderate	4 (11.1%)	3 (8.3%)	5 (17.2%)	5 (16.1%)	13 (13.5%)
Missing	1 (2.8%)	1 (2.8%)	2 (6.9%)	2 (6.5%)	5 (5.2%)

a. Data from NDA vol. 9.3, Table 16.

6. Physical Examinations

The sponsor also summarized the available data on the physical examinations performed at the 1 year follow-up. In data not shown, there were no significant differences in the growth parameters of the infants (i.e., height, weight, head circumference) or in the incidence of physical abnormalities detected on physical examination among the treatment groups (see text tables 13-15 for details).

4.2 INO-01/-02 Follow-Up (cont)

7. Mental development

The sponsor collected data on the cognitive development of the infants at time of follow-up, and no differences between the treatment groups were evident.

Table 4.2.9 Mental development at 1 year of age for infants for infants with known follow-up in INO-01/-02[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Accelerated development	2 (6%)	2 (5%)	1 (3%)	2 (6%)	5 (5%)
Normal development	21 (58%)	20 (57%)	17 (59%)	17 (55%)	54 (57%)
Mildly delayed development	7 (19%)	6 (17%)	2 (7%)	5 (16%)	13 (14%)
Significantly delayed development	2 (6%)	0 (0%)	4 (14%)	2 (6%)	6 (6%)
Missing	3 (8%)	5 (14%)	2 (7%)	3 (10%)	10 (10%)

a. Subjects were tested using the Bayley Scales of Infant Development and the standardized Mental Development Index (MDI) was calculated.

MDI ≥115	Accelerated development
85≤MDI<115	Normal development
70≤MDI<85	Mildly delayed development
MDI<70	Significantly delayed development

8. Psychomotor development

Psychomotor development was also assessed.

Table 4.2.10 Psychomotor development at 1 year of age for infants for infants with known follow-up in INO-01/-02[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Accelerated development	3 (8.3%)	4 (11%)	1 (3%)	2 (6%)	7 (7%)
Normal development	24 (67%)	20 (57%)	16 (55%)	21 (67%)	57 (60%)
Mildly delayed development	4 (11%)	2 (6%)	3 (10%)	1 (3%)	6 (6%)
Moderately delayed development	0 (0%)	3 (8.6%)	2 (6.9%)	1 (3.2%)	6 (6%)
Significantly delayed development	2 (6%)	1 (3%)	4 (14%)	3 (10%)	8 (8%)
Missing	4 (11%)	5 (14%)	3 (10%)	3 (10%)	11 (12%)

a. Subjects were tested using the Bayley Scales of Infant Development and the standardized Psychomotor Development Index (PDI) was calculated.

PDI ≥115	Accelerated development
85≤PDI<115	Normal development
70≤PDI<85	Mildly delayed development
50≤PDI<70	Moderately delayed development
PDI<50	Significantly delayed development

9. Audiology testing

No differences between the treatment groups are apparent with regard to the audiology testing at or after one year of age.

Table 4.2.11 Results of audiology testing ≥1 year of age for infants for infants with known follow-up in INO-01/-02[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
None	23 (64%)	23 (64%)	19 (66%)	18 (58%)	60 (63%)
Mild	7 (19%)	2 (6%)	4 (14%)	4 (13%)	10 (10.4%)
Major	0 (0%)	1 (3%)	0 (0%)	1 (3%)	2 (2%)
Missing	6 (17%)	10 (29%)	6 (21%)	8 (26%)	24 (25%)

a. Subjects were tested using pure-tone audiologic testing at 0.5, 1, and 2 kHz. Abnormalities were categorized according to loss of audible threshold.

Threshold ≤25 dB	None
>25 to <50 dB	Mild
≥50 dB	Major

4.2 INO-01/-02 Follow-Up (cont)

10. Overall Status

Finally, the sponsor classified the infants as normal if all of the neurologic, motor, hearing, and mental status exams summarized above were normal for a given infant. There were no treatment differences detected.

Table 4.2.12 'Normal' infants at ≥1 year of age for infants with known follow-up in INO-01/-02[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Normal	18 (50.0%)	23 (63.9%)	14 (48.3%)	15 (48.4%)	52 (54.2%)
Mild Impairment	12 (33.3%)	6 (16.7%)	6 (20.7%)	7 (22.6%)	19 (19.8%)
Severe Impairment	5 (13.9%)	7 (19.4%)	8 (27.6%)	7 (22.6%)	22 (22.9%)

a. Data from IND vol. 9.3, table 22. Normal per definitions provided by sponsor.

11. Laboratory Analyses

The sponsor performed a number of analyses of the changes in the mean lab parameters from the patients with available data. Given the small number of patients with measurements of many of the lab values, and the resultant broad confidence intervals and potential for selection bias, no clear trends towards changes in mean serum chemistries or hematology are evident (for details see NDA vol. 9.3).

Summary of long-term safety results from the INO-01/-02 trial, conducted at 12 months of age

The results of the long-term follow-up will be summarized in the Integrated Efficacy and Safety Summary below, sections 5.0 and 6.0 of this document.

5.0 Integrated Safety and Efficacy Summary For I-NO

There are three aspects of the determination of efficacy for I-NO: meeting pre-specified primary endpoints; demonstrating physiological effect; and demonstrating clinical benefit. The latter two aspects of efficacy were included in the secondary and exploratory endpoints of the three trials.

The integrated safety and efficacy summary for I-NO is broken into the following sections below:

1. Demographics.
2. Extent of Exposure.
3. Primary endpoints of the CINRGI, NINOS, INOSG and INO-01/ -02 trials.
4. Success of each trial at meeting their pre-specified primary endpoint.
5. Evidence of physiological effects from the trials.
6. Evidence of clinical benefit from the trials.
7. Review of Efficacy from Secondary (published) sources.
8. Integrated Safety Summary.
9. Conclusions and Recommendations of the Medical Reviewer.

Some of the tables and figures used in the section below are drawn from my original review of I-NO, where the reader is referred for further details of the NINOS, INOSG, and INO-01/ -02 and -03 trial results. Tables that have two heading numbers refer to my original I-NO review (with the original table number in parentheses). The INO-03 trial enrolled only 14 patients before closing, and will be included in the safety analyses only.

5.1 Demographics from the CINRGI, NINOS, INOSG and INO-01/ -02 -03 trials

5.1.1 Population Demographics

The first two tables summarize the populations enrolled in the trials. Following that, the clinical characteristics of each trial are summarized in turn. In general, the trials differed in the degree of severity of their respiratory failure (marked by oxygenation) but not in the make-up of the underlying diseases that were responsible for the hypoxia.

CINRGI Trial Demographics

Table 5.1.1.1 Population demographics of CINRGI[a].

Demographic	Placebo N=89	I-NO N=97	p Value[b]
Gender			
Male	52 (58.4%)	44 (45.4%)	0.08
Race (n (%))			
Caucasian	44 (49.4%)	40 (41.2%)	0.30
Black	33 (37.1%)	43 (44.3%)	
Hispanic	10 (11.2%)	8 (8.2%)	
Other	2 (2.2%)	6 (6.2%)	
Mean Age Since Birth (hrs ±SD)	29.9±16.5	30.0±20.2	0.95
Mean Age (±SD)[c]	38.8±2.1	39.2±1.7	0.20
Mean Weight, kg (±SD)	3.3±0.6	3.3±0.6	0.81
Apgar Scores			
1 Minute	5.4±2.8	5.2±2.5	0.69
5 Minute	7.3±2.2	7.4±1.8	0.72

a. Data from CINRGI study report, table 7-8.
b. p Value per sponsor.
c. Mean age assessed by physical exam at birth.

5.1 Demographics from the CINRGI, NINOS, INOSG and INO-01/ -02 -03 trials

NINOS, INOSG, INO-01/ -02 and –03 Trial Demographics

The demographics of the subjects in the trials submitted as part of the original NDA submission (from NINOS, INOSG, INO-01/ -02 and –03 trials) are summarized in table 5.1.2.1 below. In general, the trials all enrolled a similar population with regard to demographics (sex, ethnicity) and in the proportion of patients in each of the underlying causes of the hypoxia (MAS, RDS). The degree of hypoxia in the INO-01/ -02 trial was significantly less than in the other three trials, as judged by OI.

Table 5.1.1.2 Combined population demographics from the NINOS, INOSG, INO-01/ -02, and INO-03 trials[a].

Demographic Parameter	Control	I-NO
Total	186	271
Sex		
Male	121 (63%)	146 (54%)
Female	67 (37%)	123 (46%)
Race		
White	102 (55%)	148 (55%)
Black	34 (18%)	56 (21%)
Hispanic	31 (16%)	41 (15%)
Asian	1 (<1%)	5 (3%)
Other	13 (7%)	18 (7%)
Missing	5 (3%)	4 (1%)
Age at start of tx gas (hrs)	36.2	33.1
Birth weight (kg)	3.4	3.45
Gestational age (weeks)	39.2	39.7

a. Data from electronic datasets and NDA volume 2.29.

Details of the individual study demographics can be found in my original review, tables 5.1.2.2 to 5.1.2.4.

5.1.2 Clinical Demographics of CINRGI, NINOS, INO1, and INOSG Trials

Below are tables detailing specific clinical characteristics of the subjects in the individual studies. The reader is referred to my original reviews for full demographic details of the individual studies (e.g., concomitant medications, birth characteristics). Rather, this section focuses on the level of severity of the clinical condition found in each of the trials, especially the severity of the respiratory failure. This includes a summary of the diseases that caused the respiratory failure in each trial, along with the oxygenation parameters at baseline.

CINRGI Clinical Demographics

The two treatment groups were well-balanced with regard to the cause of the hypoxic pulmonary failure.

Table 5.1.2.1 Underlying disease leading to hypoxic respiratory failure in CINRGI[a].

Underlying Disease	Placebo N=89	I-NO N=97
Meconium aspiration	35 (39%)	34 (35%)
Pneumonia/Sepsis	21 (24%)	24 (25%)
Respiratory Distress Syndrome	8 (9%)	8 (8%)
Persistent Pulmonary Hypertension	25 (28%)	31 (32%)

a. Data from CINRGI study report, table 32.

The control group had significantly more airleak and more pulmonary hemorrhage compared with the I-NO group. The severity of the pulmonary injury on CXR was similar in the two groups.

Table 5.1.2.2 Pulmonary disease at birth in CINRGI[a].

Peri-Natal Demographic	Placebo N=89	I-NO N=97	p Value[b]
Airleak Syndrome	22 (24.7%)	11 (11.3%)	0.021
Pulmonary Hemorrhage	8 (9.0%)	4 (4.1%)	0.24
Lung Disease on CXR			
None	6 (6.7%)	4 (14.4%)	0.60
Mild	28 (31.5%)	26 (26.8%)	
Moderate	42 (47.2%)	41 (42.3%)	
Severe	13 (14.6%)	16 (16.5%)	

a. Data from CINRGI study report, table 7-8.
b. p Value per sponsor.

5.1.2 Clinical Demographics of CINRGI, NINOS, INOI, and INOSG Trials

As part of the entry criteria, all infants were to have evidence of pulmonary hypertension, and almost all infants had cardiac ECHOs. Beyond the presence of pulmonary hypertension, the treatment groups were balanced with regard to the presence or absence of the following (see sponsor's CINRGI study report tables 15, 16 for details):

1) Patent ductus arteriosus (including right-to-left and left-to-right shunts).
2) Atrial shunts (including right-to-left and left-to-right shunts).
3) Mean ejection fraction.
4) Tricuspid regurgitation.

The hemodynamics at baseline are summarized below. Note that the I-NO group had a lower mean arterial pressure. Data are shown only for those infants with available data (approximately 90% of enrolled subjects). Recall also that many of the infants were on vasoactive medications that might affect blood pressure.

Table 5.12.3 Baseline hemodynamics for patients in the CINRGI study[a].

	Placebo	I-NO	p Value
Arterial Pressure (mm Hg)	55.8±12.3	51.6±11.0	0.019
Heart Rate	157±24	152±25	0.21

a. Data from CINRGI study report, tables 18.

The next table summarizes the available data on the gas exchange at baseline in the two groups. Note the significant differences in the oxygenation and % saturation between the two groups.

Table 5.1.2.4 Baseline oxygenation status for patients in the CINRGI study[a].

	Placebo	I-NO	p Value
pH	7.44±0.13	7.46±0.13	0.25
PaO_2 (mm Hg)	54.3±36.1	77.6±68.3	0.007
$PaCO_2$ (mm Hg)	35.6±12.4	34.2±13.2	0.49
SaO_2 (%)	84.1±16.6	89.6±12.6	0.018
OI (cm H_20/ mm Hg)	43.9±22.7	35.0±20.9	0.011

a. Data from CINRGI study report, tables 18-19.

One possible explanation offered by the sponsor for this difference was that some infants had their final PaO_2 done shortly after starting the treatment gas. This occurred because, per protocol, there were two teams working on the infants, and due to the acute severity of their illness the specific timing of samples was inadvertently missed. One test of this possibility is to look at the PaO_2 and OI measured (per protocol) at 2 and 4 hours before baseline (thus well-prior to the institution of study gas). These data are summarized in the table and graph below. The pulmonary airway pressure (PAW) is also summarized as it is incorporated in the formula for determining OI (see Appendix One). The % O_2 inspired (FiO_2) did not change between time periods. Note that at all time points measured the oxygenation was better for the I-NO group, relative to the control group. Note also that the rates of change for PaO_2 and OI appear to be higher in the control group than in the I-NO group.

Table 5.1.2.5 Baseline oxygenation status for patients in the CINRGI study[a].

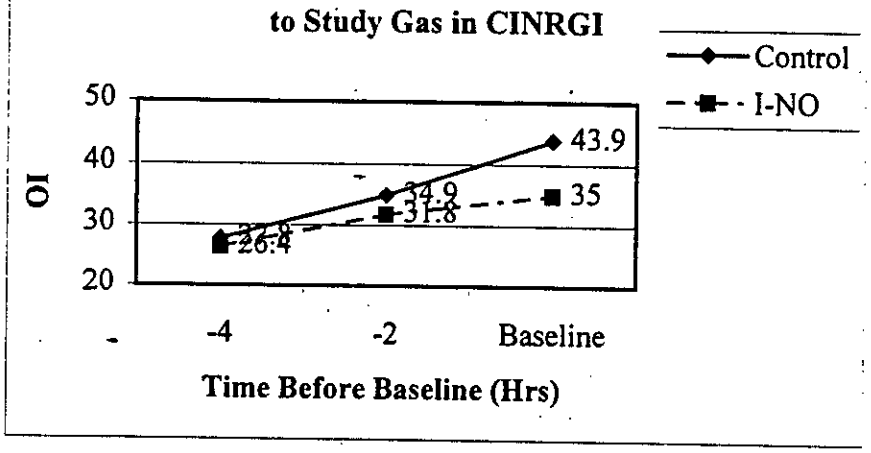
	PaO_2	Change in PaO_2[b]	OI	Change in OI[b]	P_{AW}
Control					
- 4 Hours	74.8	—	27.8	—	16.8
- 2 Hours	63.7	-11.1	34.9	+7.1	17.8
Baseline	54.3	-9.4	43.9	+9.0	19.0
I-NO					
- 4 Hours	81.8	—	26.4	—	15.5
- 2 Hours	77.6	-4.2	31.8	+5.4	16.3
Baseline	77.6	0.0	35.0	+3.2	17.6

a. Data from CINRGI study report, tables 18-19.
b. Change in PaO_2 (in mm Hg/ 2 hours) calculated as difference between timepoints and previous average value.

5.1.2 Clinical Demographics of CINRGI, NINOS, INO1, and INOSG Trials (cont)

The change in OI with time is shown below. There appears to be a consistent trend towards a higher OI in the control group, relative to the I-NO group, evident from at least 2 hours prior to baseline. Even if one assumes that the recorded baseline values are 'contaminated' by individuals receiving study gas (I-NO or control), the values at – 2 hours still suggest some baseline differences between the two treatment groups with regard to oxygenation status.

Figure 1.12.1.1 Change in OI Prior to Study Gas in CINRGI



NINOS Clinical Demographics

* Overall the NINOS trial population was balanced in their clinical characteristics, including the underlying pulmonary disease and their cardiac status at baseline.

Table 5.1.2.6 (from 6.0.1.12.1.2) Underlying disease of subjects enrolled in NINOS.

Disease	Control	Inhaled I-NO
Meconium Aspiration (% of total)	58 (48%)	58 (51%)
Idiopathic PPHN	22 (18%)	19 (17%)
Sepsis/pneumonia	24 (20%	26 (23%)
Respiratory Distress Syndrome	15 (12%)	10 (9%)
Suspected Pulmonary Hypoplasia	1 (1%)	1 (1%)
Other	1 (1%)	0 (0%)

Almost all subjects had echocardiograms (97% of placebo subjects, 98% of I-NO subjects). The results, which show that the echocardiographic characteristics of the two groups were well-balanced, are summarized below. Note that a significant percentage of the subjects in the NINOS had evidence of left to right shunt on ECHO (hence, did not have pulmonary hypertension severe enough to cause reversal of normal pressure gradient). These individuals would not have been accepted into the INO-01/ -02 or INOSG trials, as they did not meet the echocardiographic definition of PPHN.

5.1.2 Clinical Demographics of CINRGI, NINOS, INO1, and INOSG Trials (cont)

Table 5.1.2.7 (from 6.0.1.12.1.3) Echocardiographic characteristics of randomized subjects in NINOS.

	Placebo Group (n=121)	I-NO Group (n=114)	p Value[a]
Echocardiogram performed	118/121 (98%)	110/114 (97%)	0.64
Patent ductus arteriosus (PDA) identified	92/118 (78%)	88/110 (80%)	0.71
Direction of PDA shunt			
Right to left	19/92 (21%)	18/87 (21%)	0.82
Bidirectional	43/92 (47%)	37/87 (42%)	
Left to right	30/92 (32%)	32/87 (37%)	
Tricuspid regurgitation identified[b]	76/117 (65%)	63/109 (58%)	0.27
Foramen ovale (FO) shunt identified[b]	99/117 (85%)	81/108 (75%)	0.07
Direction of FO shunt[b]			
Right to left	23/99 (23%)	21/78 (27%)	0.6
Bidirectional	39/99 (39%0	25/78 (32%)	
Left to right	37/99 (37%)	32/78 (41%)	
Right ventricular function[b]			
Normal	79/110 (72%)	71/104 (68%0	0.85
Decreased	29/110 (26%)	31/104 (30%)	
'Markedly" decreased'	2/110 (2%)	2/104 (2%)	
Interventricular septum flattening[b]	45/112 (40%)	47/106 (44%)	0.53
Local diagnosis of PPHN[b]	82/117 (70%)	60/110 (63%)	0.24
Clinical diagnosis of PPHN[b]	95/117 (81%)	81/109 (74%)	0.21

a. p value calculated using chi-square or Student's t-test, as appropriate (see NDA, vol. 2.35, page 009510.
b. data shown as % of subject with available data.

The next table summarizes baseline oxygenation in the two groups. The average OI is quite high in both groups, identifying an extremely 'sick' population (the higher the OI the worse the respiratory failure). Compare this with the baseline OI in the INO-01/ -02 trial of approximately 25 and in the INOSG trial of 42-45.

Table 5.1.2.8 (from 6.0.1.12.1.4) Baseline clinical parameters of subjects enrolled in NINOS[c].

Pulmonary status at baseline	Control	Inhaled I-NO	p Value[a]
Oxygenation Index #1[b] (OI) (cm H_2O/mmHg)	45±22	43±17.6	0.46
Oxygenation Index #2 (OI)	46.3±19.9	47.3±31.3	0.76
Oxygenation Index #3 (OI)	43.1±25.5	39.8±24.6	0.43
PaO_2 (first determination) (mmHg)	45.5±13.9	46.8±15.5	0.51
A-aDO_2	615±38	614±36	0.81
Mean Airway Pressure (cm H_2O)	19±4.9	18.7±4.1	0.6

a. p value calculated using chi-square or Student's t-test, as appropriate (see NDA, vol. 2.35, page 009510.
b. Up to three OI reading could be taken prior to initiation of study gas. A majority of both groups received three OI readings (90% in placebo, 84% in I-NO group).
c. No hemodynamic data (blood pressure, heart rate) prior to randomization is available.

INO-01/ -02 Clinical Demographics

The underlying disease responsible for the hypoxemic respiratory failure in the enrolled subjects is listed below. There were larger numbers of subjects with idiopathic PPHN in the I-NO group (especially the 80 ppm group). Otherwise, the treatment groups were well-balanced in the INO-01/ -02 trials.

Table 5.1.2.9 (from 6.0.3.12.1.2) Underlying disease of the infants enrolled in INO-01/ -02.

Disease (#, % of total)	Control	Inhaled I-NO			Pooled I-NO	p Value
		5 ppm	20 ppm	80 ppm		
Meconium Aspiration	26 (41%)	26 (41%)	17 (47%)	17 (46%)	60 (53%)	0.22
Idiopathic PPHN	5 (12%)	5 (12%)	6 (17%)	13 (35%)	24 (21%)	0.03
Sepsis/pneumonia	13 (32%)	11 (27%)	9 (25%)	10 (27%)	30 (26%)	0.92
RDS	4 (10%)	4 (10%)	7 (19%)	2 (5%)	13 (11%)	0.27
Other	5 (12%)	6 (15%)	8 (22%)	8 (22%)	22 (19%)	0.54
Total	41	41	36	37	139/144 (96%)[a]	

a. 5 subjects were entered into the trial, and later found not to meet the criterion of echocardiographically proven PPHN.
b. RDS: Respiratory Distress Syndrome

5.1.2 Clinical Demographics of CINRGI, NINOS, INO1, and INOSG Trials (cont)

INO-01/ -02 (cont)

The baseline hemodynamic, pulmonary and cranial ultrasound findings were also well-matched between the four groups. Note that the average OI in the INO-01/ -02 trial was significantly lower than in either the INOSG or the NINOS trial, where the OI averaged >40.

Table 5.1.2.10 (from 6.0.3.12.1.3) Baseline clinical characteristics of subjects enrolled in INO-01/ -02.

Clinical Characteristic	Control	Inhaled I-NO			Pooled I-NO
		5 ppm	20 ppm	80 ppm	
Pulmonary status[a]					
Oxygenation Index (OI) cm H_2O/mm Hg	25.3±10.4	24.4±10.4	25.3±9.5	22.4±7	24.0±9
PaO_2	58.6±16	68.2±57	60.1±16	63.7±27	64.2±38
Peak Inspiratory Pressure (cm H_2O)	32.5±6	33.3±6	31.8±6	32.0±5	32.4±6
PH	7.48±0.12	7.52±0.11	7.47±0.13	7.51±0.09	7.50±0.11
$PaCO_2$	32.9±10	29.3±8	32.0±11	29.5±8	30.2±9
Hemodynamic status[a]					
Diastolic BP (mm Hg)	45.5±9.8	45.0±7.5	43.7±12.1	42±9	43.6±9.6
Systolic BP (mm Hg)	68.5±13	67.9±12	68.6±17	60.9±10	65.9±14
Mean BP (mm Hg)	54.5±10	54.5±8	53.5±13	49.5±9	52.6±10
Heart Rate (BPM)	148±19	152±26	153±25	151±23	152±25

a. Pulmonary and hemodynamic status, and cranial ultrasound findings taken from the baseline values at time of randomization.
b. p value calculated using chi-square test.

The next table summarizes the echocardiographic data for the infants INO-01/ -02. The echocardiographic findings were well-matched between the four groups.

Table 5.1.2.11 (from 6.0.3.12.1.1) Baseline echocardiographic findings in subjects enrolled in INO-01/ -02. Note: more than one criteria could be present in one subject.

Clinical Characteristic	Control	Inhaled I-NO			Pooled I-NO
		5 ppm	20 ppm	80 ppm	
Patent ductus arteriosus (PDA) not identified but pre- vs post-ductal O_2 difference >10%	2/41 (5%)	4/41 (10%)	2/36 (6%)	4/37 (11%)	10/114 (9%)
Right to left or bidirectional PDA shunt	34/41 (83%)	32/41 (78%)	30/36 (83%)	24/37 (65%)	86/114 (75%)
Closed ductus arteriosus with other evidence of PPHN[a]	6/41 (15%)	5/41 (12%)	5/36 (14%)	11/37 (30%)	21/114 (18%)
None of the above[c]	2/41 (5%)	2/41 (5%)	1/36 (3%)	0/37 (0%)	3/114 (3%)

a. Evidence of pulmonary hypertension in the absence of identified patent ductus arteriosus: a right to left or bidirectional shunt at the level of the foramen ovale plus either moderate to severe tricuspid insufficiency or severe tricuspid insufficiency with evidence of pulmonary systolic pressure >75 % of systemic, or posterior systolic bowing of the intraventricular septum.
b. p value by Fischer's exact chi-squared test. NS = >0.05
c. Subjects lacking all of the first three findings were protocol violators.

INOSG Clinical Demographics

The INOSG trial did not collect information about the underlying pulmonary disease causing the respiratory failure. Below is the baseline oxygenation data, which identify a very sick population.

Table 5.1.2.12 (from 6.0.2.12.1.2) Baseline clinical parameters of subjects enrolled in the INOSG trial[a].

Variable	Control	I-NO	p-value[b]
Oxygenation Index (OI) cm H_2O/mmHg	45.9±18 (range 23 to 88)	42.1±15 (9 to 75)	
PH	7.47±0.14 (range 6.99 to 7.61)	7.50±0.12 (range 7.22 to 7.69)	0.52
PaO_2	38.1±9 (range 23 to 53)	41.3±9 (range 25 to 58)	0.48
$PaCO_2$	33.5±11 (range 19 to 71)	32.4±12 (range 21 to 73)	0.78

a. Data from NDA volume 2.16, Table 7 and appendix 16.2.3.
b. p value calculated using Wilcoxon Rank Sum test or chi-square test as appropriate.

5.2 Extent of exposure (dose/duration)

A total of 375 subjects received I-NO during the trials submitted in this NDA, as listed in the demographics tables above. In the NINOS trial, four subjects in the control group and one subject in the I-NO group did not received study gas, and so are not included in this table (or the safety analysis). Additionally, 7 subjects randomized to control were administered I-NO, and 1 subject randomized to I-NO received control gas. The INO-03 trial enrolled only 14 subjects before being stopped, and so represents a small fraction of the total safety database.

Table 5.2.1 Enumeration of subjects from CINRGI, NINOS, INOSG, INO-01/ -02 and INO-03 trials exposed to I-NO.

Trial	Control	I-NO 5 ppm	I-NO 10 ppm	I-NO 20 ppm	I-NO 40 ppm	I-NO 80 ppm	I-NO 100 ppm	Combined I-NO
CINRGI	89			97				97
NINOS[a]	110	1	1	43	1	82	2	120
INOSG[b]	28					30		30
INO-01/02	41	41		36		37		114
INO-03	0			14				14
Total	275	42	1	93	1	149	2	375

a. All subjects in the I-NO group in NINOS were first exposed to 20 ppm. A subset of the subjects who did not respond were then given I-NO, 80 ppm. Small numbers of subjects also received either more, or less, then the intended 20 or 80 ppm (protocol violations).
b. Three subjects in the INO-01/ -02 trial also received the incorrect dose of treatment gas.

Exposure Data

A total of 375 infants with hypoxic respiratory failure were exposed to I-NO as part of the NDA. The demographics of the infants are summarized in their respective study reports. The studies used a population of neonates who were near-term. The trials varied with respect to the severity of the hypoxic failure and the presence or absence of documented pulmonary hypertension, but the population demographics were otherwise fairly similar between the four trials. It is critical to remember that the dose of I-NO used varied between the four major trials between 5 and 80 ppm. The small numbers of patients preclude any pooled analyses of the various doses.

In addition to the NDA population, the published literature has approximately 150-200 additional infants exposed to I-NO.

The duration of exposure in the trials in the NDA ranges from a few minutes to >100 hours. The tables below summarize their duration of exposure.

Because the duration of exposure was not normally distributed, the table below shows the median duration of exposure for each of the trials. NINOS had the longest median duration of exposure to I-NO, with one patient who received I-NO for 253 hours (14 days).

Table 5.2.2 Median duration of exposure to treatment gas (control gas or I-NO) from CINRGI, NINOS, INOSG, INO-01/ -02 and INO-03 trials[a].

Study	Control Group	I-NO Group
CINRGI	73.3 hours	27.8 hours
NINOS	21 hours	71 hours
INOSG	20 minutes	48 hours
INO-01/ -02	34 hours	38 hours
INO-03	No control group	91.2 hours

a. From NDA volume 2.50, page 339210 and from the CINRGI study report.
b. Mean duration of gas administration in CINRGI was 27.1 (control) and 40.1 (I-NO).

5.3 Primary and secondary efficacy endpoints from the CINRGI, NINOS, INOSG, and INO-01/ -02 trials

Primary efficacy endpoints

I. CINRGI Primary endpoint

1. The number of patients in each treatment group that received ECMO.

II. NINOS primary endpoint

1. Death before discharge or 120 days (whichever comes first), and/or the initiation of ECMO.

III. INOSG primary endpoint

1. Number of acute oxygenation 'successes' following 20 minutes of treatment gas.

5.3 Primary/ secondary efficacy endpoints from the CINRGI, NINOS, INOSG, and INO-01/ -02 trials (cont)

IV. INO-01/ -02 primary endpoint

1. The occurrence of one or more of the PPHN major sequelae prior to discharge:
   a. Death.
   b. Initiation of ECMO.
   c. Evidence of abnormal neurological sequelae.
   d. Bronchopulmonary dysplasia.

Secondary efficacy endpoints

I. CINRGI Secondary Endpoints

1. Improvement in arterial oxygenation, measured by arterial-alveolar oxygen ratio (a-A ratio), the alveolar-arterial oxygen gradient (A-aDO_2), the arterial partial pressure of oxygen (PaO_2), and the oxygenation index (OI) in the treatment groups.
2. Incidence of the following in the two treatment groups:
   a. Physiologic measures
      i. blood pressure,
      ii. gas exchange,
      iii. methemoglobin levels.
   b. Safety measures
      i. discharge home on O_2 and/or pulmonary medications,
      ii. neurologic abnormalities
      iii. survival to discharge.

II. NINOS Secondary Endpoints

1. Change in PaO_2 levels measured 30 minutes after initial administration of the study gas.
2. Change in mean OI levels measured 30 minutes after initial administration of the study gas.
3. Change in Aa-DO_2 levels before and 30 minutes after initial administration of the study gas.
4. Neurodevelopmental outcomes assessed at 18-24 months corrected age (data not yet submitted).
5. The average length of hospitalization among surviving infants.
6. The number of days of assisted ventilation.
7. The incidence of air leak.
8. The incidence of chronic lung disease.
9. The proportion of infants transferred for potential ECMO.

III. INOSG Secondary and post-hoc analyses

1. The number of subject deaths within 120 days and/or receipt of ECMO
2. Percentage of subjects receiving oxygen therapy at 28 days.
3. Percentage of subjects surviving.

IVa. I-NO-01/ -02 secondary endpoints

1. Physiologic response to I-NO, measured by change in OI and time-weighted OI.
2. Number of days requiring supplemental oxygen.
3. Number of days requiring mechanical ventilation.
4. Number of days in hospital (defined as to end of medically indicated hospitalization, not related to social issues).

IVb. I-NO-01/ -02 long-term follow-up endpoints (measured at 1 year follow-up examination)

1. Incidence of hearing abnormalities.
2. Incidence of developmental delay.

IVc. I-NO-01/ -02 exploratory variables

1. Postductal PaO_2.
2. Preductal O_2 saturation.
3. Postductal O_2 saturation.
4. Mean Arterial Pressure.
5. Positive Inspiratory Pressure.
6. Positive End-Expiratory Pressure (PEEP).
7. Arterial-alveolar O_2 ratio.
8. Arterial-alveolar O_2 gradient.

5.4 Success of trials in meeting pre-specified primary endpoints

Three of the four trials submitted in support of efficacy met their pre-specified primary endpoint: the NINOS, INOSG and CINRGI trials. Of these, the NINOS and CINRGI endpoints included the use of ECMO. The INO-01/ -02 trial was prematurely halted, which may have affected its power to detect a difference for its primary endpoint.

The table below summarizes the rates of the pre-specified, primary endpoints from the four efficacy trials in NDA 20-845. The populations in CINRGI and NINOS are placed in the column for their initial dose of I-NO, although they could be changed during the trials.

Table 5.4.1 Primary endpoints from the CINRGI, NINOS, INOSG, and INO-01/ -02 trials[d].

Study Endpoint	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p value
CINRGI: receipt of ECMO	51/89 (57%)		30/97 (31%)			0.001
NINOS: death before 120 days and/or initiation of ECMO	70/111 (63%)	-	57/119 (50%)			0.021[a]
INOSG: acute oxygenation success	2/28 (7%)			16/30 (53%)		0.0002[b]
INO-01/ -02: PPHN major sequelae[c]	23/41 (56%)	18/36 (50%)	21/35 (60%)	13/33 (39%)	52/104 (50%)	0.34[c]

a. p Value calculated from the subjects who actually received study gas, grouped according to the study gas actually received, using unadjusted chi-square.

b. p Value calculated using unadjusted chi-square.

c. PPHN major sequelae: death; initiation of ECMO; bronchopulmonary dysplasia; neurologic abnormalities. p Value calculated using unadjusted chi-square.

d. Data from original NDA individual study reports, sections 6.0.1, 6.0.2, and 6.0.3 and from CINRGI study review above.

It should be noted that there were significant baseline imbalances in the CINRGI trial which might a role in the results listed above. Based on post-hoc analyses accounting for these imbalances, including an analysis incorporating the different 'trends' in change in OI, all suggest that these adjustments diminish, but do not eliminate the statistical significance of the difference in the use of ECMO between placebo and I-NO in CINRGI. See my CINRGI review for details (section 3.12.5).

Similarly, the NINOS trial had a series of unblinding errors, along with a group of patients randomized to one treatment group (control) but who received I-NO. When the data are analyzed to account for these errors, I-NO still has a significant effect on the use of ECMO in NINOS (see section 4.1.1 above and my written review from 11.19.97 for details, section 6.0.1.12.3a, page 41).

The initiation of ECMO can also be compared across the trials in a search for demonstration of efficacy. The first table below shows the absolute rates of ECMO use, while the following table shows the % reductions from the control rate for each of the trials. Note the lower rate of use of ECMO in the INO-01/ -02 trial in the control group (34%), when compared with the CINRGI (57%), NINOS (55%) or INOSG (71%). The p Values are not adjusted for any baseline imbalances.

Table 5.4.2 Rate of the receipt of ECMO in the CINRGI, NINOS, INOSG, and INO-01/ -02 trials[b].

Study	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p Value[a]
CINRGI	51/89 (57.3%)		30/97 (30.9%)			0.001
NINOS[a]	66/121 (54.5%)		44/114 (38.5%)			0.014
NINOS[c]	62/112 (55%)				48/118 (41%)	0.067[c]
INOSG	20/28 (71%)			12/30 (40%)		0.0198
INO-01/ -02	14/41 (34%)	10/41 (24%)	9/36 (25%)	6/37 (16%)	25/114 (22%)	0.34

a. Based on ITT population, p value calculated using unadjusted chi-square.

b. Data from individual study reports, sections 6.0.1, 6.0.2 and 6.0.3 and from CINRGI study report above.

c. Based on 'gas received' population. p Value calculated using Cochran-Mantel-Haenszel adjusted chi-square test.

5.4 Success of trials in meeting pre-specified primary endpoints (cont)

The percent reduction in the receipt of ECMO varied between the trials and doses of I-NO from as little as a 26% reduction in the 20 ppm group in the INO-01/ -02 trial to a 53% reduction at 80 ppm in the INO-01/ -02 group. There is also no apparent dose-relationship between the I-NO dose and the receipt of ECMO.

Table 5.4.3 Percent reduction in the rate of the initiation of ECMO in the NINOS, INOSG, and INO-01/ -02 trials[a].

Study	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled
CINRGI	57.3%		30.9% (-46%)		
NINOS	55%		41% (-25%)		
INOSG	71%			40% (-44%)	
INO-01/ -02	34%	24% (-29%)	25% (-26%)	16% (-53%)	22% (-35%)

a. Percent reduction calculated as control minus I-NO/control X100.

5.4.1 Sub-Group Analyses of the Primary Endpoint

NINOS and CINRGI were analyzed to see if the effect of I-NO to reduce the use of ECMO was consistent across the various groups of patients, including groups according to the causes of respiratory failure in the newborn in these trials: Meconium Aspiration Syndrome (MAS), Respiratory Distress Syndrome (RDS), Pneumonia/Sepsis, and Primary Pulmonary Hypertension of the Newborn. The results, summarized below, suggest that factors other than the cause of the respiratory failure are also responsible for the effect of I-NO. For example, in CINRGI but not NINOS, infants with RDS had the greatest response to I-NO, while patients with PPHN showed the least difference in the use of ECMO between the treatment groups.

NINOS

In NINOS, the effect of I-NO was independent of most demographics, including sex of the infant, race, and initial OI (although the initial OI did predict somewhat the probability of successful use of I-NO). One important difference was seen, however: the use of I-NO in subjects with idiopathic pulmonary hypertension and pulmonary hypertension associated with pneumonia/sepsis was associated with a greater decrease in the rate of death and/or ECMO than in subjects with either meconium aspiration or respiratory distress syndrome (RDS). In the table below, the % of the control and I-NO subjects who met the primary endpoint is grouped according to the underlying disease responsible for the pulmonary hypertension.

Table 5.4.1.1 (from 6.0.1.12.3b.1) Incidence of subjects meeting the primary endpoint (death and/or initiation of ECMO) within specific subgroups of the NINOS study population[c].

	Control	I-NO Therapy	Relative Risk	95% C.I.[a]	p value
Analysis by etiology					
PPHN (n=41)	16/21 (76%)	6/20 (30%)	0.39	0.21-0.73	0.11
Meconium Aspiration Syndrome (MAS) (n=116)	36/59 (61%)	30/57 (53%)	0.86	0.63-1.19	
Pneumonia/sepsis (n=50)	16/24 (67%)	10/26 (39%)	0.58	0.33-1.00	
RDS (n=25)	7/15 (47%)	5/10 (50%)	1.07	0.46-2.49	
Analysis by demographics					
Delivery method					
Vaginal delivery (n=119)	43/63 (68%)	25/66 (45%)	0.65	0.47-0.9	0.433
Cesarean section (n=116)	34/58 (59%)	27/58 (47%)	0.79	0.56-1.13	
Sex					
Male (n=139)	49/75 (65%)	31/64 (48%)	0.74	0.55-0.99	0.779
Female (n=96)	28/46 (61%	21/50 (42%)	0.69	0.46-1.02	
Race					
White (n=142)	41/72 (57%)	33/70 (47%)	0.83	0.6-1.14	0.245
Non-white (n=88)	35/47 (74%)	19/41 (46%)	0.62	0.44-0.88	
Analysis by initial OI					
Initial OI 25-30 (n=53)	17/28 (61%)	7/25 (28%)	0.46	0.24-0.88	0.672
Initial OI <40 (n=126)	32/64 (50%)	24/63 (39%)	0.77	0.52-1.15	
Initial OI>40 (n=108)	45/57 (79%)	28/51 (55%)	0.70	0.53-0.91	

a. C.I.: confidence interval.
b. Includes HFOV and HFJV.
c. Data from NDA volume 2.14, Table T-1.

5.4.1 Sub-Group Analyses of the Primary Endpoint (cont)

The sponsor performed a post-hoc analysis of those infants who were assessed for the need for ECMO after starting study gas. As discussed in the section reviewing NINOS above (4.1.1), fewer I-NO treated patients in this group received ECMO and the number of infants who never met ECMO criteria were higher in the I-NO group.

Table 5.4.1.1a Time criteria for ECMO was met for sub-group with available data from NINOS[a].

	Control N=106	I-NO Therapy N=93
Met ECMO criteria prior to randomization[b]	62 (58.5%)	54 (58.1%)
Met ECMO criteria after randomization[b]	23 (21.7%)	12 (12.9%)
Never met ECMO criteria[b]	21 (19.8%)	27 (29.0%)
Total with information[c]	106 (87.6%)	93 (81.6%)

a. Data from NDA volume 9.1, table 4.
b. Calculated as a percentage of the infants with available information regarding timing of ECMO assessment.
c. Expressed as a percentage of the original randomized population.

The sponsor also analyzed the frequency of ECMO/Death according to the use of other medical interventions. Note that the infants who did not receive steroids or tolazoline did not have a response to I-NO with regard to the primary endpoint.

Table 5.4.1.2 (from 6.0.1.12.3b.1) Incidence of subjects meeting the primary endpoint (death and/or initiation of ECMO) within specific subgroups of the NINOS study population[c] (cont).

	Control	I-NO Therapy	Relative Risk	95% C.I.[a]	p value
Analysis by Specific Therapy					
Surfactant					
Used (n=168)	47/87 (54%)	31/81 (38%)	0.72	0.55-0.95	0.94
Not used (n=67)	30/34 (88%)	21/33 (64%)	0.71	0.51-0.99	
High-frequency ventilation[b]					
Used (n=130)	44/68 (65%)	29/62 (47%)	0.72	0.53-0.99	0.94
Not used (n=105)	33/53 (62%)	23/52 (44%)	0.71	0.49-1.02	
Both surfactant and high-frequency Ventilation					
Both used (n=147)	50/73 (68%)	37/74 (50%)	0.73	0.56-0.96	0.744
Both not used (n=88)	27/48 (56%)	15/40 (37.5%)	0.67	0.42-1.05	
Steroids					
Used (n=34)	10/19 (53%)	4/15 (27%)	0.51	0.21-1.23	0.433
Not used (n=201)	35/102 (34%)	67/99 (66%)	0.74	0.57-0.94	
Tolazoline					
Used (n=40)	10/17 (59%)	9/23 (39%)	0.67	0.35-1.28	0.772
Not used (n=194)	66/103 (64%)	43/91 (64%)	0.74	0.57-0.95	

a. C.I.. confidence interval.
b. Includes HFOV and HFJV.
c. Data from NDA volume 2.14, Table T-1.

CINRGI

In CINRGI, by contrast with NINOS, infants with RDS had the greatest response to I-NO, while patients with PPHN showed the least difference in the use of ECMO between the treatment groups.

Table 5.4.1.3 Infants receiving ECMO grouped by underlying disease, from the CINRGI trial[a].

	Placebo N=89	I-NO N=97
Meconium aspiration	21/35 (60.0%)	11/34 (32.4%)
Pneumonia/sepsis	14/21 (66.7%)	9/24 (37.5%)
Respiratory Distress Syndrome	7/8 (87.5%)	1/8 (12.5%)
PPHN	9/25 (36.0%)	9/31 (29.0%)
Lung Hypoplasia/ CDH	13/15 (86.7%)	9/11 (81.8%)

a. Data from CINRGI study report table 32 and 33.

5.4.1 Sub-Group Analyses of the Primary Endpoint (cont)

CINRGI (cont)

CINRGI also examined the rate of use of ECMO according to the baseline PaO_2, and found that while the largest reduction in the use of ECMO was seen in the patients with PaO_2 between 50 and 70 mm Hg, I-NO had an effect to reduce the use of ECMO numerically in all strata.

Table 5.4.1.4 Rate of ECMO stratified by baseline PaO_2[a].

	Placebo	I-NO
≤30 mm Hg	11/11 (100%)	5/6 (83.3%)
30 to ≤50	20/40 (50.0%)	13/32 (40.6%)
50 to ≤70	12/19 (63.2%)	4/25 (16.0%)
70 to ≤100	2/6 (33.3%)	3/13 (23.1%)
>100	1/5 (20%)	2/14 (14.3%)

a. Data from CINRGI study report, table 24. Subject with unknown PaO_2 at baseline not included.

The sponsor also used Cochran Mantel Haenzel to test the rate of ECMO controlling for baseline PaO_2, and reported a p Value= 0.007. Again, the majority of the effect of I-NO appeared to reside in the moderately ill infants.

Table 5.4.1.5 Rate of ECMO stratified by baseline OI[a].

	Placebo	I-NO
≤30 cm H_2O/ mm Hg	7/20 (35.0%)	9/43 (20.9%)
30 to ≤40	9/20 (45.0%)	4/15 (26.7%)
40 to ≤50	7/11 (63.6%)	6/11 (54.5%)
>50	12/24 (50.0%)	8/14 (57.%)

a. Data from CINRGI study report, table 24.

Recall also that there was an imbalance with regard to the OI in the treatment groups, and that there appeared to be a difference in the rate at which the infants were getting sicker. In this case, the I-NO patients appeared to be more stable than the control group. To address the impact of this imbalance, the sponsor performed an additional modeling, seeking to incorporate the change in the baseline. Per their analysis, incorporating this into the model does not eliminate the significant effect of I-NO to reduce the use of ECMO in CINRGI. The p-Values for this analysis are shown in the table below.

Table 5.4.1.6 Logistics model examining effect of varying baseline slope for oxygenation parameters on reduction in ECMO by I-NO[a].

Parameter	p-Value for I-NO effect on ECMO[b]	p-Value for I-NO effect on ECMO[c]
OI (n=101)	0.045	0.0025
PaO_2 (n=150)	0.0013	0.0006
FiO_2	0.0002	0.0002

a. Data from sponsor at request of Medical Reviewer. Not confirmed by FDA analysis.
b. For change in parameter prior to baseline.
c. For change in slope of change in parameter prior to baseline.

The FDA statistician also examined the issue of the effect baseline imbalances on the results seen in CINRGI, and concluded that 'the reviewer and sponsor's analyses suggest a difference in the rate of use of ECMO between the two groups even adjusted for the imbalance of baseline OI.' The reader is referred to Dr. Cui's review for further details.

Finally, CINRGI looked at the effect of I-NO in the population with congenital diaphragmatic hernia (CDH), where it was anticipated that I-NO would have little clinical efficacy, leading to their exclusion from the population used for the primary efficacy analysis. The sponsor did collect information on this sub-group separately, which is summarized below. No beneficial effects of I-NO in this population were detected.

Table 5.4.1.6 Efficacy outcomes in infants with lung hypoplasia due to CDH[a].

Clinical Event	Placebo N=15	I-NO N=11	p-Value[b]
Receipt of ECMO	13 (87%)	9 (82%)	1.00
Death within 28 days	6 (40%)	4 (36%)	1.00
Hospital Stay (days)	70±41	53±37	0.40

a. Data from CINRGI study report, table 33.
b. p Value calculated using Student's t test.

5.4.1 Sub-Group Analyses of the Primary Endpoint (cont)

In conclusion then, two trials (CINRGI, NINOS) have demonstrated a significant effect of I-NO to reduce the use of ECMO in infants with hypoxic respiratory failure. These findings are corroborated by a body of evidence, including two other trials with either methodological problems or inadequate power (INOSG, INO-01/ -02) and smaller trials reported in the literature. While this effect is consistent across the trials in the NDA as a whole, there are some inconsistencies among the subsets of the enrolled populations yet to be explained. Most important among these is the possible interaction between I-NO and certain drugs commonly used in the treatment of hypoxia in neonates. Additionally, there is a striking contrast in the outcomes for the small number of patients with RDS between NINOS and CINRGI.

5.5 Success of trials in meeting secondary efficacy endpoints: demonstrating a physiological effect of I-NO

1. Acute changes in oxygenation

The acute effect of I-NO on oxygenation, measured in all four trials, are summarized in the table below. Shown are the average changes (±standard deviation) from baseline to the first measurement after starting study gas (placebo or I-NO). All four trials reported an improvement in oxygenation following I-NO.

Table 5.5.1 Results: comparison of acute changes in oxygenation from the CINRGI, NINOS, INOSG and INO-01/ -02 trials[d].

Changes in clinical markers of oxygenation	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
CINRGI Trial[c]					
Change in PaO_2 (mmHg)	+21.6		+59.1		
Change in OI	-4.3		-10.7		
Change in A-a DO_2 (mmHg)	-23.2		-59.5		
NINOS Trial[a]					
Change in PaO_2 (mmHg)[d]	9.7±51.7		58.2±85.2		
Change in OI[d]	0.8±21.1		-14.1±21.0		
Change in A-a DO_2 (mmHg)[d]	-6.7±57.5		-60.0±85.1		
INOSG Trial[b]					
Change in PaO_2 (mmHg)	-1.9±9.6			47.4±68	
Change in OI	-2.0±15			-16±11.5	
INO-01/ -02 trial[c]					
Change in mean PaO_2	18.0±53	32.3±56	38.6±69	64.4±84	44.6±71
Change in mean OI	-1.3±7.7	-4.7±4.6	-4.3±9.6	-7.4±9.0	-5.5±8.7
Preductal O_2 saturation	0.35±4.1	0.77±3.28	0.14±3.0	0.26±3.4	0.4±3.2
Postductal O_2 saturation	0.27±4.5	1.85±3.86	1.49±4.72	1.43±3.88	1.60±4.1
Arterial-alveolar O_2 ratio	0.03±0.08	0.05±0.09	0.06±0.11	0.10±0.13	0.17±0.11
Arterial-alveolar A-aDO_2	-19.5±56	-31.6±56	-39.6±68	-63.2±81	-44.3±70

a. Shown is the mean± s.d. of the change from baseline for each parameter, measured after 30 minutes.
b. Acute effects measured at baseline and after 20 minutes.
c. Acute effects measured at baseline and after 30 minutes.
d. Variable in which the I-NO and control group means differ significantly (<0.05) using Student t-test are shaded.

2. Dose-dependency of I-NO effects on oxygenation

The INO-01/ -02 trial also suggests that this effect of I-NO to improve oxygenation is dose-dependent in the range from 5 to 80 ppm. The table below show the changes in PaO_2 following study gas administration in the INO-01/ -02 study. The change from baseline was statistically significant using one-way ANOVA (p value = 0.029).

Table 5.5.2 (from 6.0.3.12.3b.1) Acute change in PaO_2 following study gas administration in the INO-01/ -02 study.

Endpoint	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO	p Value
Increase in PaO_2 > 20 mm Hg after 30 minutes of study gas	8/40 (20%)	16/41 (39%)	15/35 (43%)	21/36 (58%)	52/112 (52%)	0.008
% increase in PaO_2 from baseline to 30 minutes	23.4%	31.8%	39.4%	50.3%	40.8%	
Change in PaO_2 from baseline to 30 minutes	17.5±52.1	31±56	38±69	62±83	55.8±79.8	0.029[a]

a. p value determined using one-way ANOVA.

5.5 Success of trials in meeting secondary efficacy endpts: demonstrating a physiological effect of I-NO (cont)

3. Durability of the effect of I-NO on oxygenation

INO-01/ -02

The long-term effects of I-NO on oxygenation were examined in the INO-01/ -02 trial, using the time-weighted OI (TWOI), a measure of the average OI/hour of study gas administration. The results suggested that I-NO has a sustained effect to improve oxygenation.

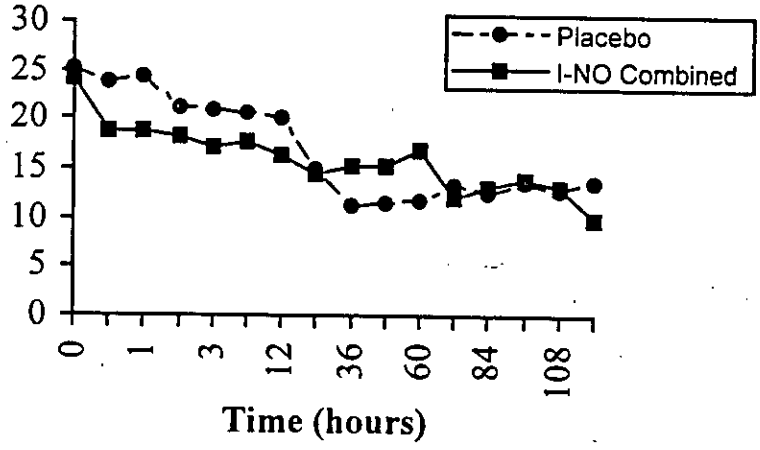
Table 5.5.3 (from 7.0.3.3) Effects of NO on oxygenation and hemodynamics beyond 30 minutes[a] in the INO-01/ -02 trial.

Endpoint	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO	p value
Change in TWOI	-1.60±8.0 (-22 to 15)	-4.67±7.7 (-25 to 14)	-4.78±10.1 (-36 to 14)	-5.59±7.4 (-24 to 8)	-5.0±8.4 -36 to 14	see note[b]

a. Data taken from baseline to beyond 30 minutes to the time of withdrawal from I-NO, from NDA volume 2.17, Table 23.
b. Repeated t test vs control group were significant (<0.05) for the 5 and 80 ppm group, as well as the pooled I-NO group.

The majority of the effect of I-NO on oxygenation was manifest in the first few hours of study gas administration, where there was a clear separation of control and I-NO groups with regards to their average OI. The figure below shows the OI measured every hour in the INO-01/ -02 trial.

Figure 7.0.3.4 Time vs. OI in the INO-01/02 Trial



CINRGI Trial

CINRGI also looked at the sustained effects of I-NO on oxygenation. The table below summarizes the changes in the mean PaO_2 through 24 hours for the subjects with available data. At all time points measured, including baseline and the earliest time-point after initiation of study gas, the infants in the I-NO group had a higher mean PaO_2. In data not shown, this effect to improve oxygenation was independent of the underlying pulmonary disease (e.g., pneumonia, RDS, MAS) except for hypoplastic lungs (CDH), where little effect of I-NO on oxygenation (or use of ECMO) was seen.

5.5 Success of trials in meeting secondary efficacy endpts: demonstrating a physiological effect of I-NO (cont)

Table 5.5.4 Arterial PaO_2 over time (mm Hg) in the CINRGI trial[a].

Time		Placebo	I-NO
Baseline	mean±sd	54.3±36.1	77.6±68.3
	median	47	53.5
	number of pts	81	90
30 Minutes	mean±sd	75.9±68.2	136.7±105.8
	median	54.5	92.5
	number of pts	82	86
1 Hour	mean±sd	100.9±102.8	141.4±104.7
	median	60.0	108
	number of pts	77	94
4 Hour	mean±sd	117.6±96.3	145.1±102
	median	74.5	101
	number of pts	62	88
12 Hours	mean±sd	130.9±93.2	147.2±79
	median	92	127
	number of pts	45	80
24 Hours	mean±sd	116.6±67	141.6±77.5
	median	90	115
	number of pts	44	73

a. Data from CINRGI study report, table 36.

The data from CINRGI were also analyzed by the FDA statistician, Dr. Cui. He concluded that the CINRGI ...'analyses fail to support the conclusion that there is a sustained treatment effect of NO on oxygenation.' He also concludes that ...'the NO treatment affected patients more rapidly as compared to the conventional therapy used (as the background therapy) in placebo.' An analysis of the CINRGI oxygenation data from Dr. Cui's draft review is found below. The reader is referred to his review for further details.

Table 5.5.4a (from Dr. Cui's review, table 2.4) Analysis of change in OI and PaO_2.

OI			
Time	Control (n, Δ)	NO (n, Δ)	Nominal p-value*
Baseline	(n=75) 43.9	(n=83) 35.0	0.0119
30 min	(n=65) –4.4	(n=71) –11.8	0.0230
1 hour	(n=69) –4.9	(n=81) –12.0	0.0679
4 hours	(n=71) –4.9	(n=82) –13.3	0.0374
12 hours	(n=71) –8.1	(n=82) –14.9	0.0943
24 hours	(n=71) –8.8	(n=82) –15.1	0.1198
PaO_2			
Time	Control (n, Δ)	NO (n, Δ)	Nominal p-value*
Baseline	(n=81) 54.3	(n=90) 77.6	0.0055
30 min	(n=75) 23.7	(n=80) 57.8	0.0093
1 hour	(n=78) 38.3	(n=89) 63.9	0.1023
4 hours	(n=79) 44.4	(n=89) 63.6	0.2422
12 hours	(n=79) 45.3	(n=89) 61.4	0.3005
24 hours	(n=79) 36.6	(n=89) 46.5	0.4874

* for group difference

In conclusion, then, the data demonstrate that I-NO has an acute effect to improve oxygenation in neonates with hypoxic respiratory failure due to any of the pulmonary diseases examined with the exception of congenital diaphragmatic hernia (CDH).This effect appears to be dose-dependent between 5 and 80 ppm, based on the data from the INO-01/ -02 trial. The majority of the effect on oxygenation appears to be in the first few hours of I-NO. This last conclusion is limited by the concomitant use of other interventions in these infants with effects on oxygenation.

5.5 Success of trials in meeting secondary efficacy endpts: demonstrating a physiological effect of I-NO (cont)

2. Effect of I-NO on pCO_2

The INOSG trial reported a significant, acute effect of I-NO on pH when comparing the initial baseline value to the value at the end of 20 minutes. No significant difference between the second baseline and the 20 minute value was detected. The second baseline value was taken after reducing the FiO_2 to 90%, just prior to starting the study gas. Thus, the comparison between the second baseline and the 20 minute value is the best comparison to use to determine the acute effect of I-NO, independent of any effect of reduced FiO_2. No effect of I-NO on pCO_2 was seen in the larger INO-01/ -02 trial, although there was an numerical decrease in pCO_2 in that trial in all dose-groups of I-NO. As shown in Figure 6.0.3.14.9, the average pCO_2 remained stable in both I-NO and control groups during chronic administration of study gas.

Table 5.5.5 (from 7.0.3.4) Results: comparison of acute changes in pCO_2 from the INOSG, and INO-01/ -02 trials (no data submitted from the CINRGI and NINOS trials)[a,d].

Changes in pCO_2	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
INOSG Trial					
Change in pCO_2 from first baseline[b]	-0.9±4.8			-4.1±7.6	
Change in pCO_2 from second baseline[b]	+0.6±10			-3.2±10	
INO-01/ -02 trial[c]					
Mean change in pCO_2	-0.75±5.5	-1.24±6.0	-1.31±3.6	-1.17±4.9	-1.24±4.9

a. Shown is the mean± s.d. of the change from baseline for each parameter.
b. Acute effects measured at first baseline and after 20 minutes of study gas.
c. Acute effects measured at second baseline and after 20 minutes of study gas.
d. Variable in which the I-NO and control group means differ significantly (<0.05) using Student's t-test are shaded.
e. Acute effects measured at second baseline and after 30 minutes of study gas.

In conclusion, no clinically significant effect of I-NO on pCO_2 was detected in two of the trials.

3. Effect of I-NO on pH

No significant effect of I-NO on pH was detected in either the INOSG or the INO-01/ -02 trials (no data were submitted from the CINRGI and NINOS trials). As shown below, the average pH remained stable in both I-NO and control groups during acute administration of study gas.

Table 5.5.6 (from 7.0.3.5) Acute effect of I-NO on pH in the INOSG and INO-01/ -02 trials[a].

Changes in pH	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
INOSG Trial[b]					
Change in pH	0.02±0.07			0.05±0.09	
INO-01/ -02 trial[c]					
Mean pH	0.02±0.06	0.02±0.06	0.02±0.04	0.01±0.05	0.02±0.05

a. Shown is the mean± s.d. of the change from baseline for each parameter.
b. Acute effects measured at baseline and after 20 minutes.
c. Acute effects measured at baseline and after 30 minutes.
d. Variable in which the I-NO and control group means differ significantly (<0.05) using Student t-test are shaded.

4. The effect of I-NO on hemodynamics and pulmonary pressures

The effects of I-NO on hemodynamics and pulmonary pressures was measured in the INOSG and INO-01/ -02 trials, which are summarized below. The INOSG trial reported an acute, significant decrease in mean systemic blood pressure only between the first baseline value and the 20 minute value. There was no significant difference between the second baseline value and the 20 minute value in the INOSG trial (not shown). The larger INO-01/ -0_2 trial found a small, non-significant decrease in mean arterial pressure in the I-NO group, and no long-term effect of I-NO on blood pressure.

There was no acute effect of I-NO on mean airway pressures detected in the INO-01/ -02 trial, as seen in the table below. There was also not a long-term trend towards a more rapid decrease in mean airway pressures in the I-NO group. Additionally, no acute or chronic effect of I-NO on the amount of PEEP required by the infants, or the average positive inspiratory pressure (PIP) was detected in the available trials (data not shown).

5.5 Success of trials in meeting secondary efficacy endpts: demonstrating a physiological effect of I-NO (cont)

Table 5.5.7 (from 7.0.3.6) Acute effect of I-NO on pulmonary and systemic hemodynamics in the INOSG and INO-01/ -02 trials.

Changes in pH	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled
INOSG Trial[b]					
Change in mean systemic blood pressure (mmHg) from first baseline[e]	+4.1±9.0			-4.6±13.2	
Change in mean systemic blood pressure (mmHg) from 2nd baseline[f]	+1.5±10			-1.0±10	
Change in heart rate (beats per minute)	+2.0±15			+1.0±10.2	
INO-01/ -02 trial[c]					
Mean Arterial Pressure (mmHg)	-0.73±10.9	-2.39±9.69	-3.22±9.4	+0.69±10.0	-1.7±9.8
Mean Systolic Pressure	-1.32±13	-3.29±12	-4.64±14	+1.86±12	-2.0±13
Mean Diastolic Pressure	-0.68±9	-1.39±8	-2.47±7.3	-0.08±8	-1.31±8
Mean Peak Inspiratory Pressure (PIP)	+0.27±1.1[b]	+0.07±0.41	+0.31±1.06	+0.03±0.16	+0.13±0.66
Positive End-Expiratory Pressure (PEEP)	+0.0±0.0	+0.02±0.16	+0.00±0.24	-0.03±0.16	+0.0±0.19
Mean Airway Pressure (P_{AW})	-0.1±1.2	+0.1±0.7	-0.1±1.4	+0.0±1.3	+0.0±1.2

a. Shown is the mean± s.d. of the change from baseline for each parameter, measured after 30 minutes.
b. Acute effects measured at baseline and after 20 minutes.
c. Acute effects measured at baseline and after 30 minutes.
d. Variable in which the I-NO and control group means differ significantly (<0.05) using Student t-test are shaded.
e. Statistics calculated using Wilcoxon Rank Test comparing first baseline value to 20 minute value.
f. Statistics calculated using Wilcoxon Rank Test comparing second baseline value to 20 minute value.

The effect of I-NO on cardiac output, pulmonary vascular resistance, systemic vascular resistance, or reduction in the right-to-left shunting of blood from pulmonary hypertension were not examined in any of the trials.

In conclusion, no clinically-significant effect of I-NO on systemic or pulmonary pressures was detected in the INO-01/ -02 or INOSG trials. No information about the effects of I-NO on the reversal of pulmonary hypertension is available from the trials in the NDA.

5. Summary of physiological effects of I-NO

The most striking, and consistent effect of I-NO is to improve oxygenation in subjects with hypoxic respiratory failure. In all of the NDA trials, there was a significant, acute improvement in oxygenation at the earliest measured timepoint. In the INO-01/ -02 trial, this improvement was dose-dependent between 5 and 80 ppm, and persisted beyond the initial measurement, compared with controls. The effect on oxygenation was also sustained numerically in the CINRGI trial, although the significance of the difference was lost within a few hours of gas initiation.

There was a nonsignficant decrease in both pCO_2 and mean systemic blood pressure acutely following I-NO administration. No long-term effect of I-NO on blood pressure, pH, or pCO_2 was seen in the INO-01/ -0_2 trial. No acute or chronic effect of I-NO on the pulmonary airway pressures was detected. No information about the pulmonary and systemic vascular resistance, or the reversal of the right-to-left shunting from pulmonary hypertension was obtained in these trials.

5.6 Success of trials in meeting secondary efficacy endpoints: demonstrating a clinical benefit for I-NO

Each of the trials measured several other endpoints, either as part of secondary endpoints or as part of the safety measurements, which could be used to argue a clinical benefit. A summary of these potential benefits is below, along with the efficacy data that was collected for each benefit. Overall, no significant beneficial effect of I-NO was detected for any of the listed endpoints.

1a. Duration of supplemental O_2 at time of discharge

The trials all collected data on the use of supplemental O_2 at the time of discharge. Overall, no trend towards a significant difference was detected.

Table 5.6.1 (from 7.0.4.1) Incidence of use of supplemental O_2 at time of discharge in the CINRGI, NINOS, INO-01/ -02 and /-03 and INOSG trials[a].

Use of O_2 at time of discharge	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p Value[b]
CINRGI	7/81 (7.4%)		4/90 (4.4%)			0.195
NINOS	15/100 (15%)				14/98 (14%)	0.89
INO-01/ -02 and /-03	6/41 (15%)	9/45 (20%)	4/43 (9%)	7/39 (18%)	20/127 (16%)	0.51
INOSG[b]	4/21 (19%)			1/27 (4%)		0.19
Pooled Results	32/243 (13.1%)				39/342 (11.4%)	

a. Data from NDA, volume 2.26 appendix 16.2.2.21, and 2.31 data listing 16.5 as well as the CINRGI study report. Data shown as % of all subjects with data. INOSG data from NDA volume 2.16, Appendix 16.2.7.

b. p values calculated using unadjusted chi-square.

Table 5.6.2 (from 7.0.4.2) Duration of supplemental O_2 therapy in the INOSG trial and INO-01/ -02 trials (no data available from the CINRGI and NINOS trials).

Duration of supplemental O_2 (days)	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p Value[a]
INOSG[b]	19±21			11±6		0.066
INO-01/ -02	6±7	5±5	5±3	6±8	5±6	0.47

a. NA = not available

b. Excludes one infant in the control group reportedly required O_2 for 445 days.

1b. Duration of supplemental O_2 at time of long-term follow-up

Long-term follow-up data were collected for the available patients in the NINOS, CINRGI, and INO-01/ -02 trials. The number of infants who required O_2 at time of follow-up (ranging from 6 months to 2 years) is summarized below. In one trial, INO-01/ -02, there were no infants in the control group who required O_2 at the time of follow-up, compared with O_2 use in 3-22% of the I-NO group. In the other two trials, the use of O_2 at the time of follow-up was balanced between the two treatment groups.

Table 5.6.3 Other medical therapies used by the subjects with long-term F/U in the CINRGI, NINOS and INO-01/ -02 trials[d].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
CINRGI[c]					
On O_2	2/48 (4.2%)	0/42 (0%)			
INO-01/ -02[a]					
Home Oxygen	0 (0%)	8 (22.2%)	1 (3.4%)	5 (16.1%)	14 (14.6%)
Mean age when O_2 was D/C'd (months)[b]	–	4.0±3.0	1.0±0.0	3.3±1.3	3.5±2.5
NINOS[c]					
Currently using O_2	0 (0%)		1 (1.2%)		
Previously used O_2	14 (16.1%)		13 (15.3%)		

a. Data from INO-01/ -02 study report, NDA vol. 9.3, Tables 11.

b. For infants who received O_2 at time of initial discharge.

c. Data from NINOS study report, NDA vol. 11.1, Table 38, 39.

d. Period of follow-up 6 months (CINRGI), 18-24 months (NINOS) and 1 year (INO-01/ -02).

e. From CINRGI study report.

5.6 Success of trials in meeting secondary efficacy endpoints: demonstrating a clinical benefit for I-NO (cont)

2. Duration of mechanical ventilation

The duration of mechanical ventilation was collected in three of the trials, and the results summarized below. No effect of I-NO to decrease the duration of ventilation was evident.

Table 5.6.4 (from 7.0.4.3) Duration of mechanical ventilation in the NINOS, INOSG and INO-01/ -02 trials[a].

Duration of mechanical ventilation (Days)	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p Value[b]
NINOS	11.1±13				12.3±14	0.47
INOSG	27±84			8±4		0.56
INO-01/ -02	8±5	9±7	8±5	10±10	9±7	0.94

a. Data from respective study reports and my original review dated 11.97.
b. p Value calculated by unadjusted unpaired t test.

3. Duration of hospitalization

No effect of I-NO on the duration of hospitalization was reported in any of the trials.

Table 5.6.5 (from 7.0.4.4) Duration of hospitalization in the CINRGI, NINOS, INOSG and INO-01/ -02 trials[a].

Duration of hospitalization (Days)	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Pooled	p Value
CINRGI	25.3+17	22.5±10				0.198
NINOS[a]	29.5±23			36.4±45		0.17
INOSG	42±91			20±8		0.24
INO-01/ -02	26±20	22±11	21±10	24±12	24±11	0.45

a. Data from my original NDA review dated 11.97 and the CINRGI study report, table 29.

4. Mortality Rate

The mortality rates reported for each of the trials are summarized below. These analyses exclude the patients with CDH, as they were only included in the CINRGI trial as a subset. No trial found a significant effect of I-NO on mortality, although the INO-01/ -02 did find a 4X increase in mortality through 28 days in the I-NO group. The pooled analysis, which is limited by the amount of follow-up available for each trial, suggests that no large adverse effect of I-NO on mortality is likely.

Table 5.6.6 (from 8.1.1.1) Incidence of death in the NINOS, INOSG, INO-01/ -02 and INO-03 studies[b].

Study	Control group	I-NO group	p Value
CINRGI (0-28 days)	5/89 (5.7%)	3/97 (3.1%)	0.48
CINRGI (0-180 days)	5/89 (5.7%)	4/97 (4.1%)	0.74
NINOS (0-120 days)[d]	20/121 (16.5%)	16/114 (14%)	0.596
NINOS (0-120 days)[c]	17/112 (15.1%)	17/118 (14.4%)	0.87
INOSG (0-445 days)	3/28 (10.7%)	2/30 (6.7%)	0.70
INO-01/ -02 (0-28 day)	1/41 (2.4%)	9/113 (8%)	0.29
INO-01/ -02 (0-1 yr)	2/41 (4.9%)	10/113 (8.8%)	0.42
INO-03 (0-28 days)	No control group	0/14 (0%)	N/A
Total[b,e]	30/279 (10.7%)	32/368 (8.7%)	0.252

a. The comparability of the incidence rates between the trials is limited by the varying length of follow-up for each trial.
b. Data from individual study reports and electronic datasets.
c. Grouped from the NINOS subjects who actually received study gas, according to the gas actually received, using unadjusted chi-square.
d. Based on NINOS ITT population, p value calculated using unadjusted chi-square.
e. This overall incidence figure includes the 0-180 day CINRGI data, the 0-120 day ITT NINOS population, the INOSG trial, the INO-01/ -02 0-1-yr population, and the INO-03 trial population. p Value using Fisher's Exact test.

5.6 Success of trials in meeting secondary efficacy endpoints: demonstrating a clinical benefit for I-NO (cont)

The data above are for infants without congenital diaphragmatic hernia, who were excluded from the NINOS, INOSG and INO-01/ -02 trials. In the CINRGI trial, these infants were enrolled as a separate stratum. Their mortality data are summarized below.

Table 5.6.7 All-cause mortality for infants with lung hypoplasia through 28 days in CINRGI[a].

	Placebo N=15	I-NO N=11
Dead	6/15 (40.0%)	4/11 (36.4%)
Alive	9/15 (60.0%)	7/11 (63.6%)

a. Data from CINRGI study report table 33. Shown for the lung hypoplasia population.

In conclusion, there was no significant effect of I-NO on mortality in any of the trials individually, and the pooled analysis, with all of its difficulties, has a numerical trend in favor of I-NO. The INO-01/ -02 trial is the one trial that found a numerical excess mortality in the I-NO group. The small number of infants with lung hypoplasia due to CDH precludes any conclusions about the effect of I-NO on mortality in this population.

5.7 Clinical effect of I-NO from the secondary data sources

Almost all of the published data on I-NO, with the exception of the papers reporting the NINOS and INOSG trial results, come from single centers administering I-NO in unblinded, uncontrolled fashion. For efficacy results, then, the studies are under-powered to detect a significant impact of I-NO on clinically relevant endpoints. Improvements in oxygenation have been reported in almost all trials. The percentage of infants who had such an improvement with I-NO has varied from 44% to nearly 100% depending on the study. In a review of the available trials on I-NO, one group estimated that 59% of neonates have an initial improvement in oxygenation, and that 60% of these infants have a sustained response (1).

The effect of I-NO on the use of ECMO and oxygenation in newborns with hypoxemic respiratory failure has been explored in other trials. Two trials were published in late 1999 that investigated the use of I-NO in hypoxic newborns. In addition to these two trials, there are a handful of older trials using I-NO in hypoxic newborns.

1. The Franco-Belgium Collaborative NO Trial Group published the results of a random, open-label trial enrolling pre-term (<33 weeks) and near-term infants with hypoxic respiratory failure to I-NO (10 ppm) or control gas, using change in oxygenation (OI) from baseline through 2 hours as the primary endpoint (2). While oxygenation improved in both groups numerically, the change was significant at <0.05 only in the near-term infants. The paper does not report the incidence of ECMO use, but implies it was similar in the two groups. The trial also purports to show a significant reduction in the number of days of ventilation for the near-term infants (a change from 7 to 6 days on average!), and in the number of days in the NICU (12±9 to 9±6 days on average).

Of relevance, the trial did measure the use of pulmonary medications (beta-agonists, steroids) and O_2 at the end of 28 days. For both the pre-term and near-term infants there was a trend towards less use of these agents in the I-NO group. No effect on mortality was detected (as expected).

2. A second trial, directed by Dr. John Kinsella, investigated the effect of I-NO (5 ppm) in a randomized, double-blind trial enrolling premature infants (≤34 weeks gestation) into two groups: control (n=48) or I-NO (n=32) (3). The primary endpoint was survival to discharge, and no difference between the treatment groups was detected 47% in controls, 52% in the I-NO group). There was also no differences detected between the two groups with respect to intracranial hemorrhage grade 2-4 (28% I-NO, 33% control), pulmonary hemorrhage (13% vs. 9%) or chronic lung disease (60% vs. 80%). The use of O_2 was greater in the control group at 36 weeks (80%) than in the I-NO group (54%).

3. Finally, there are a number of smaller trials published prior to 1999: One randomized, controlled, open-label trial failed to demonstrate a reduction in the use of ECMO in subjects receiving I-NO (4). This trial enrolled a relatively small numbers of subjects (23 control, 26 I-NO). In a trial using historical controls (16 total subjects), a small decrease in ECMO following I-NO was suggested (5), while another using a cross-over design (17 total subjects) found no effect of I-NO on ECMO use (6). Another investigator, in an unblinded study, estimated that NO apparently cut out a 15% segment from the patient group' (30 total subjects) who would otherwise have received ECMO (7). The death rates in each of the trials was low, so no statement of an I-NO effect can be inferred from these papers. No other clinical benefits of I-NO have been demonstrated from any of the published trials.

6.0 Integrated Safety Summary for CINRGI, NINOS, INO-01/ -02 and INOSG Trials

6.1 General Comments about Adverse Event collection

The trials that comprise the NDA had varying approaches to adverse event collection. The INO-01/ -02 and CINRGI trials collected adverse events more or less like is usual, although no Serious Adverse Events were collected. These two trials, in addition, collected specific information about selected adverse events felt to be of particular relevance to the population (i.e., airleak syndrome, seizures). The NINOS and INOSG trials were investigator-derived, and collected only specific adverse events.

Of these specific adverse events, there were several adverse, which, if they occurred with less frequency in the I-NO group, could be taken as supportive of a beneficial clinical effect of I-NO. A list of these events, followed prospectively in one or more clinical trials, includes:

1) Air Leak Syndrome (ALS);
2) Bronchopulmonary dysplasia;
3) Chronic lung disease, including lung disease during the 1 year after the initial discharge from the hospital;
4) Reactive airways disease;
5) Abnormal neurological development, including neurologic development 1 year after the initial discharge from the hospital;
6) Seizures;
and 7) Clinically significant bleeding, including interventricular and intracranial hemorrhage.

This list also collects the majority of the potential toxicities of I-NO identified in my initial review, with the exception of increased methemoglobin and NO_2 concentrations. As such, what follows is the integrated safety summary for the four trials submitted as part of the NDA. This summary will be broken into the following headings:

1. Acute Pulmonary Injury
2. Chronic Pulmonary Injury
3. Acute Neurological Injury
4. Chronic Neurological Injury
5. Laboratory Adverse Events
6. Miscellaneous Other Adverse Events

The data from each of the four trials submitted to the NDA that is relevant to the given adverse event will be reviewed in turn. The reader is referred to my original review of the NINOS, INOSG and INO-01/ -02 trials, dated 11.19.97, for a discussion of the individual adverse events reported in those trials, arranged by body system. The reader is also referred to my review of CINRGI, in this packet, for a discussion of the individual adverse events reported in the CINRGI trial, arranged by body system.

6.2 Acute pulmonary injury

There are at least two potential mechanisms for pulmonary toxicity of I-NO. First, I-NO can impair surfactant production in rats exposed to 100 ppm I-NO for 24 hours. An effect of I-NO to inhibit surfactant has been suggested in human neonates. A decrease in surfactant could potentially make the lungs of the infant less compliant, increasing the risk of pneumothoraces and airleak syndrome.

Second, during co-administration of O_2 and I-NO, these two gases may potentiate each other's toxic effects. Exposure to high concentrations of O_2, as in the present trials, can lead to increased superoxide formation in the lung, which in turn stimulates the formation of peroxy-nitrite from I-NO. These highly-reactive intermediate species can interact with pulmonary proteins, including catalase and surfactant, to induce lung injury.

The reader is referred to section 8.2.7.2 from my original consult for additional information on this class of adverse events.

CINRGI

At the time of discharge, the incidence of lung injury was assessed by the investigators as summarized below. A numerical trend towards less use of O_2 and pulmonary medications was noted. In data not shown, the incidence of reported adverse events such as bronchitis and stridor were rare, and no striking imbalance between the treatment groups was seen.

6.2 Acute pulmonary injury (cont)

Table 6.2.1 Pulmonary disease at discharge in CINRGI[a].

	Placebo	I-NO	p Value
Chronic lung disease	11/82 (13.4%)	3/92 (3.3%)	0.023
Discharged on home O_2	7/81 (8.6%)	3/90 (3.3%)	0.195
Discharged on pulmonary medications	6/81 (7.4%)	4/90 (4.4%)	0.520
Discharged on O_2 or pulmonary medications	10/89 (11.2%)	6/97 (6.2%)	0.296

a. Data from CINRGI study report, table 28. Data shown only for those infants with available data.

NINOS

Pulmonary injury could manifest in any of the following adverse events captured at the time of discharge in the NINOS trial. Note the higher incidence of airleak during the administration of I-NO.

Table 6.2.2 (from 6.0.1.13.1.2) Pulmonary disease during the NINOS trial[a].

Characteristic	Placebo Group (n=121)	I-NO Group (n=114)	p Value[a]
Air leak syndrome during study gas administration[b]	7/121 (6%)	12/110 (11%)	0.23
Airleak syndrome during and after study gas[d]	19/121 (16%)	21/110 (19%)	–
Pulmonary hemorrhage	4/110 (4%)	2/107 (2%)	0.43
Chronic lung disease[c]	15/121 (12%)	16/114 (14%)	0.85
Discharge home on O_2	15/100 (15%)	14/98 (14%)	0.89

a. Unless otherwise noted, the data shown is for adverse events which occurred after randomization. P-value calculated using chi-square test.

b. Incidence of pulmonary leak occurring up to 24 hours after discontinuation of study gas. Does not include 21 subjects (12 control, 9 I-NO) who developed air leak >24 hours after discontinuation of study gas.

c. Chronic lung disease (CLD) defined as O_2 >21% required at 28 days of age with abnormal chest x-ray.

d. Incidence of pulmonary leak occurring up to time of discharge, starting after initiation of study gas.

INO-01/-02

The table below summarizes the results of the specified safety parameters relating to pulmonary disease at the end of hospitalization ascertained by the investigators using pre-specified definitions. There were no significant differences between control and I-NO groups for any of the endpoints.

Table 6.2.3 (from 6.0.3.13.1.1) Pulmonary disease from INO-01/-02.[a]

Changes in safety endpoints	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Incidence of air leak syndrome[f]	13/41 (32%)	9/41 (22%)	11/33 (33%)	10/36 (28%)	30/110 (27%)
Incidence of BPD[g]	5/40 (13%)	7/39 (18%)	3/32 (9%)	3/34 (9%)	13/105 (12%)
Subjects requiring O_2 at 28 days	6/41 (15%)	9/41 (22%)	3/33 (9%)	6/36 17%)	18/110 (16%)
Subjects with reactive airways disease at 28 days	1/40 (3%)	3/38 (8%)	1/30 (3%)	1/34 (3%)	5/102 (5%)

f. Air leak syndrome includes the occurrence of any one of the following: interstitial emphysema; pneumomediastinum; pneumopericardium; and pneumothorax. Subjects with more than one event were counted only once in the total.

g. Bronchopulmonary dysplasia defined as: use of supplemental O_2 at 28 days of life in the presence of an abnormal CXR, or the use of bronchodilators suggesting severe reactive airway disease.

There was, however, a greater number of infants with pneumothoraces identified as adverse events by the investigators in the I-NO groups. Pneumothoraces as adverse events were more reported more often in all of the dose-groups of I-NO.

Table 6.2.3a (from 8.2.7.2.4) Incidence of subjects with pneumothoraces identified by investigators as adverse events in INO-01/-02 and /-03 trials.

Adverse Event	Control n=41	I-NO 5 ppm n= 45	I-NO 20 ppm n=44	I-NO 80 ppm n=39	I-NO Combined n=128
Pneumothorax	1 (2%)	4 (9%)	3 (7%)	3 (8%)	10 (8%)

6.2 Acute pulmonary injury (cont)

Pneumothorax and pneumopericardium were also identified as Serious Adverse Events for several of the infants in the I-NO group, and in no control subject in the INO-01/ -02 trail. Once again, these were more common in both the 20 and 80 ppm groups.

Table 6.2.3b (from 8.2.7.2.8) Serious adverse events within the Air Leak Syndrome in INO-01/ -02.

Study Group	Subject #	Adverse Event	Withdrawn from Study?	Outcome
Control	None			
I-NO 5 ppm	01-11012	Pneumothorax	No	Died
I-NO 20 ppm	01-03025	Pneumothorax	No	Sequelae
	01-11005	Pneumothorax	No	Died
		Pneumopericardium		
I-NO 80 ppm	01-06006	Tension pneumothorax	No	Died
	01-11011	Bilateral pneumothoraces	No	Died
		Pneumopericardium	No	Died

INOSG

Finally, the smaller INOSG trial collected minimal information about the incidence of acute lung injury.

Table 6.2.4 (from 6.0.2.13.1.1) Results: incidence of specific adverse events in INOSG trial.

Changes in Safety Endpoints	Control (n=28)	I-NO Therapy (n=30)
Bronchopulmonary dysplasia	N/A	N/A
Days of supplemental O_2	40±95	12±7
Corrected days of supplemental O_2[d]	19±21	11±6
% of subjects on O_2 at day 28	4/21 (19%)	1/27 (4%)
Air leak	N/A	N/A

c. N/A Not available as part of the NDA package and/or not collected.

d. One subject was reported to require O_2 for 445 in the control group. Data for other infants were missing. This row calculates the need for O_2 from available data, not including the outlier subject.

In conclusion, the data are variable with regard to the possible acute association of I-NO with pulmonary toxicity. First, the NINOS trial reported an increase in the incidence of pneumothoraces during study gas administration. In addition, more pneumothoraces, and more Serious Adverse Events related to them, were reported as adverse events in the INO-01/ -02 trial. These pieces might suggest an effect of I-NO to decrease compliance of the lung, perhaps accounting for the increase in pneumothoraces (among other mechanisms).

Data against a significant association with acute lung injury comes from the use of O_2 and pulmonary medications at the time of discharge in CINRGI and INO-01/ -02, which did not differ markedly between the treatment groups. Further, the incidence of 'chronic lung disease', as assessed by the investigators in CINRGI and INO-01/ -02 at the time of hospital discharge, was not different in the treatment groups.

In conclusion, while there are some provocative data suggesting a potential injury in small numbers of patients exposed to I-NO, the data insufficient to either demonstrate or exclude an acute lung injury associated with I-NO. Additional data are required.

6.3 Chronic Pulmonary Injury

Chronic lung injury was collected in various ways in three trials as well, with follow-up that ranges between 6 months (CINRGI) and 18-24 months (NINOS). In this discussion, and in the discussion of chronic neurologic injury, it is essential to remember that the degree of follow-up for each of the trials was <90% for almost all endpoint, and <50% for some endpoints of interest. This will be apparent by comparing the numbers in the denominators of the results with the number of patients enrolled in each of the trials. This obviously introduces potential biases (both positive and negative) in the interpretation of the results.

CINRGI

At both 6 and 12 months there was a numerical trend for fewer patients to be on O_2 and pulmonary medications. CINRGI enrolled 89 placebo patients and 97 I-NO patients.

Table 6.3.1 Six-month follow-up data from the CINRGI trial[a].

Pulmonary Adverse Events	Placebo	I-NO
On O_2	2/48 (4.2%)	0/42 (0%)
On pulmonary medications	7/48 (14.6%)	3/42 (7.1%)

a. Data from CINRGI study report, table 38.

Fewer patients have 12-month follow-up data available.

Table 6.3.2 Twelve-month follow-up data from the CINRGI trial[a].

Pulmonary Adverse Events	Placebo	I-NO
On O_2	0/36 (0%)	0/32 (0%)
On pulmonary medications	6/36 (16.7%)	4/32 (12.5%)

a. Data from CINRGI study report, table 39.

NINOS

NINOS collected data on the use of pulmonary medications, O_2, and other breathing devices. Both treatment groups appear fairly comparable. NINOS enrolled 121 placebo patients and 114 I-NO patients.

Table 6.3.3 Medication used by the subjects with long-term F/U in the NINOS trial[a].

	Control N=88	I-NO N=85
Taking medications regularly	23 (26.1%)	20 (23.5%)
Diuretics	0 (0%)	1 (1.2%)
Bronchodilators	7 (8.0%)	9 (10.6%)

a. Data from NDA vol. 11.1, Table 37.

Table 6.3.4 Other medical therapies used by the subjects with long-term F/U in the NINOS trial[a].

	Control N=88	I-NO N=85
Currently using apnea monitor	0 (0%)	1 (1.2%)
Previously used apnea monitor[b]	8 (9.2%)	9 (10.6%)
Currently using O_2	0 (0%)	1 (1.2%)
Previously used O_2[b]	14 (16.1%)	13 (15.3%)
Currently on ventilator/CPAP	0 (0%)	1 (1.2%)

a. Data from NDA vol. 11.1, Table 38, 39.
b. Refers to those patients who used a given therapy after discharge but before the long-term F/U visit.

INO-01/ -02

In the INO-01/ -02 trial, infants in the I-NO group used respiratory medications at a higher rate than did the control group at the time of follow-up. INO-01/ -02 enrolled 41 placebo patients and 104 I-NO patients.

Table 6.3.5 Post-discharge medications at the one-year follow-up visit in the INO-01/ -02 trial[a].

	Placebo N=36	I-NO 5 ppm N=36	I-NO 20 ppm N=29	I-NO 80 ppm N=31	Combined I-NO N=96
Taking medications	5 (13.9%)	12 (33.3%)	11 (37.9%)	9 (29.0%)	32 (33.3%)
Respiratory medications	2 (5.5%)	6 (16.7%)	6 (20.7%)	2 (6.5%)	14 (14.6%)
Diuretics	0 (0%)	0 (0%)	2 (6.9%)	0 (0%)	2 (2.1%)

a. Data from NDA vol. 9.3, Tables 9.