6.0.1.13.2c Subject deaths (cont)

The individual patient deaths are listed in the table below. No death narratives for the individuals are available.

Table 6.0.1.13.2c.2 (from table 8.1.1.2) Deaths in the NINOS study[a,b].

Trial	Subject	Received ECMO?	Time of Death (days)	Description[b,c]
Control group				
	3-A05	Yes	12	Multi-organ failure, withdrawal of support
	3-A08	No	2	Severe hypoxia
	7-01	Yes	6	Severe intracranial hemorrhage
	10-A05	No	2	Refractory pulmonary hypertension
	12-A13	Yes	9	Severe intracranial hemorrhage, withdrawal of support
	14-A02	Yes	20	Suspected sepsis/infection
	15-A08	Yes	12	Alveolar-capillary dysplasia
	51-A11	Yes	16	Alveolar-capillary dysplasia
	52-A02	No	2	Suspected sepsis/infection
	52-A09	No	3	Left ventricular failure
	52-A14	No	3	RDS
	54-A03	No	1	Suspected sepsis/infection
	54-A14	No	5	Severe pulmonary hypertension
	55-A05	Yes	14	Polycystic kidneys
	56-A08	Yes	1	Proven sepsis/infection
	57-A01	No	2	Pulmonary hypoplasia
	58-A03	No	1	Proven sepsis/infection
	59-A03	No	5	Suspected sepsis/infection
	59-A08	Yes	3	Severe intracranial hemorrhage
	60-A02	No	43	Broncho-pulmonary dysplasia
I-NO group				
	3-A04	No	20	Respiratory failure
	5-A07	Yes	16	Severe CNS ischemia
	5-A14	No	4	Suspected sepsis/infection
	5-A20	Yes	1	RDS
	5-A25	Yes	60	'Thrombi', Withdrawal of support
	12-A11	Yes	15	Alveolar-capillary dysplasia
	15-A09	Yes	5	Proven sepsis/infection
	51-A08	Yes	18	Pulmonary lymphangiectasia
	52-A04	No	2	Meconium aspiration
	51-A06	Yes	5	
	55-A09	No	10	Withdrawal of support
	55-A21	Yes	136	Proven sepsis/infection
	56-A14	No	1	Suspected sepsis/infection
	57-A02	No	1	Proven sepsis/infection
	58-A01	No	1	Surgical death
	59-A02	No	8	RDS

a. Any death prior to 120 days is included in the NINOS data.
b. Study subjects are identified by center # and patient # (e.g., 05-A04).
c. Cause of death from electronic datasets of summary clinical data.

6.0.1.13.3 Long-term safety results of the NINOS trial

Data on the neurodevelopmental outcomes of the survivors is to be collected at 18 to 24 months corrected age. No interim results are available.

6.0.1.14 NINOS Efficacy Summary

Trial Design

This was a multi-center, multi-national, double-blind, placebo-controlled trial to evaluate the efficacy of I-NO in the treatment of term and near-term infants with hypoxic respiratory failure.

Subjects with hypoxic respiratory failure (see inclusion and exclusion criteria) were randomized to receive either O_2 (no flow of I-NO) or I-NO, 20 ppm for up to 336 hours (14 days). A total of 121 control and 114 I-NO subjects were enrolled.

Subjects who responded fully to treatment gas (either control or I-NO) were continued on the 'low-flow' study gas. For the subjects who received control gas, 17/117 (14.5%) had a full response. In the 20 ppm I-NO group, 57/113 (50.4%) had a full response (p value vs. control <0.001).

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, and their response measured after another 30 minutes. For the subjects who received control gas, none had a full response (0%) to high-flow control gas (O_2). In the 80 ppm I-NO group, 1/17 (6%) had a full response (no statistical comparison possible).

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 3 times. If no positive response was observed after 3 repeat trials (a total of 4 trials), the subject was labeled a non-responder. Despite this detailed repeat trial protocol, only 3 subjects in the control group (3%) and 2 in the I-NO group (2%) underwent re-initiation of study gas.

Primary and Secondary Endpoints

Primary endpoint

The incidence of death before discharge or 120 days (whichever comes first), and/or the initiation of ECMO between placebo- and I-NO-treated subjects.

The primary endpoint included one part looking at an unquestioned clinical benefit (reduction in mortality) and a component with a less-clear clinical benefit (initiation of ECMO). The results (see below) were completely driven by the reduction in the percentage of infants who received ECMO.

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.
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.

The secondary endpoints can be broken into three groups: 1) measures of acute effects of I-NO on oxygenation; 2) measures of clinical outcomes measured at time of discharge; and 3) long-term neurodevelopmental outcomes.

Number of subjects/ randomization

A total of 250 subjects were planned for enrollment. A total of 235 enrolled: 121 subjects in the control group and 114 in the I-NO group. While the trial was multi-center, three centers accounted for 37% of the enrolled infants (Wayne State University, Stanford University/Packard Children's Hospital, and Baylor Hospital/Texas Children's Hospital).

Two teams were used to accomplish the blinding in the trial. The first team consisted of the patient caregivers, who were blinded to the treatment gas being administered. The second team consisted of a least one unblinded investigator, who was responsible for all activities that revealed the treatment gas. These activities included maintenance of the bedside stock of treatment gas, daily calibration of the gas blender, and recording the methemoglobin, I-NO, and NO_2 levels.

6.0.1.14 NINOS Efficacy Summary (cont)

Inclusion/ Exclusion Criteria

NINOS has several important differences from the INOSG and INO-01/ -02 trials with regard to the subjects included in the trial.

First, subjects did not have to have echocardiographic proof of pulmonary hypertension. Indeed, 19% of control and 26% of I-NO infants did not have the clinical diagnosis of PPHN (see table 6.0.1.12.1.3). Additionally, 37% of the control subjects and 41% of the I-NO subjects had left-to-right shunting of blood across the patent foramen ovale.

Second, infants who had previously received surfactant and/or high-frequency ventilation were not excluded from the trial (see table 6.0.1.12.1.6). Over 70% of the infants in both groups had received surfactant, and over 30% had received high-frequency ventilation.

Congenital diaphragmatic hernia (CDH) was not an exclusion criteria in the NINOS trial, although those subjects were not included in the subjects for the primary analysis. This contrasts with the INOSG trial and INO-01/ -02 trial, where CDH was an exclusion criteria. No data on the effects of I-NO in the CDH population were submitted with this NDA.

The impact of these differences in the NINOS trial was that even critically ill neonates, who were already receiving maximal standard therapy, were eligible for enrollment. In distinction, the INO-01/ -02 trial excluded infants who had recently received surfactant or high-frequency ventilation, and required that the infant have PPHN prior to enrollment. For this reason, they had to be stable enough that the investigator was not forced to start these interventions while the enrollment process went on (including the determination of PPHN by ECHO). Additionally, the investigators in the INO-01/ -02 trial may have selected only 'less-ill' infants for consideration for that trial, preferring to start other, established, therapies for the critically ill infants. These differences are reflected in the significantly higher OI in the NINOS trial, relative to the INO-01/ -02 trial (averaging 22-25 in the INO-01/ -02 trial versus 42-44 in the NINOS trial (see tables 6.0.3.12.1.3 and 6.0.1.12.1.4).

Dosage/ Administration

Of the infants enrolled in the trial, 117 infants received control gas and 113 received I-NO, 20 ppm. In the I-NO group, 57/113 of the infants responded initially to 20 ppm I-NO, while 55/113 infants did not have a full response to I-NO 20 ppm, and so were administered I-NO 80 ppm.

Individuals in both groups received treatment gas promptly after randomization, save for one individual in the placebo group who started treatment gas 43 hours after randomization: 26.3 minutes was the mean time to start of treatment gas in the control group and 29.3 minutes in the I-NO group. Over 50% of the infants in both groups started treatment gas in <15 minutes.

Five individuals did not receive study gas after enrollment in the trial. Another seven individuals received I-NO after being randomized to receive control gas. These individuals are listed in section 6.0.1.12.3a above, along with an analysis of the results according the actual gas received.

Finally, isolated individuals received non-standard amounts of I-NO. The table below lists the subjects in the NINOS trial by the concentration of I-NO actually received.

Table 6.0.1.14.1 (from table 8.0.3.1) Enumeration of subjects from NINOS according to study gas received[b].

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
NINOS[a]	110	1	1	50	1	55	2	120

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. Does not include the 4 control and 1 I-NO infants who were randomized but did not receive study gas (see section 6.0.1.12.3a).

Duration/ Adjustment of Therapy

In the NINOS trial, the median duration of exposure to control gas was 21 hours, compared with 71 hours for the I-NO group. This reflects the higher fraction of control infants who were discontinued from study gas after failing to increase their PaO_2.

6.0.1.14 NINOS Efficacy Summary (cont)

Statistical Considerations

There are several statistical issues which need to be addressed with regards to the NINOS trial. The first is the pivotal efficacy analysis, comparing the incidence of death and/or initiation of ECMO in the control and I-NO groups. Based on the sponsor's primary analysis, shown in Table 6.0.1.12.2d.2, there was a highly significant advantage for the group who received I-NO, such that the trial was stopped early and a clinical alert issued. This analysis, which was intent-to-treat, included several subjects who were randomized but never received study gas, as well as subjects who were randomized to control, but received I-NO. This latter group, listed in section 6.0.1.12.3a above, included a large number of subjects who later went on to die and/or receive ECMO. When an analysis is performed according to the gas actually received, the p value for the difference between the two groups is significantly diminished.

Second, the use of an arbitrary p value <0.05 as the threshold for significance needs to be re-thought. The trial had three interim analyses. Under such circumstances, the p value for significance at the end of the trial must be adjusted downwards to a p value of 0.044.

Third, there was evidence of a center effect, as detected by variability in the rate of the primary endpoint among the centers. Correction of this variability can be performed by analyzing the data using the Cochran-Mantel-Haenszel test, rather that the unadjusted chi-square analysis.

Finally, one can analyze the data for the primary endpoint from the NINOS trial using the Cochran-Mantel-Haenszel test, separating the subjects according to the study gas they actually received, and excluding the subjects who did not receive any study gas. The results of such an analysis are presented in the section below for the primary endpoint and for the incidence of ECMO.

Patient Demographics & Baseline Characteristics

The baseline data for the NINOS trial are summarized in tables 6.0.1.12.1.1 to 6.0.1.12.1.6. Overall, the two groups were well-balanced with regards to their demographics and baseline characteristics.

Disposition of Subjects

A larger percentage of the subjects screened for the NINOS trial were enrolled when compared with the INO-01/ -02 trial. From a personal conversation with the principle investigator of the NINOS trail, Dr. Ehrenkranz, a large fraction (>50%) of subjects who were evaluated were ultimately randomized. This contrasts with the INO-01/ -02 study, where only 12% of the screened infants were randomized.

Table 6.0.1.12.2c shows the treatments received in addition to study gas after randomization. The two groups were well-matched with regard to the other therapies they received in addition to study gas, including HFOV/HFJV, surfactant and alkalinization.

Protocol Violations & Deviations

The protocol violations and deviations are listed in Table 6.0.1.12.2b.1 above. The two most significant violations were the infants who were randomized but did not receive study gas, and the infants who received I-NO after being randomized to control gas. These infants are discussed above in section 6.0.1.12.3a. The fact that all of the infants who received the wrong study gas were randomized to receive placebo, and instead received I-NO, raises the possibility that the treatment was unblinded somehow for these infants. There is no other evidence of unblinding for these subjects, who all received ECMO and/or died. The 8 subjects came from 7 different centers, all of whom administered I-NO and control gas to other infants without reported protocol violation.

Concomitant Therapies used after Trial Initiation

As summarized in table 6.0.1.12.2c.1, the two treatment groups were well-balanced with regard to the concomitant therapies received.

Analysis of Primary and Secondary Efficacy Outcomes (see Table 6.0.1.12.2d.2)

1. Incidence of death and/or initiation of ECMO

The table below summarizes the results of the NINOS trial from the primary and secondary endpoints, based on either the Intent-to-Treat study population or on population according to the actual gas received. In the latter population, those infants who were randomized but did not receive study gas are eliminated from analysis.

Table 6.0.1.14.2 Incidence of primary endpoint (death and/or ECMO) in NINOS trial.

Primary Endpoint	% of control subjects	% of I-NO subjects	p value
ITT population	77/121 (63.6%)	52/114 (45.6%)	0.006[a]
'Gas received' population[b]	71/112 (63%)	56/118 (47.4%)	0.015[a]
'Gas received' population[b]	71/112 (63%)	56/118 (47.4%)	0.022[c]

a. p value calculated using unadjusted chi-square

b. Subjects who did not receive any study gas were excluded from the analysis, while the remaining subjects were classified according to the actual gas received.

c. p value calculated using Cochran-Mantel-Haenszel adjusted chi-square test.

6.0.1.14 NINOS Efficacy Summary (cont)

Analysis of Primary and Secondary Efficacy Outcomes (see Table 6.0.1.12.2d.2) (cont)

2. Incidence of death.

No difference in the rate of death was detected between the two groups. This was true both for the ITT population as well as the population analyzed according to the gas actually received.

Table 6.0.1.14.3 Incidence of death in NINOS trial.

Death	% of control subjects	% of I-NO subjects	p value[a]
ITT population	20/121 (16.5%)	16/114 (14%)	0.596
'Gas received' population[b]	17/112 (15%)	17/119 (14.2%)	0.869

a. p value calculated using unadjusted chi-square

b. Subjects who did not receive any study gas were excluded from the analysis, while the remaining subjects were classified according to the actual gas received.

3. Initiation of ECMO

Significantly more subjects in the control group received ECMO using the ITT population. If the populations were corrected to reflect the actual gas received, however, the p value for the difference became less significant. Correcting for center effect, using the pre-specified Cochran-Mantel-Haenszel adjusted chi-square test, the reduction in ECMO is not significant. Given the three interim looks used in the trial, Dr. Nuri recommends using 0.044 as the cut-off for nominal significance.

Table 6.0.1.14.4 Incidence of ECMO in NINOS trial.

Initiation of ECMO	% of control subjects	% of I-NO subjects	p value
ITT population	66/121 (54.5)	44/114 (38.5%)	0.014[a]
'Gas received' population[b]	62/112 (55%)	48/118 (41%)	0.026[a]
'Gas received' population[b]	62/112 (55%)	48/118 (41%)	0.067[c]

a. p value calculated using unadjusted chi-square.

b. Subjects who did not receive any study gas were excluded from the analysis, while the remaining subjects were classified according to the actual gas received.

c. p value calculated using Cochran-Mantel-Haenszel adjusted chi-square test.

4. Meeting criteria for ECMO

Importantly, there was no significant difference in the number of subjects who met the criteria for ECMO between the two groups.

Table 6.0.1.14.5 Incidence of meeting criteria for ECMO in NINOS trial.

Met criteria for ECMO	% of control subjects	% of I-NO subjects	p value[a]
ITT population	83/121 (69%)	67/114 (59%)	0.12
'Gas received' population[b]	76/111 (68%)	72/119 (60.5%)	0.208

a. p value calculated using unadjusted chi-square

b. Subjects who did not receive any study gas were excluded from the analysis, while the remaining subjects were classified according to the actual gas received.

The reasons for subjects meeting the ECMO criteria but not receiving it are listed in table 6.0.1.12.2d.5. The most common reason was 'Improved', which occurred more frequently in the infants receiving I-NO. Unfortunately, no further details are available for the crucial time period between when an infant was evaluated and 'met' the criteria for ECMO, and when the decision was made either to go to ECMO or not. In discussions with Dr. Ehrenkranz, the NINOS principle investigator, there was no set time when the infants were evaluated for ECMO criteria. Thus, some infants were evaluated prior to initiating study gas, while other infants were evaluated after significant time on study gas had elapsed. The time the evaluation took place was also not recorded. The effect of this missing data is to make interpretation of this discrepancy between meeting criteria and receiving ECMO impossible to resolve.

5. Survivor endpoints.

There was no significant difference between the two groups in either the length of hospital stay or assisted ventilation (see Table 6.0.1.12.2d.2). The length of hospital stay was numerically longer in the I-NO group (36.4±45 in the I-NO group versus 29.5±23 in the control group).

6.0.1.14 NINOS Efficacy Summary (cont)

Analysis of Primary and Secondary Efficacy Outcomes (see Table 6.0.1.12.2d.2) (cont)

6. Acute changes in oxygenation parameters.

The next table summarizes the oxygenation endpoints specified in the NINOS trial. The results clearly show that I-NO has a significant effect to improve oxygenation acutely.

Table 6.0.1.14.6 Oxygenation endpoints from NINOS trial[a]

Acute Changes in Oxygenation	Control (n=121)	I-NO Therapy (n=114)	p value
Change in PaO_2 (mmHg)	9.7±51.7	58.2±85.2	<0.001
Change in OI	0.8±21.1	-14.1±21.0	<0.001
Change in A-a DO_2 (mmHg)	-6.7±57.5	-60.0±85.1	<0.001

a. Taken from the ITT population, measured as change from baseline to 30 minutes after start of study gas.

Not all subjects, however, responded to I-NO. A larger percentage of the control subjects who had an acute improvement ultimately went on to ECMO and/or death (53 vs 24 %), although the numbers of full responders in the control group were small. Subjects who had no acute response to study gas in both groups were at higher risk for the primary endpoint (death and/or ECMO).

Table 6.0.1.14.7 Subjects who met primary endpoint grouped by response to low -flow study gas[a].

Study	Control Group	I-NO 20 ppm
NINOS low-flow full-responders	9/17 (53%)	14/57 (25%)
NINOS low-flow partial or non-responders[b]	67/100 (67%)	36/55 (65%)

a. Data from NDA volume 2.14, page 029508. Data shown only for evaluable subjects.

b. NINOS defines a full response to study gas as an increase in PaO_2 >20 mmHg, and a partial response as an increase of 10-20 mmHg. This row includes those subjects who did not have an increase in PaO_2>20 mmHg (full response).

Subgroup & post-hoc analyses of the NINOS trial results

The sponsor performed several post-hoc analyses, looking at the effect of I-NO in several subgroups of the NINOS population. The endpoint used was the number of subjects who met the primary endpoint of the NINOS trial. These results are summarized in table 6.0.1.12.3b.1. The significant results of the analysis include:

1) the use of I-NO in subjects with idiopathic pulmonary hypertension and pulmonary hypertension associated with pneumonia/sepsis is 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 graph 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.

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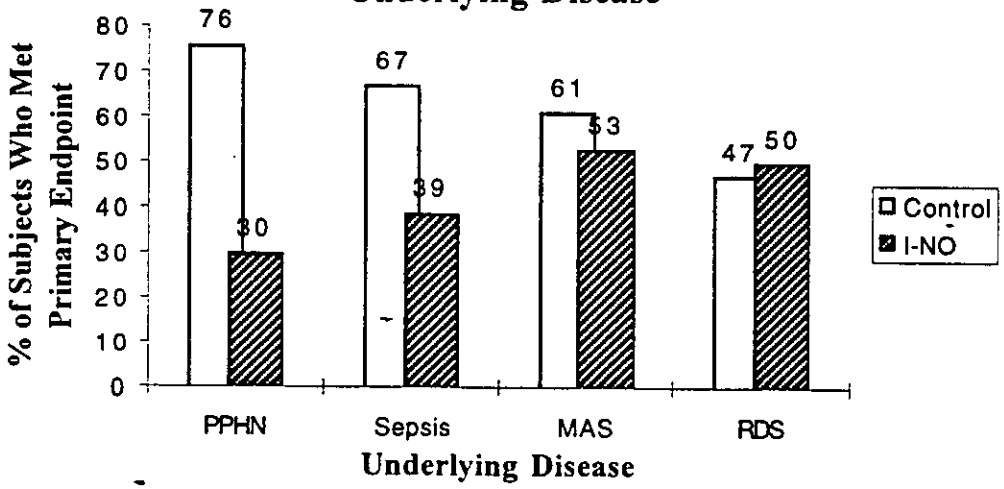
6.0.1.14 NINOS Efficacy Summary (cont)

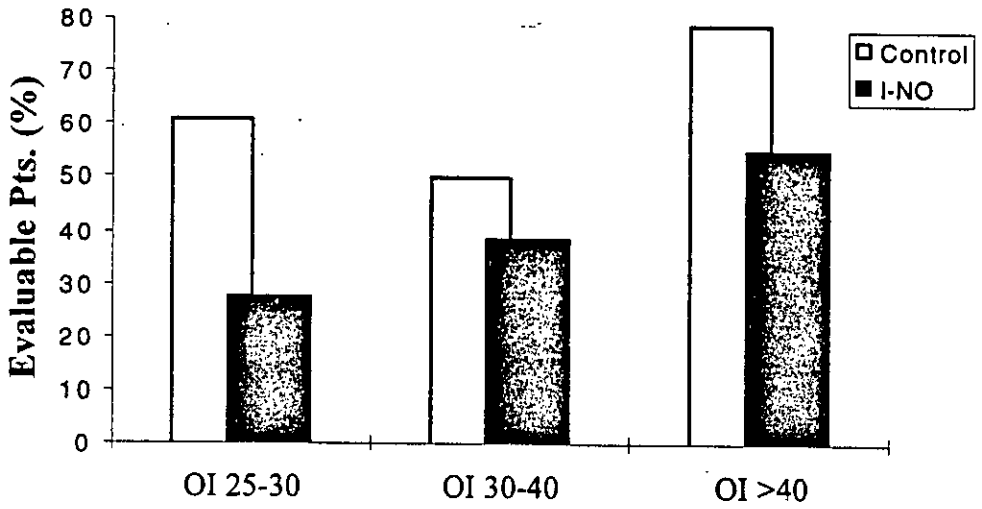
Analysis of Primary and Secondary Efficacy Outcomes (see Table 6.0.1.12.2d.2) (cont)

Figure 6.0.1.14.8 Primary Endpoint According to Underlying Disease



2) the response to I-NO may also depend on the severity of the overall clinical condition of the infant and/or duration of disease when I-NO was initiated. In the graph below, the rate at which the subjects met the primary endpoint is grouped according to the baseline OI of the subjects. Subjects with the lowest OI who received I-NO had the lowest rate of meeting the primary endpoint.

Figure 6.0.1.14.9 Primary Endpoint by Baseline OI

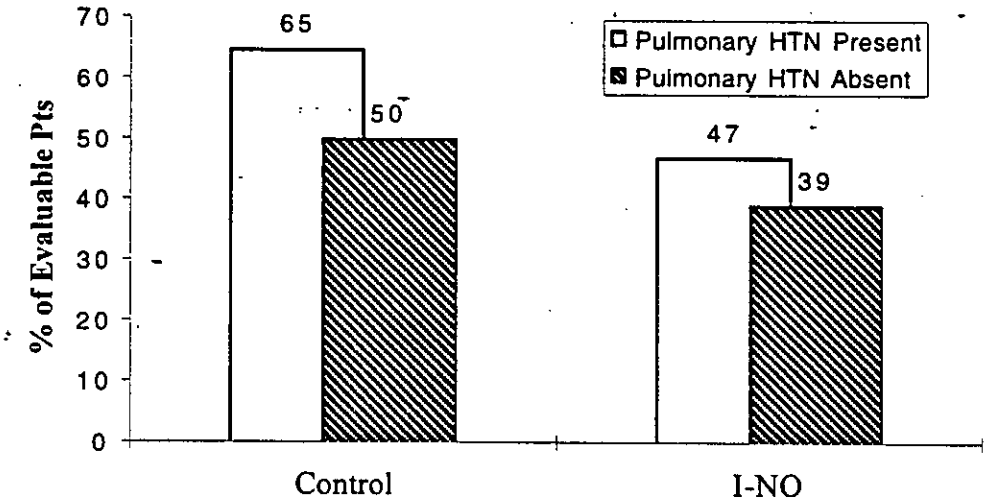


6.0.1.14 NINOS Efficacy Summary (cont)

Analysis of Primary and Secondary Efficacy Outcomes (see Table 6.0.1.12.2d.2) (cont)

3) in the second graph below, the subjects are grouped according to the presence or absence of echocardiographically demonstrated PPHN. There was a 27% reduction in the risk for meeting the primary endpoint when pulmonary HTN was present (65 to 47%), compared with 22% in the group without baseline evidence of pulmonary hypertension (50 to 39%).

Figure 6.0.1.14.10 Primary Endpoint by Baseline ECHO



4) the use of surfactant appears to have lowered the percentage of subjects who met the primary endpoint, independent of an effect of I-NO. In the presence of surfactant, 47/87 control subjects met the primary endpoint (54%) compared with 30/34 subjects who did not receive surfactant (88%).

5) No differences in the response rate to I-NO related to either sex or race were detected (see table 6.0.1.12.3b.1).

6.0.1.14 NINOS Efficacy Summary (cont)

Overall Efficacy Summary for the NINOS Trial

Several patient benefit parameters were prospectively defined and followed. There were no statistically significant differences between the two groups with regards to any of the following endpoints: incidence of death, meeting criteria for need for ECMO; length of hospital stay; duration of assisted ventilation; duration of continuous positive airway pressure (CPAP); duration of either low or high-dose O_2 therapy; the incidence of Air Leak Syndrome; the incidence of chronic lung disease; or the number of infants discharged home on O_2 therapy.

The NINOS trial met its primary endpoint with a p value of 0.022 according to the analysis favored by this reviewer, which corrected for the protocol violations and for the center effect. The group that received I-NO also had a lower incidence of initiation of ECMO, although this decrease was also not of overwhelming significance (p=0.026 using the unadjusted chi square test, p=0.067 using Cochran-Mantel-Haenszel adjusted chi-square test.). The use of I-NO in subjects with idiopathic pulmonary hypertension and pulmonary hypertension associated with pneumonia/sepsis is 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).

There was no significant difference between the control and I-NO groups in terms of meeting the pre-specified criteria for needing ECMO. Given the available information, the most likely reason infants who met the criteria for ECMO improved is an improvement in OI, secondary to the effect of I-NO on oxygenation. In fact, failure to improve from the average baseline OI, in either group, met criteria for transfer to ECMO (see section 6.0.1.9). The concern is that if the primary reason the subjects did not receive ECMO was that they 'improved' clinically, and 'improvement' was a reflection of the decreased OI caused by I-NO, then the reduced need for ECMO was nothing more than a reflection of improved oxygenation. It could be argued that by delaying the transfer of a given subject for ECMO, by improving oxygenation and reassuring the clinician ('pink' babies are better than 'blue' babies), I-NO allows other, effective therapies time to work. In this scenario, I-NO has no clinically beneficial effect, beyond reassurance of the clinician.

Another piece of evidence suggesting that the acute effect of I-NO on oxygenation was responsible for the decreased rate of use of ECMO is seen in table 6.0.1.12.3d, where the rate of ECMO and/or death is grouped according to the initial response to study gas. Infants in both control and I-NO groups who did not have an improvement in their oxygenation after 30 minutes ultimately went on to receive ECMO and/or die at almost exactly the same rate.

Finally, if there is another beneficial effect of I-NO beyond oxygenation, an improvement in other markers of clinical outcome (i.e., duration of hospitalization or ventilation) might be expected to be detected (none were).

1. I-NO acutely improves oxygenation in a substantial fraction of neonates exposed. In the NINOS study, 50% of I-NO subjects and 17% of control subjects had an increase of >20 mm Hg in their PaO_2 after 30 minutes.
2. I-NO use is associated with a significant decrease in the incidence of both death before 120 days and/or the initiation of ECMO. In the NINOS trial, there was a reduction in 29% in the rate of initiation of ECMO in the I-NO group for the intent to treat population, and 25% in the 'gas received' population.
3. I-NO appears to have a more substantial impact on subjects with idiopathic PPHN and pneumonia/sepsis with regards to the rates of death and/or ECMO.
4. I-NO appears to have a similar impact on subjects with and without echocardiographically-proven PPHN with regards to the rates of death and/or ECMO.
5. No effect of I-NO on several efficacy parameters was detected: incidence of death, meeting criteria for need for ECMO; length of hospital stay; duration of assisted ventilation; duration of continuous positive airway pressure (CPAP); or the duration of either low or high-dose O_2 therapy.

6.0.1.15 NINOS Safety Summary

Primary and Secondary Endpoints involving Safety

In addition to the incidence of death and ECMO, which were included in the efficacy summary above, several of the secondary endpoints measured safety endpoints, including:

1. Methemoglobin levels.
2. Inhaled NO_2 concentrations.
3. Incidence of chronic air leak (pneumothorax, pneumopericardium, pneumoperitoneum, pneumomediastinum, interstitial emphysema).
4. Incidence of bronchopulmonary dysplasia (defined as O_2 >21% required at 28 days of age with abnormal chest x-ray) or reactive airway disease (defined as requiring bronchodilators at discharge).
5. Incidence of intracranial abnormalities and risk factors for abnormal neurological sequelae (seizures, intraventricular hemorrhage and brain infarct).
6. Incidence of pulmonary or gastrointestinal hemorrhage.
7. The average length of hospitalization among surviving infants.
8. The number of days of assisted ventilation.
9. The proportion of infants transferred for potential ECMO.
10. Neurodevelopmental outcomes assessed at 18-24 months corrected age.

6.0.1.15 NINOS Safety Summary (cont)

Protocol Violations & Deviations (see Table 6.0.1.12.2b.1)

1. Wrong gas given

The most critical protocol violations involved the infants who were randomized to receive control gas, and instead received I-NO. These seven infants, along with the one infant who received both I-NO and control gas, were discussed in section 6.0.1.12.3a, including an analysis of the data corrected for the gas actually received by each of the infants.

2. Overdose

a. Two infants received >80 ppm I-NO.

Two subjects in the NINOS trial were given 100 ppm I-NO inadvertently. One infant developed an elevated NO_2 level, ultimately received ECMO and was discharged home requiring supplemental O_2.

1. Subject #54-A02: this 3.7 kg female received I-NO for PPHN with meconium aspiration syndrome, with a baseline OI of 23. After responding to 20 ppm I-NO, she was continued on study gas for 159 hours (day 5), at which time her dose was inadvertently increased to 100 ppm for approximately 36 minutes. While no coincident methemoglobin and NO_2 levels were obtained, her higher recorded levels were 2.2% methemoglobin on day 3 and 0.4 ppm NO_2 on day 1. She was weaned with difficulty, did not receive ECMO, and was discharged, with no chronic lung disease or excess bleeding or other major organ dysfunctions.

2. Subject #3-A02: this 2.9 kg female received I-NO for PPHN with pneumonia/sepsis, with a baseline OI of 56 and 26. She had a partial response to I-NO 20 ppm, and no response to I-NO 80 ppm. An improper flow-meter setting led to her exposure to I-NO 101 ppm for approximately 1 hour. Her methemoglobin level at that time was 6% and her NO_2 level was 5.1 ppm. Study gas was weaned down to 20 ppm and the NO_2 fell to 3.4 ppm. After 14 hours more, she received ECMO for persistently elevated A-aDO_2. She survived, but was discharged to home on O_2.

b. One infant received I-NO for more than 240 hours.

3. Subject #51-A12: this female received I-NO for PPHN with meconium aspiration. She had a partial response to I-NO 20 ppm. At 240 hours, the infant was on 20 ppm, and was ultimately weaned after a total of 253 hours and 25 minutes. Her maximum NO_2 and methemoglobin levels were 0.1 and 3.6 respectively. The subject received high-frequency ventilation while receiving study gas, and developed both pneumothorax and periventricular leukomalacia. Chronic lung disease was also diagnosed, after the infant required ventilation for 16 days.

3. Unblinded subjects

There was no significant difference in the reported rate of unblinding between the two groups.

Safety Outcomes

1. Methemoglobin levels.

There was a clear association between the dose of I-NO and the risk of elevated methemoglobin levels, as was seen in table 6.0.1.13.2b.1.

In the NINOS trial, the mean peak methemoglobin level was significantly higher in the I-NO group. During the first 12 hours of exposure to 80 ppm I-NO, the peak in the control group was 1.2±0.8, versus 2.0±1.5 in the I-NO group ($p<0.001$).

In the NINOS trial, methemoglobin was defined as >5%, and a total of 11 subjects (4 controls, 7 I-NO) had their study gas decreased because their methemoglobin levels exceeded this level. All of these subjects continued on study gas at lower flow rate. No subject was discontinued because of methemoglobin >10%. No short-term adverse outcomes were identified that can be linked to the elevated methemoglobin levels.

2. Inhaled NO_2 concentrations.

In the NINOS trial, the average peak NO_2 level was significantly higher in the I-NO group than in the control group (see table 6.0.1.13.2a.1).

There was a clear association between the dose of I-NO and the risk of elevated methemoglobin levels. The mean peak NO_2 level occurring in the first 12 hours was 0.0±0.3 in the control group and 0.8±1.2 in the I-NO group, excluding the infants who received the wrong study gas.

In the NINOS trial, one subject receiving I-NO 80 ppm had a NO_2 level >7.0 % during the trial. Subject #55-08, a Caucasian male, had a peak level of 9.1, and the subject underwent a successful wean of study gas, without withdrawal from the trial. He received ECMO and was discharged home with chronic lung disease.

6.0.1.15 NINOS Safety Summary (cont)

3. Incidence of chronic air leak syndrome.

Table 6.0.1.12.1.5 shows that a smaller % of infants in the control group had ALS prior to study gas administration than in the I-NO group (21 vs. 18%). However, more subjects developed ALS during study gas administration in the I-NO group (11% vs. 6% in the control group, see Table 6.0.1.13.1.2). A higher % of I-NO infants also developed ALS, looking just at those infants who developed ALS after staring I-NO (19% in the I-NO group vs. 16% in the control group).

4. Incidence of chronic lung disease (CLD) (see table 6.0.1.12.2d.2).

Both groups had similar rates of chronic lung disease at 28 days or time of discharge (12% in the control group, 14% in the I-NO group). There was also no significant difference in the percentage of subjects who were discharged home on supplemental O_2 (15% of controls, 14% of I-NO group).

5. Incidence of intracranial abnormalities and risk factors for abnormal neurological sequelae (seizures, intraventricular hemorrhage and brain infarct).

No differences in the rates of any intracranial abnormalities were noted in the safety database (see table 6.0.1.13.1.2). A higher % of subjects had seizures requiring therapy in the control group (17%) compared with the I-NO group (11%).

6. Incidence of pulmonary or gastrointestinal hemorrhage (see table 6.0.1.12.2d.2).

Pulmonary hemorrhage after initiation of study gas occurred in 4/110 control subjects (4%) and 2/107 (2%) of I-NO subjects. GI bleeding occurred in 1% of both groups.

7. The average length of hospitalization among surviving infants (see table 6.0.1.12.2d.2).

There was no significant difference in the duration of hospitalization between the two groups. The I-NO infants were hospitalized for a numerically longer period of time (36.4±45 in the I-NO group vs. 29.5±23 days in the control group).

8. The number of days of assisted ventilation (see table 6.0.1.12.2d.2).

There was no significant difference in the duration of mechanical ventilation between the two groups. The I-NO infants were ventilated for a numerically longer period of time (12.3±14 days in the I-NO group vs. 11.1±23 days in the control group).

9. The proportion of infants transferred for potential ECMO (see table 6.0.1.12.2d.2).

There was no significant difference in the percentage of subjects who met criteria for transfer to ECMO between the two groups. Fewer I-NO infants met the criteria for transfer to ECMO (59% in the I-NO group, versus 69% of the control infants).

10. Neurodevelopmental outcomes assessed at 18-24 months corrected age.

No data was submitted on this endpoint. Follow-up data is expected to be completed in the first part of 1998.

Overall safety summary for the NINOS trial

Some aspects of the safety database for the NINOS trial are to be discussed in section 8.1, and have not been included here.

1. Subjects receiving I-NO 20 ppm are at significant risk for elevated methemoglobin levels and NO2 levels. These levels, in the NINOS trial, were not high enough to cause the discontinuation of any subjects from I-NO.

2. There was an excess rate of Air Leak Syndrome during the administration of I-NO in the NINOS trial. There were no other signs of acute pulmonary toxicity, such as the development of chronic lung disease, including bronchospastic lung disease, and the need for supplemental O_2 at time of discharge. The issue of pulmonary toxicity of I-NO will be discussed in section 8.2.7.2 as well.

3. No excess GI, pulmonary or CNS bleeding was identified in the I-NO group. This is an especially important piece of data, as the NINOS trial was the only trial to prospectively identify bleeding as an adverse event of interest.

4. No effect of I-NO on neurologic adverse events was identified in the NINOS study. The long-term follow-up data is not yet available for the NINOS trial.

6.0.1.16 NINOS Reviewer's Conclusions

The NINOS trial demonstrated that I-NO administration is associated with a significant reduction in the incidence of death and/or the initiation of ECMO. This was the prospectively-defined, primary endpoint of the study. This combined endpoint was driven exclusively by the reduced incidence of initiation of ECMO in the I-NO group. This effect of I-NO may reflect the improvement in oxygenation caused by I-NO.

No other clinically beneficial effect of I-NO was detected.

With regards to safety, the trial was not designed to collect all adverse events, and some important safety information is not available to this reviewer. No effect of I-NO on overall mortality was detected. There is a possible association between I-NO administration and pulmonary toxicity. The administration of I-NO was also associated with higher peak NO_2 and methemoglobin levels. No other safety concerns were raised in the NINOS database.

6.0.2 INOSG Study

6.0.2.1. Title of Study: Inhaled Nitric Oxide and persistent pulmonary hypertension of the newborn (PPHN)

6.0.2.2. Site(s) of Investigation and Investigators

Table 6.0.2.2.1 INOSG study sites and investigators.

Investigator	*Site of Investigation*
Jesse Roberts, Jr. MD (Medical Monitor)	Massachusetts General Hospital
Warren Zapol, MD	Massachusetts General Hospital
Jeffrey Fineman, MD	Univ. of California a San Francisco
Frederick Morin, III, MD	SUNY Buffalo
Michael Zayek, MD	SUNY Buffalo
Phillip Shaul, MD	Univ. of Texas Southwestern Med. Center
Stephen Rimar, MD	Yale University
Ian Gross, MD	Yale University
Michael Schreiber, MD	University of Chicago
Richard Polin, MD	University of Chicago

6.0.2.3. Background

This study was performed under an individual IND held by Dr. Zapol. The original IND was submitted 10.4.91, for compassionate use of I-NO in a single infant. A separate protocol is also submitted for use of NO in adult respiratory distress syndrome (ARDS). Both protocols were reviewed and approved by R. E. Keenan, MD. The current protocol was submitted 8.13.93 as part of a meeting held between the FDA and Drs. Roberts and Zapol, in conjunction with the Zeneca company. No review of this protocol is recorded. This IND is no longer active, having been withdrawn by the FDA 5.21.97 for failure by Dr. Zapol to submit the required Annual Reports.

6.0.2.4. Study Design

This multi-center, double-blind, placebo-controlled trial was designed to..."assess the effects of 20 minutes of inhaled I-NO on oxygenation in neonates with severe PPHN." (INOSG Study Report, NDA submission volume 2.16, page 056308).

In a document identified as the INOSG protocol, the specific aims were stated differently: "This investigation will examine whether low levels of inhaled nitric oxide gas will reverse systemic hypoxemia and reduce morbidity in patients with persistent pulmonary hypertension of the newborn (PPHN) when compared with conventional medical and ventilator therapy. In addition, we will demonstrate that inhaled NO does not produce systemic hypotension nor significantly elevate methemoglobin levels (NDA submission volume 2.16, appendix 16.1.1, page 066808).

Subjects with hypoxemia and a diagnosis of PPHN (see inclusion and exclusion criteria) were randomized to one of two groups: 1) a control group who received N_2 (no flow of I-NO); and 2) a treatment group who received inhaled I-NO, 80 ppm. Markers of respiration (oxygenation index, PaO_2, $PaCO_2$, pH) were measured at baseline and again after 20 minutes. Because the introduction of treatment gas reduced the maximum deliverable amount of O_2 from 100% to 90%, two baseline readings were collected (Baseline one, FiO_2 =100%; Baseline two, FiO_2 =90%). If the second baseline PaO_2 has decreased >15% or >25 mmHg, the subject was returned to FiO_2 =100% and was considered a treatment failure (see below).

After 20 minutes of treatment gas inhalation, a determination whether there has been 'failure of treatment' is made by the clinician, based on the following criteria.

Treatment Success

1) In the INOSG study report submitted as part of the NDA, the subject was considered to have responded to the study gas (a 'success') if:

1. the oxygenation index (OI) was <40;
2. the PaO_2 was >55 mmHg;

and 3. the mean systolic pressure was >40 mmHg (NDA volume 2.16, page 057708).

2) In the original INOSG protocol, the subject was considered to have responded to the study gas (a 'success') if they did not meet the criteria for Treatment Failure (NDA volume 2.16, page 067008- 067108, originally submitted 8.13.93).

6.0.2.4. Study Design (cont)

Treatment Failure

The subjects is considered to be a treatment failure if:

1. if the second baseline PaO_2 has decreased >15% or >25 mmHg (see above);
2. if the postductal PaO_2 has decreased by 15% from $baseline_2$ value, or the OI >40;
3. if, at any time, the mean systolic blood pressure <40 mmHg;
4. if the OI >40 at any time after the first 20 minutes; or
5. if the patient dies.

If the subject was considered not to be a treatment failure, an attempt to decrease the amount of treatment gas administered was made twice per day, provided the PaO_2 did not drop >15% or fall to <55 mmHg. Both groups could receive all other conventional therapies for hypoxic respiratory failure, including surfactant and HFJV/HFOV. Infants were followed to death or discharge to home. Each center's guidelines determined the use of surfactant, the mode of ventilation, the use of tolazoline and therapies to maintain arterial pressure, induce alkalosis, or provide sedation and analgesia.

6.0.2.5 Primary and Secondary Endpoints

Primary Endpoints

1. Number of acute oxygenation 'successes' (or the absence of treatment failure) following 20 minutes of treatment gas.

2. Percentage of subjects receiving ECMO therapy.

The second 'primary endpoint' was not part of the original protocol, and no mention of an ECMO endpoint exists in either the original IND protocol submitted by Dr. Zapol, dated 8.13.93, or in the original protocol submitted by Ohmeda (NDA, vol. 2.16, page 060308). Attempts to discuss this with the principle investigator were not successful.

Secondary and Post-Hoc Analyses

1. A comparison of the number of subject deaths within 120 days and/or receipt of ECMO in the two groups was added to the analysis following the results of the NINOS trial (NDA, vol. 2.16, page 060308).

The sponsor states that data on the receipt of ECMO, death and oxygen therapy at 28 days was collected on all subjects 'by review of the medical records prior to the unblinding of the trial.' (NDA, vol. 2.16, page 057808).

2. Percentage of subjects receiving oxygen therapy at 28 days.
3. Percentage of subjects surviving.

6.0.2.6 Number of Patients/ Randomization

A total of 60 subjects were planned for enrollment. A total of 58 enrolled: 28 subjects in the control group and 30 in the I-NO group (80 ppm). No information is available about the number or the clinical state of the subjects screened but not enrolled.

Table 6.0.2.6.1 Enrollment in INOSG by site.

Site	Control	I-NO	Total
SUNY, Buffalo	8 (14%[a])	10 (17%)	18 (31%)
Univ. of Chicago	1 (2%)	4 (7%)	5 (9%)
Children's Hospital of Philadelphia	3 (5%)	1 (2%)	4 (7%)
Massachusetts General Hospital	1 (2%)	0 (0%)	1 (2%)
Univ. of Texas at Dallas	4 (7%)	5 (9%)	9 (16%)
University of California at San Francisco	8 (14%)	7 (12%)	15 (26%)
Yale University	3 (5%)	3 (5%)	6 (10%)
Total	28 (48%)	30 (52%)	58 (100%)

a. patient number expressed as percent of total.

Randomization

Following entry into the study, subjects were randomized to receive either control gas (N_2) or I-NO 80 ppm in blinded fashion. Two teams were used to accomplish the blinding in the trial. The first team consisted of the patient caregivers, who were blinded to the treatment gas being administered. The second team consisted of a least one unblinded investigator, who was responsible for all activities that revealed the treatment gas. These activities included maintenance of the bedside stock of treatment gas, daily calibration of the gas blender, and recording the methemoglobin, I-NO, and NO_2 levels.

6.0.2.7 Inclusion/Exclusion Criteria

Inclusion Criteria for Primary Entry Into Trial

1. Hypoxemia defined as a postductal $PaO_2 < 55$ mmHg on two consecutive determinations at least 30 minutes apart while breathing at $FiO_2 = 100\%$.
2. A diagnosis of pulmonary hypertension, including evidence of right to left shunting by echocardiogram and/or upper vs. lower body oxygen tension/saturation difference.
3. The infant must have an indwelling post-ductal arterial line.
4. Term neonate (≥ 37 weeks and a birth weight of ≥ 2500 grams.

Exclusion Criteria for Primary Entry Into Trial

1. A gestational age less than 37 weeks.
2. Birth weight <2500 grams.
3. Uncorrected hypotension defined as a mean systemic blood pressure <40 mmHg measured through an intra-arterial catheter.
4. Uncorrected hyperviscosity defined as a hematocrit greater than or equal to 70% within 24 hours or birth.
3. Previous treatment with high frequency jet or oscillatory ventilation or ECMO.
4. Known structural congenital heart disease, other than patent ductus arteriosus or patent foramen ovale.
5. Congenital diaphragmatic hernia.
6. Phenotype consistent with a lethal chromosomal abnormality.

Criteria for use of ECMO

Initiation of ECMO occurred at the discretion of the individual investigator. No criteria were defined to guide transfer to ECMO and no information regarding the clinical state of the infant at the time of transfer was collected.

6.0.2.8 Dosage/Administration

Subjects were randomized to receive either I-NO 80 ppm or control gas based on the inclusion/exclusion listed above. If they were determined to be a 'success' the subject 'may continue exposure (to I-NO) for as long as there is a direct benefit' (NDA vol. 2.16, page 067008). An attempt to decrease the amount of treatment gas administered was made twice per day, provided the PaO_2 did not drop >15% or fall <55 mmHg. The treatment gas was to be weaned in 10 ppm increments. No upper limit for the duration of I-NO exposure was specified.

The treatment gases were administered by mixing either NO or placebo (N_2) with carrier N_2 using a gas blender system. The study mixture was introduced into the inspiratory limb of the breathing circuit of a standard, continuous flow, pressure limited, time-cycled ventilator using a flow meter. To limit the formation of NO_2, the residence time of NO and O_2 together within the breathing circuit were minimized by keeping the flow rate of the gas >10 liters/minute. As a consequence of this high-flow of study gas, the maximum FiO_2 during study gas administration was 90%. The exhaled gases were collected and discharged into the hospital vacuum system.

6.0.2.9 Duration of Study

The treatment gas therapy was continued in those subjects for which the initial treatment was considered a 'success'. An attempt to decrease the amount of treatment gas administered was made twice per day, provided the PaO_2 did not drop >15% or fall to <55 mmHg. Subjects were monitored for up to 204 hours after initiation of treatment for the pulmonary efficacy parameters (e.g., PaO_2, pH, $PaCO_2$, OI), and were followed clinically until discharge from hospital or death. The stated median duration of therapy for the two groups are summarized in the table below.

Table 6.0.2.9.1 Median duration of exposure to treatment gas (control gas or I-NO) from the INOSG trial[a].

Study	Control Group	I-NO Group
INOSG	20 minutes	48 hours

a. From NDA volume 2.50, page 339210.

6.0.2.10 Safety and Efficacy Parameters Measured

Efficacy Parameters

1. Hemodynamic parameters--diastolic blood pressure, systolic blood pressure, heart rate.
2. Oxygenation parameters--pH, PaO_2, OI, $PaCO_2$, pre- and post-ductal O_2 sat/$TCPPO_2$.
3. Respiratory parameters--respiratory rate, peak inspiratory pressure, positive end expiratory pressure (PEEP), inspiratory to expiratory ratio (I:E), mean airway pressure, FiO_2 required, and $PaCO_2/FiO_2$ index.
4. '(V)asopressor requirements in the two patient groups.' (IND protocol submission, page 0011).

6.0.2.10 Safety and Efficacy Parameters Measured (cont)

Safety Parameters

"Standard adverse events were not collected." (NDA volume 2.16, page 060808).

The following safety parameters were collected: incidence of death; incidence of elevated methemoglobin levels; and incidence of systemic hypotension.

6.0.2.11 Statistical considerations

The Cochran-Mantel-Haenszel chi-square test was used to test for differences between the two groups after 20 minutes of exposure to study gas. The proportion of subjects who required ECMO rescue and the proportion of subjects who died were also compared using Cochran-Mantel-Haenszel. P-values ≤0.05 were considered statistically significant. No adjustment was made to the nominal significance level for testing over multiple endpoints.

A two-sided t-test was used to assess group comparability for mean age at start of treatment, gestational age, birth weight, and Apgar score.

A Cochran-Mantel-Haenszel, stratified for investigative sites, was used to compare the treatment groups for bias with regards to sex, race, meconium staining, blood cultures, priscoline infusion, pneumothorax evacuation, HCO_3 pretreatment, and surfactant pretreatment.

6.0.2.12 INOSG Efficacy Endpoint Outcomes

6.0.2.12.1 Subject demographics and baseline characteristics

Demographics

The table below shows the baseline demographics of the INOSG trial. The two groups were well-matched with regard to their demographics.

Table 6.0.2.12.1.1 Demographics of INOSG subjects[b].

Variable	Control n=28	I-NO n=30	p-value[a]
Sex			
Male	18 (64%)	16 (53%)	0.43
Female	10 (36%)	14 (47%)	
Race			
White	13 (46%)	11 (37%)	0.72
Black	4 (14%)	8 (27%)	
Hispanic	4 (14%)	7 (23%)	
Asian	2 (7%)	1 (3%)	
Other	2 (7%)	2 (7%)	
Missing	3 (11%)	1 (3%)	
Age at start of tx gas (hrs)	31.6±29.5 (2.7 to 147 hrs)	38.6±42.6 (0.6 to 189.4 hrs)	0.5
Birth weight (kg)	3.56±0.62 (2.5 to 4.68 kg)	3.44±0.58 (2.4 to 4.77 kg)	0.49
Gestational age (weeks)	40.1±1.2 (37 to 42)	39.8±1.5 (37 to 42)	0.39

a. p value calculated using chi-square test.
b. Data from NDA volume 2.14.

Underlying disease of subjects enrolled in INOSG

No data on the underlying disease of the subjects is available. A similar number of subjects in the two groups had meconium staining below the vocal cord, which is one of the criteria for meconium aspiration syndrome (see appendix one).

Baseline clinical characteristics of subjects enrolled in INOSG

The table below shows the baseline characteristics of the subjects in the trial. Note that the subjects in the I-NO group had a significantly higher diastolic blood pressure at baseline. The following data of interest are not available and/or were not collected: echocardiographic parameters; the age of the mother; the incidence of complications at birth; the rate of cesarean sections; and underlying cause of PPHN.

6.0.2.12.1 Subject demographics and baseline characteristics (cont)

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

Variable	Control	I-NO	p-value[b]
Diastolic BP (mmHg)	41±13 (range 3 to 60)	49±10 (range 32 to 68)	0.02
Systolic BP (mmHg)	66±15 (range 30 to 97)	75±15 (range 55 to 112)	0.07
Mean BP (mmHg)	54±10 (range 31 to 69)	60±12 (range 38 to 88)	0.08
Heart Rate (beats per minute)	163±22 (range 120 to 202)	160±18 (range 122 to 192)	0.38
Apgar at 1 minute	3.7±2.7 (range 0 to 9)	4.4±2.5 (range 1 to 9)	0.30
Apgar t 5 minutes	5.9±2.6 (range 1 to 9)	6.3±2.5 (range 1 to 9)	0.59
Apgar at 10 minutes	5.8±2.9 (range 1 to 9)	5.9±1.9 (range 2 to 8)	0.94
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
Peak Inspiratory Pressure (cm H_2O)	36±8	35±7	0.8
Inspiration:Expiration (I:E)	1.41±0.55	1.41±0.63	0.79
Bicarbonate Therapy	9/28 (32%)	10/30 (33%)	0.96
Surfactant	5/28 (18%)	4/30 (13%)	0.87
Meconium below vocal cords	17/28 (61%0	15/30 (50%)	0.50
Tolazoline infusion	2/28 (7%)	1/30 (3%)	0.55
Dopamine infusion, ≥5 µg/kg/min	20/28 (71%)	23/30 (70%)	0.64
Dobutamine infusion, ≥5 µg/kg/min	8/28 (29%)	9/30 (30%)	0.64

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.

6.0.2.12.2 Disposition of subjects

6.0.2.12.2a Subject selection

No information regarding the number of subjects screened versus the number enrolled into INOSG is available.

6.0.2.12.2b Protocol Violations and Deviations

No systematic information regarding protocol violations during the INOSG trial were available for review.

Two unidentified subjects treated unsuccessfully with control gas were then treated with open-label I-NO in violation of the study protocol. "Although these subjects responded successfully to inhaled nitric oxide, only the acute response results gathered during their initial treatment with the control gas are reported." (NDA volume 2.14, page 067508).

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6.0.2.12.2c Concomitant therapies used after randomization in the INOSG trial

The only available information regarding concomitant therapies used in this trial regards the effect of changes in administered FiO_2 which were required as part of the protocol.

Effect of lowered FIO_2

Because the introduction of study gas meant that the delivered O_2 decreased from 100% to 90%, a second baseline blood gas and set of hemodynamic parameters was obtained prior to the initiation of study gas. If the subject did not tolerate the decrease FIO_2 they were returned to 100% FIO_2, and considered a treatment failure. The results are shown in the table below. No information is available regarding any subjects who were withdrawn for lack of tolerance to 90% O_2.

Small (albeit statistically significant) changes occur in the blood pressure and PaO_2 between Baseline 1 and Baseline 2 values in the I-NO group. No effect on pH or pCO_2 was detected.

Table 6.0.2.12.2c.1 Effect of lowered FIO_2 on hemodynamic and pulmonary parameters in INOSG.

Parameter	Control Group			I-NO Group			
	Baseline 1	Baseline 2	Change	Baseline 1	Baseline 2	Change	p value[a]
PaO_2	38±9.1	39.5±9	1.5	41.3±9.4	39.8±9.8	-1.5	0.001
Mean BP[b]	54±11	55.6±10	2.0	60±12.2	56±10	-3.8	0.002
Diastolic BP	41±13	45.8±9	4.6	49.5±10	47±10	-2.4	0.004
Heart rate (BPM)	163±21	159±25	-4	158±18	160±21	2	0.054
pH	7.47±0.14	7.49±0.13	0.03	7.50±0.12	7.52±0.11	0.02	0.725
$PaCO_2$	33±11	28±32	-1.7	32.4±12	31.5±11	-0.93	0.71
Pre-Ductal O_2/ $TCPPO_2$ (%)	88.6±9.6	88.5±9.8	-0.08	85.9±11.6	86.2±10	0.3	0.67
Post-Ductal O_2/ $TCPPO_2$ (%)	84.4±10	85.1±10	1.0	82.4±16	83.1±14	0.76	0.825

a. Wilcoxon Rank Sum test used to compare median differences between control and I-NO groups.
b. the Baseline 1 value for the I-NO groups is higher than for the control group (p=0.061 using non-paired t-test).

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6.0.2.12.2d Primary Efficacy Analysis from the INOSG Trial Data

Primary Endpoints

The table below summarizes the results of the INOSG trial from the primary and secondary endpoints. There was a significant difference between the two groups with respect to the incidence of the primary endpoint specified in the original protocol. Two other endpoints, need for ECMO and need for ECMO and/or death, also differed in their incidence between the control and I-NO groups. There was no significant difference in the incidence of death or in the duration of hospitalization.

Table 6.0.2.12.2d.1 Results: primary and secondary endpoints from INOSG trial[b].

	Control (n=28)	I-NO Therapy (n=30)	p value[b]
Combined Primary Endpoint[a]: Oxygenation 'success'			
Success	2/28 (7%)	16/30 (53%)	0.0025
Failure	26/28 (93%)	14/30 (47%)	
Second Primary Endpoint[c]: ECMO therapy			
Received ECMO	20/28 (71%)	12/30 (40%)	0.016
Did not receive ECMO	8/28 (29%)	18/30 (60%)	
Primary Endpoint from NINOS trial[d]			
Met endpoint	21/28 (75%)	13/30 (43%)	0.014
Did not meet endpoint	7/28 (25%)	17/40 (57%)	
Secondary Endpoints			
Incidence of subject death	3/28 (11%)	2/30 (7%)	0.58
Incidence of subjects receiving external O_2 after 28 days with available data[g]	4/28 (14%)	1/30 (3%)	0.14
Incidence of subjects receiving external O_2 after 28 days[g]	11/28 (39%)	4/30 (13%)	0.024

a. Primary endpoint: incidence of 'success' in each group after 20 minutes (see section 6.0.2.4 above.

b. p value determined by using unadjusted chi-square test.

c. Second 'primary' endpoint: incidence of subjects receiving ECMO therapy.

d. NINOS endpoint analysis added post hoc, following results of the NINOS trial were published: incidence of death before discharge or 120 days and/or initiation of ECMO.

e. Secondary endpoints are all measured after 20 minutes of study gas, and compared with baseline I value, unless otherwise noted.

f. Events taken from surviving subjects only.

g. The number of subjects requiring external O_2 after 28 days is missing data from several subjects, making interpretation of the data problematic. Shown are the values for the total evaluable and the entire population (counting missing as requiring O_2).

Acute physiological changes after administration of study gas

The sponsor also measured the acute effects of I-NO on several physiological parameters. The first table shows the analyses submitted by the sponsor, comparing the first baseline (before the decrease in FiO_2) and the value at the end of 20 minutes. Several significant effects of I-NO were noted using this analysis.

The following tables show the data for the first and second baselines, and for the 20 minute value. The only significant difference between the second baseline and the 20 minute value was the increased PaO_2 seen in the I-NO group.

Table 6.0.2.12.2d.2 Physiological changes following 20 minutes of study gas in the INOSG trial.

Changes in clinical markers (from baseline to 20 minutes)	Control (n=28)	I-NO Therapy (n=30)	p value[b]
Change in PaO_2 (mmHg)	-1.9±9.6	47.4±68	0.001
Change in average OI levels	-2.0±15	-16±11.5	0.001
Change in A-aDO_2	N/A	N/A	
Change in pH	0.02±0.07	0.05±0.09	0.052
Change in pCO_2	-0.9±4.8	-4.1±7.6	0.022
Change in mean systemic blood pressure from first baseline to 20 minutes (mmHg)	4.1±9.0	-4.6±13.2	0.002
Change in heart rate (beats per minute)	2.0±15	1.0±10.2	0.827

b. p value calculated using Wilcoxon Rank Sum Test of median difference of changes between control and I-NO groups.

6.0.2.12.2d Primary Efficacy Analysis from the INOSG Trial Data (cont)

Acute physiological changes after administration of study gas (cont)

Table 6.0.2.12.2d.3 Acute effects of I-NO on blood pressure in the INOSG[a].

	Control	I-NO 80 ppm
Mean systemic arterial pressure (mmHg)		
Baseline one	53.6±10	59.8±12
Baseline two	55.6±10	56±10
20 minutes of study gas	57.2±12	55.0±10
Mean systolic pressure (mmHg)		
Baseline one	65.5±15	74.9±15
Baseline two	70.0±13	67.9±10
20 minutes of study gas	71.9±14	67.1±12
Mean diastolic pressure (mmHg)		
Baseline one	41.2±13	49.5±10
Baseline two	45.8±10	47.2±10
20 minutes of study gas	47.0±11	46.5±10

a. INOSG data from NDA, volume 2.16, Tables T-1 to T-3.

Table 6.0.2.12.2d.4 Acute effects of I-NO on heart rate in the INOSG[a].

Heart rate (BPM)	Control	I-NO 80 ppm
Baseline one	163±22	158±18
Baseline two	159±25	160±21
20 minutes of study gas	164±24	160±19

a. INOSG data from NDA, volume 2.16, Tables T-4.

Table 6.0.2.12.2d.4 Acute effects of I-NO on pH in the INOSG[a].

pH	Control	I-NO 80 ppm
Baseline one	7.47±0.14	7.50±0.53
Baseline two	7.49±0.13	7.52±0.11
20 minutes of study gas	7.49±0.13	7.55±0.10

a. INOSG data from NDA, volume 2.16, Tables T-5.

Table 6.0.2.12.2d.5 Acute effects of I-NO on PaO_2 in the INOSG[a].

PaO_2	Control	I-NO 80 ppm
Baseline one	38.1±9	41.3±9
Baseline two	39.5±9	39.8±10
20 minutes of study gas	36.1±10	88.7±70

a. INOSG data from NDA, volume 2.16, Tables T-6.

Table 6.0.2.12.2d.6 Acute effects of I-NO on $PaCO_2$ in the INOSG[a].

$PaCO_2$	Control	I-NO 80 ppm
Mean systemic arterial pressure (mmHg)		
Baseline one	33.5±11	32.4±12
Baseline two	31.8±11	31.5±11
20 minutes of study gas	32.6±12	28.3±9.7[b]

a. INOSG data from NDA, volume 2.16, Tables T-7.

b. Two-tailed p value equals 0.24 using Wilcoxon Rank Sum Test comparing the I-NO baseline two and 20 minute values.

Long-term physiological changes

Individual subject data was submitted only for the subjects in the I-NO group who responded to I-NO. In the absence of a comparator group, no interpretation of the long-term changes can be performed.

6.0.2.13 Safety comparisons

6.0.2.13.1 Comparison of defined safety parameters up to 28 days

The reported the incidence rates for selected adverse events in the INOSG trial are in the table below. Those serious clinical events which were collected for the NINOS and INO-01/ -02 trials which are not included in the INOSG trial are also noted.

Table 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)	p value[b]
Methemoglobinemia 0-2.5%	N/A[c]	11 (37%)	
Methemoglobinemia 2.5-5.0%	N/A	6 (20%)	
Methemoglobinemia ≥5%	N/A	3 (10%)	
Elevated NO_2 level			
Duration of ECMO	5.3±2.5	5.0±0.7	0.442
Incidence of adverse events among survivors:			
Bronchopulmonary dysplasia	N/A	N/A	
Duration of hospitalization	42±91	19.78±8.5	0.243
Number of days of assisted ventilation	27±84	7.9±4.4	0.56
Days of supplemental O_2	40±95	12±7	0.04
Corrected days of supplemental O_2[d]	19±21	11±6	0.066
% of subjects on O_2 at day 28	4/21 (19%)	1/27 (4%)	0.09
Air leak	N/A	N/A	
Interventricular hemorrhage and other CNS events	N/A	N/A	
GI bleeding	N/A	N/A	

a. Of the three safety parameters specified in the protocol (incidence of death, need for external O_2 after 28 days, and methemoglobinemia), two were included in the table above under primary endpoints. The remainder of the parameters were submitted by the sponsor as 'secondary analyses' or 'other acute physiologic changes.'

b. p value determined by using chi-square test or Student's t-test as appropriate.

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.

6.0.2.13.2. Comments on specific safety parameters

6.0.2.13.2a NO_2 levels

No information on NO_2 levels was submitted as part of the NDA.

6.0.2.13.2b Methemoglobin levels

Peak methemoglobin levels averaged 3.8±4.0 during the first twelve hours of therapy, and 5.3±5.8 at any time during the trial for the subjects in the I-NO group with available data.

Three of the infants had peak methemoglobin levels >5% during the trial.

1. One infant (UT@D-1) was a female infant who responded to I-NO, 80 ppm, with a decrease in OI. Her methemoglobin level was 13.2 after 12 hours, and 8.7 after 24 hours. That is the last recorded methemoglobin level, and the infant later died without receiving ECMO.

2. Another infant (UCSF-5), had a methemoglobin level of 18.2% at 24 hours of I-NO therapy. 'Because the patient's oxygenation had improved, inhaled NO was continued.' (NDA volume 2.16, page 060908). The I-NO was decreased from 80 to 10 gradually, and the methemoglobin level declined over time to 1.3% after 144 hours. The infant survived without ECMO or need for supplemental O_2.

3. The third infant (Buf-S1) had a methemoglobin level of 9.0 after 24 hours on I-NO 40-80 ppm. Reduction of the I-NO to 10 ppm reduced the level to 1.5 after 108 hours. The infant was weaned successfully, and survived without receiving ECMO or need for supplemental O_2.

6.0.2.13.2b Methemoglobin levels (cont)

Table 6.0.2.13.2b.1 Peak Methemoglobin levels from the INOSG trial.

Changes in safety endpoints	Control	Combined I-NO
Peak methemoglobin level during first 12 hours of study gas	N/A	3.8±4.0 (n= 11)
Peak methemoglobin level at any time	N/A	5.3±5.8 (n= 11)
Peak methemoglobin level at any time[a]		
0.0 - 1.0%	N/A	2/11 (18%)
1.1 - 2.0	N/A	2/11 (18%)
2.1- 4.0	N/A	2/11 (18%)
>4.0	N/A	5/11 (45%)

a. Eleven of 16 subjects who received I-NO, and none of the control subjects, have data.

6.0.2.13.2c Subject deaths

Overall, 3/28 (11%) and 2/30 control infants 7%) died during the trial ($p = 0.665$).
Case summaries for the five infants were reviewed and the available data summarized below.

Table 6.0.2.13.2c.1 Deaths in the I-NO groups from the INOSG study[a].

Trial	Subject	Received ECMO?	Time of Death (days)	Description[b,c]
Control				
	Buf-8	Yes	5	Heart failure Pneumothorax Withdrawal of support
	CHOP-S2	Yes	3	Anoxic brain injury Withdrawal of support
	YALE-4	No	3	N/A
I-NO				
	Buf-17	Yes	N/A	N/A
	UT@D-1	No	3	Pneumomediastinum

a. Data from sponsor correspondence dated 8.22.97.

6.0.2.13.3. Long-term safety results of the INOSG trial.

No long-term follow-up data are available.

6.0.2.14 INOSG Trial Efficacy Summary

Study Design and Blinding

The INOSG trial was originally designed to evaluate the acute effect of I-NO on oxygenation and hemodynamics in infants who had PPHN. At a later date, other endpoints were added, including the incidence of ECMO and an analysis of the NINOS primary endpoint. While the sponsor states that these additions were submitted to the FDA prior to unblinding, no evidence of these submissions exist in the FDA files.

Subjects were randomly assigned to receive either control gas (N_2) or I-NO 80 ppm. Two sets of investigators were used during the trial to maintain blinding. There are strong suggestions that the blinding was not complete beyond the first 20 minutes. First, two subjects who failed to improve on control gas were treated later with I-NO. The fact that the investigators were able to determine the treatment received initially by the infants means that, by definition, their blind was broken. These two individuals were not included in the analysis of the endpoints beyond 20 minutes (the original protocol was for 60 subjects, 58 were included for analysis).

Second, the only individual subject data beyond 20 minutes submitted as part of the NDA was for the subjects who were judged acute successes after receiving I-NO. No long-term data was submitted for the control subjects, including two subjects in the control group who were judged an acute success (Buf-14 and Buf-3). These two subjects should have been followed (in blinded fashion) as the successes were in the I-NO group, and long-term data should have been submitted for them. Repeated attempts to discuss this issue with the principle investigators of the study were unsuccessful. The absence of this data suggests that the investigators were able to select which subjects to follow, and chose to follow only the acute 'successes' who received I-NO. This, of course, requires unblinding the therapy.

6.0.2.14 INOSG Trial Efficacy Summary (cont)

Study Design and Blinding (cont)

Third, the sponsor states that the median duration of exposure to control gas was 20 minutes. This would seem to imply that either no infant received control gas after 20 minutes, or that the two infants in the control group who were successes received gas for a longer period of time that was exactly balanced by the shorter than 20 minute exposures in several other infants. The latter seems unlikely. If all control infants, but not all I-NO infants, were withdrawn from gas after precisely 20 minutes, then the blinding was necessarily broken.

Number of Patient/Randomization

Table 6.0.2.6.1 above summarizes the enrollment in INOSG by site. SUNY and University of California-San Francisco together account for 56% of the total patients enrolled.

Primary and Secondary Endpoints

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

This original endpoint emphasized the acute physiological effects of I-NO. Since the administration of study gas necessitated a reduction in FiO_2 from 100% to 90%, the trial first examined the effect of this decrease, prior to study gas administration. This important clinical detail was only examined in this trial, and is discussed below.

The determination of success largely reflected improved oxygenation. This is due to the definition used to determine 'success,' which included changes in OI, PaO_2, and systolic BP. First, no subject was considered a failure due to low systolic blood pressure. Second, only small changes in OI were required for the subject to be a 'success.' The subjects in the I-NO group would be called 'success' if the average OI fell from 42.1 to 39.8 (a change of only 5%), and the PaO_2 rose from 41.3 to 55 (a change of 33% (see section 6.0.2.4 for definitions and table 6.0.2.12.1.2 for the baseline characteristics of the two groups). For the control group, larger changes from baseline were required in order for a 'success': the OI had to fall from 45.9 to 39.8 (a 13% change), and the PaO_2 had to rise from 38.1 to 55 (a change of 44%).

2. Percentage of subjects receiving ECMO therapy.

As discussed above, the second endpoint was not part of the original protocol, and no mention of an ECMO endpoint exists in either the original IND protocol submitted by Dr. Zapol, dated 8.13.93, or in the original protocol submitted by the sponsor. Like the NINOS trial, initiation of ECMO, rather than qualification for ECMO based on pre-specified criteria, was the endpoint. We have no information regarding the clinical state of the individuals requiring ECMO in this trial, including when ECMO was performed.

Secondary and Post-Hoc Analyses

3. A comparison of the number of subject deaths within 120 days and/or receipt of ECMO in the two groups was added to the analysis following the results of the NINOS trial. This endpoint, and it's clinical relevance, is discussed extensively in section 6.0.1. 14 of the NINOS trial review. Interpretation of this endpoint in the INOSG trial is also complicated by the questions of blinding, and the lack of data concerning the control subjects as well as the 'failures' in the I-NO group. Information such as time to receipt of ECMO and oxygenation status at the time of ECMO is also lacking.

4. Percentage of subjects receiving oxygen therapy at 28 days.

Data on 4 control and 1 I-NO subject is missing.

5. Percentage of subjects surviving.

Number of subjects/ randomization

A total of 60 subjects were proposed in the protocol, and 58 were enrolled. Two subjects were withdrawn from the long-term analysis because they received I-NO as a protocol violation after failing to respond to control gas.

Inclusion/ exclusion Criteria

The criteria used in this trial are similar to those in the INO-01/ -02 and /-03 trials, and require the presence of echocardiographically verified PPHN. While surfactant was not excluded, no previous high frequency jet ventilation (HFJV) or high frequency oscillatory ventilation (HFOV) was permitted before entry into the trial.

Dosage/ administration

The device used to administer I-NO was similar to that used in the other trials.

6.0.2.14 INOSG Trial Efficacy Summary (cont)

Duration/ adjustment of therapy

No information regarding the duration of treatment with study gas is available. Once a subject was identified as a 'failure' study gas was to be weaned. This implies that all but two control subjects were discontinued from study gas shortly after 20 minutes. No follow-up information about these two 'successes' is available. As discussed above, the investigators stated that the median time of exposure to control gas was 20 minutes, although 2 of the infants should have received study gas for longer periods of time per the protocol.

Statistical considerations

Dr. Nuri had the following comments regarding the statistics in the INOSG trial.

1) The protocol did not provide an explanation of how the conclusion that, using an overall alpha (α) of 0.05, only 30 subjects would be needed in each treatment group to obtain a 95% power ($\beta = 0.05$). To reach this high degree of certainty, the sponsor needed to specify the difference he was proposing to detect in the success rate between the two groups (this was not stated).

2) The protocol stated that 'the first interim analysis will occur after 20 patients have been enrolled in the study. At that point, the organizing committee will determine the number of interim analyses and the associated a- and b-values.' Details of this proposed analysis were not reported. Instead, the sponsor's report (NDA volume 2.37, Appendix 16.1.9) stated that an non-pre-specified interim analysis was performed after 50 subjects were enrolled in the study, and on that basis the study was stopped. Finally, the study ultimately reported on the results of 58, not 50, subjects. These discrepancies are not clarified in the NDA.

3) The sponsor proposed a single primary endpoint in the original protocol, and at some unspecified later date added a second primary endpoint (the percent of subjects who received ECMO). No statistical adjustment was proposed to the listed p-value for the second 'primary' endpoint.

Patient demographics & baseline characteristics

The two trial groups were well-matched in terms of age, race, and sex, as shown in table 6.0.2.12.1.2. The I-NO group starts out with a higher blood pressure, reflecting the significantly higher diastolic blood pressure of the control group at baseline. The mean baseline OIs in the trial (42-45) identify an extremely 'sick' population.

Some data of interest is not available, including: the age of the mother; the incidence of complications at birth; or the rate of cesarean sections; and the underlying disease which caused the PPHN (i.e., meconium aspiration, sepsis); and the echocardiographic parameters of the subjects. The infants were well-matched with regard to the percentage with meconium below the vocal cords. Knowing the underlying disease state of the infant is quite important, as the NINOS trial suggests that the response rate of the subjects may depend on the underlying cause of their PPHN (see tables 6.0.1.12.3b.1 and 6.0.1.12.3c.1 in the NINOS review).

Disposition of subjects

All but two of the control subjects were deemed failures (93% failure rate), and were discontinued from study gas. In the I-NO group, 14/30 infants were considered 'successes' after 20 minutes (53% failure rate). Per protocol, the two control infants who were successes were to have been followed in blinded fashion along with the 'successes' in the I-NO group (14 infants).

Protocol violations & deviations

No specific information is available as to protocol violations. Two subjects who received open-label I-NO after failing to improve on control gas were discussed previously.

Concomitant therapies used after trial initiation

1. Decreased FiO_2 during study gas administration

The INOSG trial examined the acute effect of decreasing the FiO_2 from 100 to 90%, which was required for administration of study gas.

As shown in table 6.0.2.12.1.3, there were small changes in hemodynamic and oxygenation parameters that followed the reduction of FiO_2 from 100 to 90%. There was, however, no trend towards significant decreases in PaO_2 or BP. No subject was reported to be discontinued from the study due to significant decreases in either PaO_2 or blood pressure between baseline one (FiO_2 100%) and baseline two (FiO_2 90%). This is an important set of data, as it is the only trial in the NDA to systematically examine the effect of lowered FiO_2 in the study population. It suggests that this decrease in FiO_2 does not lead to any dramatic deterioration in the infant's hemodynamic or pulmonary status, which might make initiation of I-NO more problematic.

6.0.2.14 INOSG Trial Efficacy Summary (cont)

Concomitant therapies used after trial initiation (cont)

2. O_2 Therapy

The duration of oxygen therapy was recorded for all infants. One infant required O_2 for ~~~3 days in the control group. Excluding that individual, the average need for supplemental O_2 was 19±21 days in the control group and 11±6 days in the I-NO group (p value =0.066).

3. Surfactant, jet and oscillatory ventilation

No data is available on the use of these therapies during the INOSG trial.

Analysis of Primary and Secondary Efficacy Outcomes

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

The original primary endpoint was physiological: the number of 'successes' following study gas administration, reflecting improvements in oxygenation and hemodynamics. As discussed above, the primary determinant of 'success' was the acute change in oxygenation. The subjects in the I-NO group had a significantly higher PaO_2 following 20 minutes of therapy (47.8 mmHg increase in the I-NO group, -1.9 mmHg decrease in the control group, p value <0.0001). Driven by this result, significantly more subjects in the I-NO group were judged successes (2/28 in the control group, 16/30 in the I-NO group).

2. Percentage of subjects receiving ECMO therapy.

This endpoint was added after the original protocol was submitted, and suffers from the questions regarding blinding, and absence of primary data for any of the control subjects. These issues were discussed above. Given these constraints, I view any long-term results from this study to be of supportive, rather than pivotal value. Having said this, it is difficult to envision an endpoint such as initiation of ECMO being heavily influenced by the knowledge of prior therapies, although it might influence the time it took the investigators to transfer the infant to ECMO. Such data is not available.

With these caveats, administration of I-NO was associated with a reduction in the percentage of infants who received ECMO significantly: 20/28 (71%) in the control group versus 12/30 (40%) in the I-NO group (p = 0.016).

There was no significant difference in the duration of ECMO between the control and I-NO groups (see Table 6.0.2.13.1.1).

Secondary and Post-Hoc Analyses:

3. Number of subject deaths within 120 days and/or receipt of ECMO in the two groups

This analysis, added after the results of the NINOS trial were known, has the same difficulties discussed in the first two endpoints concerning blinding and missing data.

Overall, 21/28 infants in the control group (75%) and 13/30 infants in the I-NO group met this combined endpoint (p = 0.014).

4. Percentage of subjects surviving.

The duration of follow-up for the deaths reported is not stated in the report. Additionally, no narrative description or detailed case report form for the infant deaths is available. Case summaries were obtained for the five deaths, giving some details of the causes of death (included above). Two deaths occurred in subjects who did not receive ECMO (Yale-4 in the control group, UT@D-1 in the I-NO group).

Overall, 25/28 controls (89%) and 28/30 (93%) infants in the I-NO group survived (p = 0.665).

Overall Efficacy Summary

The database used in this trial to determine efficacy is limited by the questions of blinding and the absence of primary data, especially for the control subjects. Certain baseline data used to exclude enrollment bias (especially baseline echocardiographic data and underlying cause of PPHN) are also missing. There area additional statistical deficiencies in the trial, including missing data and the use of un-specified interim analyses. Because of these deficiencies, the long-term results reported for the trial must be viewed cautiously.

For the one pre-specified endpoint, the short-term effect of I-NO on oxygenation is clear and convincing: I-NO at 80 ppm causes an acute and highly significant increase in PaO_2, without having a significant hemodynamic effect.

A second endpoint was added after the initiation of the trial, regarding the use of ECMO. In this analysis, long-term, administration of I-NO was associated with a reduction in the rate at which infants underwent ECMO. Because of the deficiencies noted above, I consider this result to have occurred during an open-label period of I-NO administration. Insufficient data are available to comment on the factors used by the investigators to guide the decision to initiate ECMO.

6.0.2.14 INOSG Trial Efficacy Summary (cont)

Overall Efficacy Summary (cont)

This reduction in the need for ECMO, however, was not coupled to any other markers of clinical improvement. There was no difference in overall mortality, and no effect of I-NO was detected on any other 'hard' endpoints' of clinical benefit: duration of hospitalization, need for supplemental oxygen at 28 days, or number of days on assisted ventilation. The clinical benefit of avoidance of ECMO will be discussed extensively in the overall efficacy summary (section 7.0), but such as claim might be expected to be based on improved patient outcome, as measured by the 'hard' endpoints listed above. No such effect was detected in this trial.

6.0.2.15 01-02 Safety Summary

Data on overall adverse events were not collected in this trial. Instead, specific adverse events were identified and data was collected. These events included: incidence of methemoglobinemia (I-NO group only); vital status; and the duration of treatments (ECMO, hospitalization, supplemental O_2, ventilation). No data was collected on other adverse events, including several which might be reasonably relevant to the safety of I-NO in this population: elevated NO_2 levels; intracranial hemorrhage (including interventricular hemorrhage); bronchopulmonary dysplasia and air leak syndrome; seizures; and clinically significant bleeding. Finally, case report forms were not available for any infants. At my request, the sponsor submitted case summaries for the 5 deaths, which form the database for individual deaths in the INOSG trial.

Of the safety parameters followed prospectively in the INOSG trial, two were included in the efficacy discussion above (vital status and receipt of ECMO). The incidence of methemoglobinemia, need for external O_2 after 28 days, duration of hospitalization and ventilation, and effect on vital signs will be discussed below.

Analysis of Safety Outcomes (not discussed during the efficacy review)

1. Methemoglobinemia (see Tables 6.0.2.13.1.1 and 6.0.2.13.2b.1).

The only available data for methemoglobin levels comes from the responding I-NO subjects. In this group, peak methemoglobin levels averaged 3.8±4.0 during the first twelve hours of therapy, and 5.3±5.8 at any time during the trial.

Three of the infants had peak methemoglobin levels >5% during the trial(UT@D-1, UCSF-5, and Buf-S1). Of these, one infant is of note.

1) Subject UT@D-1 was a female infant who responded to I-NO, 80 ppm, with a decrease in OI. Her methemoglobin level was 13.2 after 12 hours, and 8.7 after 24 hours. That is the last recorded methemoglobin level, prior to the development of pneumomediastinum and death.

2. Duration of hospitalization (see Table 6.0.2.13.1.1).

There was a non-significant, numerical decrease in the number of days of hospitalization in the I-NO group (42±91 in the control, 20±8 in the I-NO group, p=0.24).

3. Number of days of assisted ventilation (see Table 6.0.2.13.1.1).

There was a non-significant, numerical reduction in the average number of days of ventilation in the I-NO group (27±84 in the control, 8±4in the I-NO group, p=0.56).

4. Days of supplemental O_2 (see Table 6.0.2.13.1.1).

Including only those subjects with available data, 4/21 subjects in the control group (19%) and 1/27 in the I-NO group (4%) required supplemental O_2 after 28 days (p = 0.17). One infant in the control group reportedly required O_2 for 445 days in the control group. Excluding that individual, the average need for supplemental O_2 was 19±21 days in the control group and 11±6 days in the I-NO group (p value =0.066).

There was no significant difference in the number of infants who required O_2 at 28 days in the two groups (4/21 in the control group, 1/27 in the I-NO group, p=0.17).

5. Acute changes in hemodynamics and physiological parameters in the INOSG trial (see Table 6.0.2.12.2d.2).

a. Changes in oxygenation and pulmonary gas exchange.

I-NO had an acute effect to improve both PaO_2 and OI at the end of the first 20 minutes of exposure. For the PaO_2, the mean change from baseline to 20 minutes was -1.9±9.6 mmHg in control, and +47.4±68 mmHg in the I-NO group. For the OI, the mean change from baseline to 20 minutes was -2.0±1.5 in control, and -16±11.5 in the I-NO group. No long-term data is available. In this trial, 53% of neonates responded to I-NO with an improvement in oxygenation.

I-NO had an acute effect to decrease pCO_2 significantly. The mean change from baseline to 20 minutes was -0.9±4.8 mmHg in control, and -4.1±7.6 mmHg in I-NO group. No long-term data is available.

I-NO had no significant effect on pH at the end of 20 minutes.

b. Changes in vital signs

I-NO had an acute effect to lower mean systemic blood pressure. The mean change from the second baseline to 20 minutes was +1.4 mmHg in control group, and -1.0±13.2 mmHg in the I-NO group.

I-NO had no significant acute effect on heart rate.

6.0.2.15 01-02 Safety Summary (cont)

Overall safety summary for INOSG

The safety database is missing many elements from what would normally be submitted as part of a pivotal trial in an NDA. Few data on specific adverse events were collected, and data on several key adverse events was not collected. Data is missing on any control subject after the first 20 minutes of study gas administration, with the exception of vital status, receipt of ECMO, and duration of specific supports (O_2, ventilation, ECMO).

1. Methemoglobin levels

Subjects receiving I-NO 80 ppm are at significant risk for elevated methemoglobin levels (10% of the responding I-NO group had levels ≥5%, see table 6.0.2.115.1).

2. Acute effect on vital signs

Exposure to I-NO was associated with a significant decrease in mean systemic pressure at the end of 20 minutes. Overall, this equaled an 8 mmHg decrease relative to control subjects. No further information regarding the effects of I-NO on blood pressure were collected.

3. Effect on duration of hospitalization, duration of supplemental O_2, duration of ECMO, and duration of assisted ventilation.

I-NO had no significant effect on any of the safety endpoint. Administration of I-NO was associated with receiving numerically fewer days of hospitalization, ventilation, and supplemental O_2.

Absent the data listed above, the long-term safety of I-NO cannot be derived from this study, beyond the absence of a detectable effect on mortality. In that regard, it is relevant to note that one infant in the I-NO group died after developing pneumomediastinum. The possible link between I-NO and Air Leak Syndrome (including pneumomediastinum) will be discussed extensively in section 8.2.7.2.

6.0.2.16. INOSG Reviewer's Conclusions

1. Because of the difficulties in blinding discussed above, the long-term data on the use of ECMO and other therapeutic modalities from the INOSG trial must be interpreted as unblinded. Within this constraint, the trial suggests the I-NO administration is associated with a decreased use of ECMO. The results of the INOSG trail should be viewed as supportive, rather than pivotal, with regard to the hypothesis that I-NO reduces the need for ECMO.
2. No effect of I-NO on other aspects of clinical efficacy was demonstrated (mortality or the duration of hospitalization, ventilation, supplemental O_2 or ECMO).
3. I-NO administration, at a dose of 80 ppm, is associated with a significant improvement in oxygenation in a substantial fraction of neonates.
4. With regards to safety, the INOSG trial is inadequate to address any aspects of long-term safety beyond mortality rate.
5. There is acute, significant effect of I-NO to lower mean systemic blood pressure.
6. There is an acute, significant effect of I-NO to lower pCO_2, but not pH.
7. The study suggests that a significant number of subjects who receive I-NO 80 ppm will develop elevated methemoglobin levels.

6.0.3 INO-01/ -02 Study
(Ohmeda INO-01/ -02)

6.0.3.1 Title of Study: A double-blind, randomized, placebo-controlled, dose-response study of inhaled nitric oxide in the treatment of persistent pulmonary hypertension of the newborn .

6.0.3.2 Site(s) of Investigation and Investigators

Table 6.0.3.2.1 Ohmeda INO-01/ INO-02 study sites and investigators[a,b].

Investigator	Site of Investigation
Michael Damask MD (Medical Monitor)	
Richard Straube MD (Medical Monitor)	
J. Kattwinkel MD	University of Virginia
P. Griffin MD	"
R. Hillyard MD	Children's Hospital of Orange County
G. Dudell MD	San Diego Children's Hospital
M. Evans MD	"
M. Ogino MD	"
D. Davidson MD	Schneider Children's Hospital
A. Steele MD	"
R. Koppel MD	"
T. Pauly MD	University of Kentucky
J. Walker MD	"
E. Barefield MD	University of Alabama/Birmingham
V. Karle MD	"
M. Shwer MD	St. Joseph's Hospital, AZ
D. Hamburg MD	"
J. Fiascone MD	Floating Hospital for Children at New England Medical Center
J. V. McDonald MD	Legacy Emanuel Hospital and Health Center
S. Brudno MD	Medical College of Georgia
K. C. Sekar MD	Children's Hospital of Oklahoma
M. A. McCaffree	"
G. L. Dreyer MD	St. John's Mercy Medical Center
B. Goldenberg MD	Loyola University Medical Center
M. Weis MD	"
V. Bhutani MD	Pennsylvania Hospital
B. T. Blooom MD	Wesley Medical Center
R. Brilli MD	Children's Hospital Medical Center
E. M. Bifano MD	Crouse Irving Memorial Hospital
D. Hakanson MD	"
J. H. Khan MD	Children's Hospital of the King's Daughters
D. E. Mayock MD	University of Washington
G. J. Redding MD	"
S. Block MD Bch	Bowman Gray School of Medicine
M. Cohen MD	Newark-Beth Israel Medical Center
D. Vidyasagar MD	University of Illinois Hospital
R. Bhat MD	"

a. This list includes those sites which did not enroll any subjects in the trial (italicized).
b. Data from NDA volume 2.13, section 8.1.2.

Table 6.0.3.2.1 Ohmeda INO-01/ INO-02 study sites and investigators (cont).

Investigator	Site of Investigation
T. A. Merritt MD	University of California- Davis
B. W. Goetzman MD	"
E. M. Fajardo MD	Oschner Clinic
M. Hiatt MD	St. Peter's Medical Center
M. T. Carbone MD	"
T. Hegyi MD	"
George Lambert MD	"
Barry Weinberger MD	"
Anne Koons MD	"
A. Napolitano MD	All Children's Hospital
D. T. Escoto MD	"
A. Corbet MD	Santa Rosa Children's Hospital
D. Auerbach MD	Arnold Palmer Hospital for Children
G. Alexander MD	"

6.0.3.3 Background

8.26.93 & 11.19.93	pre-IND meetings.
12.13.93	INO-01/ -02 trial protocol submitted.
4.21.94	INO-01/ -02 study initiation.
6.24.96	Early termination of INO-01/ -02 study.
10.27.96	Pre-NDA meeting.
6.17.97	NDA submission to the FDA.
7.28.97	Submission of long-term safety data from INO-01/ -02 trial to FDA.

Protocols INO-01 and INO-02 were identical in design, but had different institutions participating. Ultimately, both trials were stopped due to difficulties with accrual following the announcement that the NINOS trial had been stopped for overwhelming efficacy. In the submitted NDA, the INO-01 and INO-02 databases have been combined, and will be evaluated together in this review. This format was agreed upon in advance by the FDA.

6.0.3.4 Study Design

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.' (NDA submission, volume 2.17, page 077708).

In the original IND, the first objective was stated as follows: 'The primary clinical objective of this study is to demonstrate an acute, sustained increase in oxygenation and decrease in the intensity of ventilatory support in patients treated with nitric oxide.' (Protocol INO-01/Amendment 6, page 5).

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. (continued on next page)

6.0.3.4 Study Design (cont)

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 for PPHN after discontinuation of the study gas, at the discretion of the investigator. These might include ECMO or 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.

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.

6.0.3.5 Primary and Secondary Endpoints

Primary endpoints

The incidence of one of the following events (called PPHN major sequelae):

1. Death;
2. Initiation of ECMO;
3. Evidence of abnormal neurological sequelae (intraventricular hemorrhage, brain infarct, or the presence of seizures);
4. Bronchopulmonary dysplasia [defined as O_2 >21% required at 28 days of age with abnormal chest x-ray or reactive airway disease (defined as requiring bronchodilators at discharge)].

After the results of the NINOS trial were published, the sponsor added a 'primary analysis' comparing the incidence of death before 120 days and/or initiation of ECMO.

Secondary endpoints

1. Physiologic response to I-NO, measured by change in OI and time-weighted OI (TWOI).
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).

Long-term follow-up endpoints (measured at 1 year follow-up examination)

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

"Exploratory variables" (per the sponsor)

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

6.0.3.6 Number of Patients/ Randomization

A total of 320 subjects were planned for enrollment, 80 subjects in each of 4 categories: placebo (N_2); 5 ppm I-NO; 20 ppm I-NO; and 80 ppm I-NO. From 1282 subjects screened, a total of 155 were randomized (12% of the screened subjects), of which 69 successfully completed study therapy and were weaned off the treatment gas.

6.0.3.6 Number of Patients/ Randomization (cont)

Table 6.0.3.6.1 Enrollment in INO-01/ -02 by site.

Site	Control	I-NO			Total
		5 ppm	20 ppm	80 ppm	
University of Virginia	3	2	2	2	9
Children's Hospital of Orange County	1	1	0	1	3
San Diego Children's Hospital	7	8	7	7	29
Schneider Children's Hospital	3	2	2	2	9
University of Kentucky	1	2	1	2	6
University of Alabama	2	3	2	2	9
Children's Health Center in Arizona	3	2	2	2	9
Floating Hospital for Children	1	0	0	0	1
Legacy Emanuel Hospital and Health Center	2	2	2	0	6
Medical College of Georgia	0	1	1	0	2
Children's Hospital of Oklahoma	3	4	4	4	15
Pennsylvania's Hospital	1	1	1	0	3
Wesley Medical Center	1	0	0	0	1
Crouse Irving Memorial Hospital	1	2	2	1	6
Children's Hospital of the King's Daughters	1	0	0	0	1
Bowman Gray	0	0	0	1	1
Newark-Beth Israel Medical Center	2	1	2	2	7
Univ of California, Davis	0	0	0	1	1
Ochsner Clinic	2	1	2	2	7
East Carolina University School of Medicine	2	2	2	2	8
Presbyterian/St. Lukes Medical Center, Denver	1	1	0	1	3
Christ Hospital	0	1	1	1	3
Duke University	2	3	1	2	8
Univ of South Dakota	2	1	1	2	6
Santa Rosa Children's Hospital	0	1	1	0	2
Total	41	41	36	37	155

Randomization

Two teams were used to accomplish the blinding in the trial. The first team consisted of the patient caregivers, who were blinded to the treatment gas being administered. The second team consisted of a least one unblinded investigator, who was responsible for all activities that revealed the treatment gas. These activities included maintenance of the bedside stock of treatment gas, daily calibration of the gas blender, and recording the methemoglobin, I-NO, and NO_2 levels.

6.0.3.7 Inclusion/Exclusion Criteria

Inclusion criteria for primary entry into INO-01/ -02 trial

1. Hypoxemia defined as a postductal PaO_2 between 40 and 100 mm Hg on more than one determinations while breathing an FiO_2 =100%.
2. Mechanical ventilation with a $P_{AW} \geq$ 10 cm H_2O.
3. A diagnosis of pulmonary hypertension, including the following criteria:
   a. Evidence of a right to left or bidirectional cardiac shunt at the level of the patent ductus arteriosus or formen ovale.
   b. Pre- and Post-ductal O_2 saturation difference >10% (when the ductus can be visualized) or
   c. Right-to-left, or bidirectional shunting at the foramen ovale together with one of the following indicators of pulmonary hypertension:
      i. Moderate to severe tricuspid insufficiency jet with Doppler evidence of systolic pulmonary artery pressure ≥75% of systemic pressure.
      ii. Posterior systolic bowing of the intraventricular septum.
4. The infant must have an indwelling arterial line.
5. Term neonate: >37 weeks and a birth weight of greater than or equal to 2500 grams (>2000 grams if >39 weeks gestation).
6. Neonate <72 hours old.
7. Informed parental consent.

6.0.3.7 Inclusion/Exclusion Criteria (cont)

Exclusion criteria for primary entry into trial

1. Failure to meet any inclusion criterion.
2. Intraventricular hemorrhage (Grade 2-4) or subarachnoid hemorrhage.
3. Mean systemic arterial BP <35 mm Hg after volume or vasopressor therapy.
4. Uncorrected polycythemia (central hematocrit >70%).
5. Cardiac lesions other than patent formen ovale, patent ductus arteriosus, or tricuspid regurgitation.
6. No evidence for pure left-to-right shunt.
7. Previous therapy with surfactant.
8. Use of high frequency jet or oscillatory ventilation (HFJV/ HFOV). More than 6 hours of HFJV/ HFOV at referring institution or any use of HFJV/ HFOV within 2 hours of treatment gas.
9. Clinical diagnosis of pulmonary hypoplasia, including congenital diaphragmatic hernia.
10. Phenotype consistent with a lethal chromosomal abnormality.
11. Intravenous vasodilator therapy (Tolazoline) beginning after inclusion criteria are met.
12. Uncontrollable coagulopathy and/or serious bleeding.
13. Enrollment in any investigational drug or other interventional study.

Criteria for use of ECMO:

Transfer of subjects to ECMO occurred at the discretion of the individual investigator. No criteria for use of ECMO were stipulated.

6.0.3.8 Dosage/Administration

Subjects are randomized to receive either I-NO, 5, 20 or 80 ppm or placebo (N_2). Subjects were assigned a site-specific, sequential patient number at time of randomization and were allocated to one of the groups based on that number. The sponsor chose I-NO doses based on the available animal data as well as two small clinical studies(6, 12, 80). The upper limit of 80 ppm was chosen out of concern for the report of methemoglobin and NO_2 accumulation at higher doses reported to occur in some subjects.

Because of the hazards associated with potentially high NO_2 levels and the potential for methemoglobinemia, it was decided that an unmasked investigator would monitor these parameters during the trial. To maintain a double-blind study, therefore, it was necessary to establish two clinical teams. The unmasked investigators were responsible for all activities and data collection that could potentially indicate which treatment gas was being administered. All other personnel caring for the infant remained masked as to the treatment gas identity.

The treatment gases were administered via inhalation using an Ohmeda inhaled NO delivery device (Ohmeda MSD Inc.), connected to an Infant Star ventilator (Infrasonics) and a Fisher-Paykel MR-730 heated humidifier (Fisher-Paykel Healthcare). The NO for inhalation was manufactured by BOC Specialty Gases from raw NO supplied [redacted] BOC Specialty Gases. To provide different doses of I-NO to subjects using the same delivery device and settings (for blinding purposes), multiple cylinder concentrations of NO were supplied to sites, where the gas was diluted 1:20 by the delivery device. To limit the formation of NO_2, the residence time of NO and O_2 together within the breathing circuit were minimized by keeping the flow rate of the gas >10 liters/minute. The exhaled gases were collected and discharged into the hospital vacuum system.

The necessity for high-flow of the study gas to minimize the residence time of the I-NO with O_2 meant that subjects breathing I-NO had a maximum inspired FiO_2 of 90%, which was decreased from the 95-100% FiO_2 the subjects received prior to initiating study gas.

6.0.3.9 Duration of Study

Treatment gas was administered as soon as possible after a patient met all of the screening parameters. Once started, study gas was administered until any one of the following occurred:

1. the subject was discontinued from the study because they met 'treatment failure' criteria, defined as meeting any one of the following:
   a. PaO_2 <40 mm Hg at the beginning and end of a 30 minute period not due to a correctable mechanical problem;
   b. mean systemic arterial pressure <35 mm Hg after volume or vasopressor therapy;
   c. the subject died;
   d. the subject's methemoglobin >7% on two consecutive time points at least 30 minutes apart; or
   e. the subject's NO_2 level was persistently > 3 ppm for 30 minutes.

6.0.3.9 Duration of Study (cont)

2. the subject had treatment gas prematurely discontinued for any of the following reasons:
   a. the parent(s) withdrew consent;
   b. the patient is found to have an exclusion criteria;
   c. the delivery or monitoring of I-NO malfunctions and cannot be corrected within 8 hours; or
   d. the principle investigator considered withdrawal to be in the best interest of the child.
3. the subject received 14 days of study gas.
4. the criteria for weaning of treatment gas are met, defined as all of the following:
   a. FiO_2 <0.60;
   b. P_{AW} <10 cm H_2O; and
   c. postductal PaO_2 ≥60 mm Hg.

If a subject met the criteria for weaning, or if the methemoglobin/ NO_2 levels were too high, the treatment gas was weaned in 20% decrement to 0%. Decreases occurred every 30 minutes to 4 hours, so long as the infant continued to meet criteria for weaning. In case of poor oxygenation, the gas could be increased up to 20%.

Elevated methemoglobin level was defined as >7% at two consecutive time points at least 30 minutes apart. Elevated NO_2 level was defined as > 3 ppm for 30 minutes.

6.0.3.10 Safety & Efficacy Parameters Measured

Efficacy Parameters

1. Incidence of neonatal deaths.
2. Incidence of ECMO rescue.
3. Incidence of risk factors for abnormal neurological sequelae (intraventricular hemorrhage and brain infarct). Measured by brain scan or head ultrasound prior to discharge.
4. Incidence of bronchopulmonary dysplasia (defined as O_2 >21% required at 28 days of age with abnormal chest x-ray) or reactive airway disease (defined as requiring bronchodilators at discharge).
5. Exposure to I-NO (ppm per hour)
6. Duration of treatment gas.
7. Proportion of subjects who receive the following: ECMO; high-frequency jet ventilation (HFJV); surfactant; vasodilator therapy other than I-NO.
8. Ventilatory parameters: OI; PaO_2; $PaCO_2$; pH; % oxygen saturation; postductal PaO_2; preductal O_2 saturation; postductal O_2 saturation; Arterial-alveolar O_2 ratio; Arterial-alveolar O_2 gradient; arterial pH; time-weighted OI.
9. Hemodynamic parameters: systolic blood pressure (SBP); diastolic blood pressure (DBP); mean arterial pressure (MAP).
10. Ventilation parameters: positive end-expiratory pressure (PEEP); positive inspiratory pressure (PIP); mean airway pressure (P_{aw}); FIO_2.

Safety Parameters

1. Methemoglobin levels.
2. Inhaled NO_2 concentrations
3. Adverse events
4. Routine lab chemistries and blood counts.
   Labs and hematology were collected at baseline and again within 12 hours of discontinuation of I-NO.
   The following laboratories were not collected as part of the submitted NDA database: electrolytes (Na^+, K^+, Cl^-, HCO_3^-); coagulation parameters; and urinalysis.
5. Hemodynamics (heart rate, blood pressure).
   Electrocardiograms were not collected as part of the NDA database.
6. Incidence of air leak (pneumothorax, pneumopericardium, pneumoperitoneum, pneumomediastinum, interstitial emphysema).
7. Incidence of bronchopulmonary dysplasia (BPD).
8. Requirement for supplementary O_2 at time of discharge or 28 days.
9. Incidence of seizures.

10. Surviving infants were to have a 1 year examination including the following safety assessments: vital status; medical history; neurologic assessment; audiology (using BAER); and a developmental test.

6.0.3.11 Statistical considerations

For statistical comparison between the groups, all subjects were grouped according to the occurrence of one of the primary endpoints (death, adverse neurologic event, ECMO, adverse pulmonary event) or none of the above. The number of subjects in these 5 categories were then compared for each of the 4 groups (placebo, I-NO 5, 20 and 80 ppm) and for the efficacy parameters using the Cochran-Mantel-Haenszel chi-squared test.

Analysis of covariance was used to compare baseline and 30 minute values for the exploratory endpoints (see 'Exploratory endpoints' above). It will also be used to compare the values for the baseline with times prior to first weaning and with times 6, 12, and 24 hours after gas discontinuation.

Dose-response for each dose of I-NO was to be analyzed by ANOVA on the following parameters:

1) total exposure to I-NO (ppm per hour, measured as the area under the I-NO vs. time curve);
2) total duration of treatment gas;
and 3) exposure to I-NO until weaning parameters are met.

After discontinuation of the study gas, the data from the 3 doses of I-NO would be pooled and compared to data from the placebo group.

The company estimated the trial size by first estimating the rates for the major PPHN sequelae among the total population in the U.S. with PPHN (2000 subjects per year):

1. Death, 8%.
2. Abnormal brain scan/head ultrasound or seizures, 20%.
3. ECMO rescue, 28%.
4. Bronchopulmonary dysplasia or reactive airway disease, 2%.

Because the mortality rate was low (8%), the sponsor calculated that even a reduction of 2/3 in this rate would require an unattainably large sample size (approximately 1800 subjects). The sponsor then calculated the sample size using a proposed reduction in OI and number of days of supplemental O_2 of 50%. The original protocol thus called for 24 subjects in each of 4 arms (96 total). This number was later increased to 160 subjects (40 per arm). This sample estimate was based on a Monte Carlo simulation for the Cochran-Mantel-Haenszel test assuming the placebo rates as 8% for death, 20% for abnormal neurological sequelae, 28% for ECMO, and 2% for broncho-pulmonary dysplasia. The corresponding I-NO group rates, then, were 5%, 10%, 14% and 1% respectively. 'It was assumed that each of the comparisons were at the 0.05 significance level using an 80% power.' (NDA, vol. 2.17, page 081908).

6.0.3.12 Efficacy endpoint outcomes

6.0.3.12.1 Subject demographics and baseline characteristics

The demographics of the enrolled subjects in INO-01/ -02 are listed below. There are no significant demographic differences between the groups.

Table 6.0.3.12.1.1 Demographics of the 155 subjects enrolled in trial INO-01/ -02 at baseline[a].

Demographic Characteristic	Control	Inhaled I-NO			Pooled NO
		5 ppm	20 ppm	80 ppm	
Total	41	41	36	37	114
Sex					
Male	27 (66%)	19 (46%)	20 (56%)	19 (51%)	58 (51%)
Female	14 (34%)	21 (54%)	16 (44%)	18 (49%)	56 (49%)
Race					
White (%)	20 (49%)	24 (59%)	18 (50%)	17 (46%)	59 (52%)
Black (%)	11 (27%)	7 (17%)	9 (25%)	9 (24%)	25 (22%)
Hispanic (%)	10 (24%)	8 (20%)	8 (22%)	4 (11%)	20 (18%)
Asian (%)	0 (0%)	1 (2%)	0 (0%)	3 (8%)	4 (4%)
Other (%)	0 (0%)	1 (2%)	1 (3%)	4 (11%)	6 (5%)
Age (hours)	26±18	22±14	24±16	27±20	24±17
Birth Weight (kg)	3.40±0.5	3.5±0.5	3.4±0.6	3.4±0.5	3.40±0.5
Gestational age (weeks)	39.7±1.8	40.2±1.5	39.3±1.6	39.9±1.5	39.8±1.6
Mother's age (years)	27.1±6.3	25.6±6.0	26.0±6.9	25.8±6.6	25.8±6.4

a. Data shown is mean ± standard deviation, and is from NDA volume 2.17 page 083608.

6.0.3.12.1 Subject demographics and baseline characteristics (cont)

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).

Table 6.0.3.12.1.2 Underlying disease of the infants enrolled in INO-01/ -02.

Disease	Control	Inhaled I-NO			Pooled I-NO	p value
		5 ppm	20 ppm	80 ppm		
Meconium Aspiration (% of total)	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
Repertory Distress Syndrome	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.

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 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	p value[b]
		5 ppm	20 ppm	80 ppm		
Birth characteristics						
Cesarean section	25/41 (61%)	23/41 (56%)	23/36 (64%)	24/36 (67%)	70/113 (62%)	0.80
Complicated delivery	14/39 (36%)	12/41 (29%)	11/36 (31%)	11/36 (31%)	34/113 (30%)	0.92
Apgar at 1 minute	5±3	6±3	5±3	5±3	5±3	
Apgar at 5 minutes	7±2	8±2	6±2	7±2	7±2	
Apgar at 10 minutes	7.5±1	6.9±2	6±2	6.6±2	6.6±2	
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	
Positive End-Expiratory Pressure (cm H_2O)	5.1±1.6	4.9±0.8	5.3±1.6	4.8±1.1	5.0±1.2	
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 (beats per minute)	148±19	152±26	153±25	151±23	152±25	
Findings on cranial ultrasound						
Normal	37/41 (90%)	40/41 (98%)	32/36 (89%)	33/37 (89%)	105/114 (92%)	0.45
Abnormal	4/41 (10%)	1/41 (2%)	4/36 (11%)	4/37 (11%)	9/114 (8%)	
Brain infarct	0	0	0	0	0	
Intraventricular hemorrhage	2/41 (4%)	0/41 (0%)	3/36 (8%)	1/37 (3%)	4/114 (4%)	0.28
Periventricular leukomalacia	0	0	0	0	0	
Ventriculomegaly	1/41 (2%)	0	0	0	0	

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.

6.0.3.12.1 Subject demographics and baseline characteristics (cont)

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

Table 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	p value[b]
		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%)	NS
Right to left or bidirectional PDA shunt	34/41 (83%)	32/41 (78%)	30/36 (83%)	24/37 (65%)	86/114 (75%)	NS
Closed ductus arteriosus with other evidence of PPH[Na]	6/41 (15%)	5/41 (12%)	5/36 (14%)	11/37 (30%)	21/114 (18%)	NS
None of the above[c]	2/41 (5%)	2/41 (5%)	1/36 (3%)	0/37 (0%)	3/114 (3%)	NS

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 (see below).

The next table summarizes the therapies used by the subjects prior to entry into the study. The therapies used were well-matched between the four groups.

Table 6.0.3.12.1.5 Specific therapies used by subjects in the INO-01/ -02 trial prior to randomization.

Therapy	Control	Inhaled I-NO			Pooled I-NO
		5 ppm	20 ppm	80 ppm	
Mechanical ventilation	32/38 (84%)	29/36 (81%)	24/33 (73%)	23/33 (70%)	76/102[a] (75%)
HFJV	0/38 (0%)	0/36 (0)%	3/33 (9%)	0/33 (0%)	3/102 (3%)
HFOV	1/38 (3%)	1/36 (3%)	1/33 (3%)	3/33 (10%)	5/102 (5%)
Resuscitation required	36/41 (88%)	39/41 (95%)	31/36 (86%)	32/37 (86%)	102/114 (89%)

a. The ventilation received by the subjects was not available for all subjects (102/114 had records available).

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6.0.3.12.2 Disposition of subjects

6.0.3.12.2a Subject selection

A total of 1282 subjects were screened for entry into INO-01/ -02, and 155 were entered (12%). The reasons for screened subjects not being enrolled in INO-01/ -02 are summarized below. Subjects were excluded from INO-01/ -02 if they had received surfactant (12%) or HFJV/HFOV for more than 6 hours of HFJV/ HFOV at referring institution or within 2 hours of treatment gas (9%). Subjects without echocardiographic evidence of PPHN (i.e., left-to right shunts on ECHO) were also excluded in this trial, and 19% of screened infants were rejected because of this criterion. This was the most common reason for rejection after failure to meet hypoxia criterion.

Table 6.0.3.12.2a.1 Reasons for screened subjects not being enrolled in INO-01/ -02.

Inclusion Criteria Not Met	# of subjects from INO-01/ -02	Exclusion Criteria Violated on Entry	# of subjects from INO-01/ -02
Total patients screened	1282	Total patients screened	1282
No echocardiographic evidence of PPHN	244 (19%)	Prematurity (<37 weeks)	94 (7%)
Postductal PaO_2 <40 mm Hg or PaO2 >100 mm Hg	336 (26%)	Birth Weight <2500 grams (<2000 if >39 weeks gestation)	53 (4%)
Age >72 hours	59 (5%)	Intracranial hemorrhage	5 (0.4%)
Gestation <37 weeks	103 (8%)	Mean systemic BP <35 mm Hg	3 (0.2%)
Birth Weight <2500 grams (<2000 if >39 weeks gestation)	59 (5%)	Exclusionary cardiac lesions	81 (6%)
Intra-arterial line not present	22 (2%)	Surfactant therapy	157 (12%)
Informed consent not obtained	58 (5%)	High-frequency ventilation	118 (9%)
		Lethal physical or chromosomal abnormality	49 (4%)
		Pulmonary hypoplasia or congenital diaphragmatic hernia	10 (8%)
		Vasodilator tx after entry criteria met	1 (0.1%)
		Uncontrollable coagulopathy	15 (1%)
		Uncorrected polycythemia	0 (0%)
		Enrolled in other investigational trials	155 (12%)
Patients randomized	155 (12%)	Patients randomized	155 (12%)

6.0.3.12.2b Protocol Violations and Deviations in the INO-01/ -02 trial

The table below list the reported protocol violations and deviations reported by the sponsor:

Table 6.0.3.12.2b.1 Entry criteria violations and protocol violations for the INO-01/ -02 study.

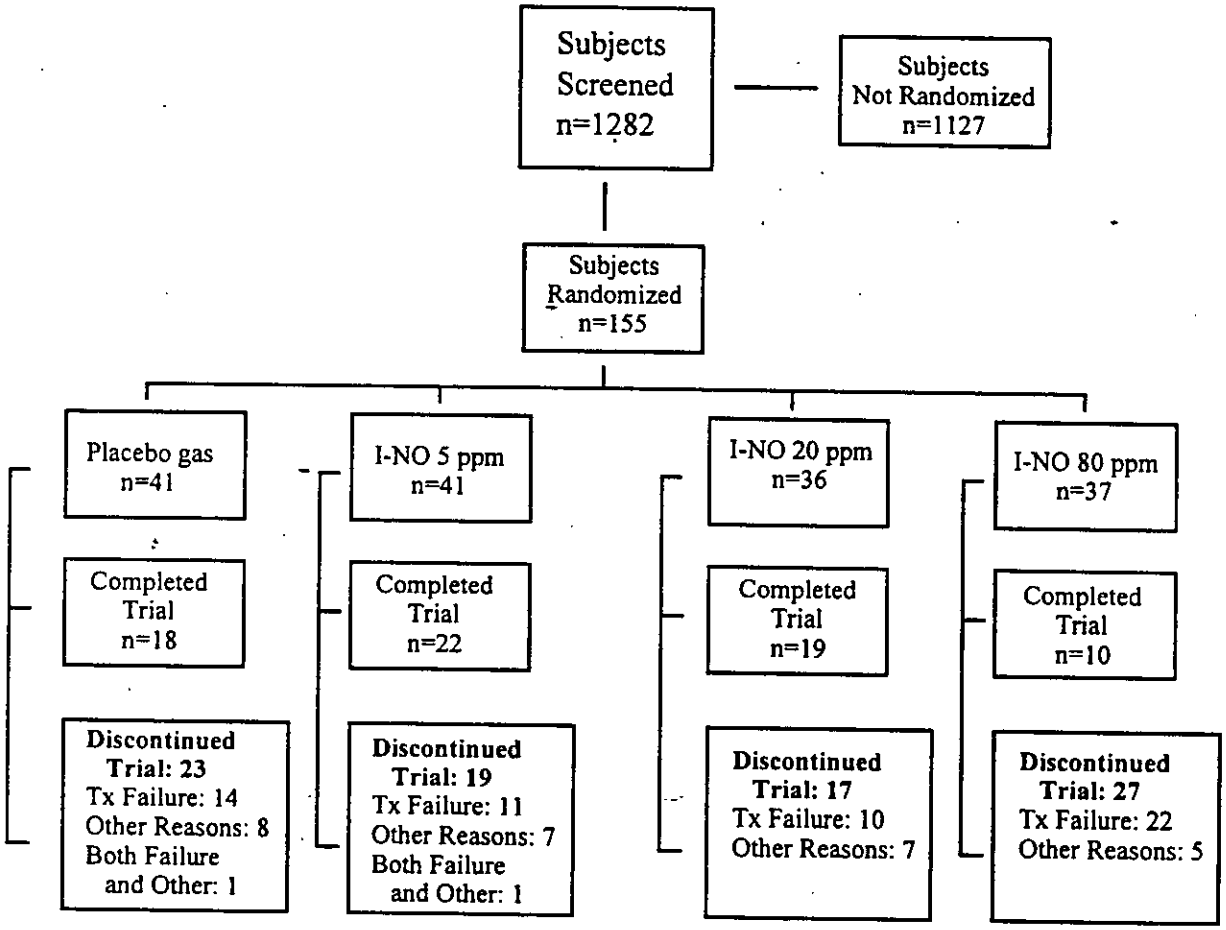
Characteristic	Placebo Group	I-NO Group	Subject #
Received 1/2 vial of surfactant prior to enrollment		80 ppm	01-01005
Had a preductal not postductal arterial access		5 ppm	01-04007
ECHO showed septal flattening not bowing	0 ppm		01-07007
ECHO showed mild not moderate-to-severe tricuspid insufficiency		5 ppm	01-07008
ECHO showed septal flattening not bowing	0 ppm		01-08001
Received HFJV prior to enrollment		80 ppm	02-06001
ECHO showed septal flattening not bowing		5 ppm	02-11004
ECHO showed no foramen ovale shunt		20 ppm	02-17001

6.0.3.12.2c. Concomitant therapies used after randomization in the INO-01/ -02 trial

6.0.3.12.2d Results of the INO-01/ -02 trial

The subjects in each response category of the study are given in the figure below.

Figure 6.0.3.12.2d.1 Disposition of Subjects in the INO-01/ -02 trial



The disposition of the 155 subjects successfully randomized into INO-01/ -02 is shown below in tabular form:

Table 6.0.3.12.2d.2 Outcomes of subjects entered into the INO-01/ -02 trial[b].

	Placebo	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO	p value[a]
Total # of subjects treated	41	41	36	37	114	
Successful weaning of treatment gas within 14 days	18 (43%)	22 (54%)	19 (53%)	10 (27%)	51 (45%)	0.07
Early discontinuation of therapy	23 (56%)	19 (46%)	17 (47%)	27 (73%)	63 (55%)	0.07
Discontinuation due to treatment failure only	14 (34%)	11 (27%)	10 (28%)	22 (59%)	43 (38%)	0.02
Discontinuation due to other reasons only	8 (19.5%)	7 (17%)	7 (19%)	5 (13%)	19 (17%)	0.80
Discontinuation due to both treatment failure and other reasons	1 (2%)	1 (2%)	0 (0%)	0 (0%)	2 (1%)	

a. p value calculated using Fischer's exact chi-square test on 4 subject groups.
b. Data from NDA volume 2.17, Table 26.

From Figure 6.0.3.12.2d.2, 'Treatment Failure' is the most common reason for subjects to discontinue the INO-01/ -02 trial. The specific reasons for subjects being labeled a treatment failure are listed in the next table. Note the high incidence of treatment failure due to methemoglobin levels >7%, which occurred only in the 80 ppm. In the absence of this cause of treatment failure (13 subjects), the incidence of treatment failure in the 80 ppm group was 9/37 (24%).

6.0.3.12.2d Results of the INO-01/ -02 trial (cont)

Table 6.0.3.12.2d.3 Reasons for 'Treatment Failure' in the INO-01/ -02 trial[a].

	Placebo	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO	p value
Cardiopulmonary instability	14/41 (34%)	11/41 (27%)	9/36 (25%)	9/37 (24%)	29/114 (25%)	0.29
PaO_2 <40 mm Hg	14/41 (34%)	9/41 (22%)	9/41 (25%)	8/37 (22 %)	26/114 (23%)	0.16
Mean arterial pressure <35	0/41 (0%)	2/41 (5%)	0/36 (0%)	1/37 (3%)	3/114 (3%)	0.30
Laboratory Adverse Event	0/41 (0%)	0/41 (0%)	0/36 (0%)	14/37 (38%)	14/114 (12%)	0.02
Methemoglobin level >7%	0/41 (0%)	0/41 (0%)	0/36 (0%)	13/37 (35%)	13/114 (11%)	0.02
NO_2 level repeatedly >3ppm	0/41 (0%)	0/41 (0%)	0/36 (0%)	1/37 (3%)	1/114 (1%)	0.55
Other	2/41 (5%)	1/41 (2%)	1/36 (3%)	2/37 (5%)	4/114 (4%)	0.70
Any Treatment Failure	15/41 (37%)	12/41 (29%)	10/36 (28%)	22/37 (39%)	44/114 (39%)	0.82

a. Data from NDA volume 2.17 page 087508.

The next table shows the reasons for discontinuation other than treatment failure which lead to withdrawal of subjects from study gas. 'Investigator Decision' was the most common cause in all treatment groups.

Table 6.0.3.12.2d.4 Reasons for premature discontinuation other than treatment failure in the INO-01/ -02 trial[a].

	Placebo	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Delivery & monitoring device malfunction	2 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Investigator Decision	8 (20%)	8 (20%)	7 (19%0	5 (14%)	20 (18%)
Total # of subjects discontinued for other than tx failure	9 (22%)	8 (20%)	7 (19%)	5 (14%)	20 (18%)

a. Data from NDA volume 2.17 page 087608.

Incidence of Primary Endpoints in the INO-01/ -02 trial

The table below summarizes the pivotal efficacy data for the INO-01/ -02 trial. Shown are the incidences of the primary endpoint, and the incidence rates for the PPHN sequelae that comprised the primary endpoint. Also shown is the primary endpoint from the NINOS trial (added as a primary endpoint in the INO-01/ -02). No significant differences between the control and I-NO groups were detected.

Table 6.0.3.12.2d.5 Results: Incidence of primary endpoints from INO-01/ -02[a].

Endpoint	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO	p value
Primary endpoint[b]	23/41 (56%)	18/36 (50%)	21/35 (60%)	13/33 (39%)	52/104 (50%)	0.34
Death (within 28 days)	1/41 (2%)	2/40 (5%)	4/36 (11%)	3/37 (8%)	9/113 (8%)	0.44
Initiation of ECMO	14/41 (34%)	10/41 (24%)	9/36 (25%)	6/37 (16%)	25/114 (22%)	0.34
Neurological sequelae	10/39 (26%)	5/34 (15%)	11/32 (34%)	7/31 (23%)	23/97 (24%)	0.35
Bronchopulmonary dysplasia	5/40 (13%)	9/38 (24%)	3/31 (10%)	3/34 (9%)	15/103 (15%)	0.23
Death or initiation of ECMO[c]	16/41 (39%)	11/40 (28%)	14/36 (39%)	8/37 (22%)	33/113 (29%)	0.25

a. Data from NDA volume 2.17, pages 084708 to 085508, and electronic datasets.

b. Primary endpoint: incidence of one of the PPHN major sequelae: death; initiation of ECMO; acute neurologic abnormalities; or development of bronchopulmonary dysplasia. See primary endpoints above for definitions.

c. Primary endpoint from the NINOS trial, added to the protocol prior to the breaking of the blind: death before discharge or 120 days (whichever comes first) and/or the initiation of ECMO.

There increased deaths in the I-NO group when compared with the control group, both numerically and when expressed as a % of the total. This difference was not statistically significant (p=0.29 for control vs. combined I-NO group). Two other infants died before 120 days (the cut-off in the NINOS trial): one in placebo and one in 20 ppm I-NO group. If these two are factored in, the difference between control and the combined I-NO group is still not statistically significantly (p = 0.51).

6.0.3.12.2d Results of the INO-01/ -02 trial (cont)

Incidence of Primary Endpoints in the INO-01/ -02 trial (cont)

The table below summarizes the secondary endpoints for the INO-01/ -02 trial. I-NO groups which differ from control significantly (<0.05) using Student t-test of group means are shaded. No differences between the control and I-NO groups with regards to the three 'clinical benefit' endpoints was detected.

Table 6.0.3.12.2d.6 Results: Secondary Endpoints from INO-01/ -02[c].

'Exploratory' Variables	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Changes in clinical markers (from baseline to 30 minutes)[a,]					
Mean OI[c]	-1.3±7.7	-4.7±4.6	-4.3±9.6	-7.4±9.0	-5.5±8.7
Mean PaO_2[c]	18.0±53	32.3±56	38.6±69	64.4±84	44.6±71
Time-weighted OI[c]	See below[b]				
Clinical benefit endpoints					
Days requiring supplemental O_2[d]	6±7	5±5	5±3	6±8	5±6
Days requiring mechanical ventilation[d]	8±5	9±7	8±5	10±10	9±7
Days in hospital[d]	26±20	22±11	21±10	24±12	24±11
Duration of gas until ECMO[e]	10.6±10.5				22.4±27

a. Shown is the mean± s.d. of the change from baseline for each parameter, measured after 30 minutes. Data from NDA volume 2.18 Tables T-1 and T-7.

b. Time-weighted OI will be evaluated as part of the long-term effects (>30 minutes) of I-NO below.

c. Secondary endpoints identified by the sponsor, measured after 30 minutes and compared with baseline value.

d. Secondary endpoints identified by the sponsor, measured at the end of hospitalization.

e. Records the average duration of study gas received by the infants in both groups that ultimately received ECMO.

The table below summarizes the 'exploratory' endpoints for the INO-01/ -02 trial named by the sponsor. Variable for which the I-NO group differ from the control significantly (<0.05) are shaded. No differences between the control and I-NO groups with regards to the three 'clinical benefit' endpoints was detected. Several of these parameters would be expected to change slowly; thus, no acute change would be expected. There was, however, a significant improvement in the OI, from 24.0±9.2 to 18.7±11.5 in the combined I-NO group after 30 minutes (a 22% decrease). This is similar in magnitude to the 29% increase in FiO_2 (77 to 108 mm Hg) and a 7.6% decrease in the mean A-aDO_2 (573 to 529) which also occurred within the first 30 minutes of I-NO therapy. Other parameters of oxygenation also improve acutely (postductal O_2 saturation, mean PaO_2). There was no significant change in the mean PCO_2 or pH from baseline.

Table 6.0.3.12.2d.7 Results: comparison of acute changes in the specified 'exploratory variables' from INO-01/ -02 trial[a,c].

'Exploratory' Variables	Control	I-NO 20 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Changes in clinical markers (from baseline to 30 minutes)[a,b]					
Mean OI	-1.3±7.7	-4.7±4.6	-4.3±9.6	-7.4±9.0	-5.5±8.7
Mean PaO_2	18.0±53	32.3±56	38.6±69	64.4±84	44.6±71
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
Mean pCO_2	-0.75±5.5	-1.24±6.0	-1.31±3.6	-1.17±4.9	-1.24±4.9
Mean pH	0.02±0.06	0.02±0.06	0.02±0.04	0.01±0.05	0.02±0.05
Changes in hemodynamic parameters					
Mean Arterial Pressure	-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
Changes in pulmonary parameters[b]					
Mean Peak Inspiratory Pressure (PIP)[b]	0.27±1.1	0.07±0.41	0.31±1.06	0.03±0.16	0.13±0.66
Positive End-Expiratory Pressure (PEEP)[b]	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
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 O_2 gradient (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. The long-term effects of I-NO on the clinical markers will be examined below.

c. Variable in which the I-NO and control group means differ significantly (<0.05) using Student t-test are shaded.

6.0.3.12.3 Sub-group and Post-hoc Efficacy Analyses of the INO-01/ -02 Trial Results

6.0.3.12.3a Effects of I-NO on physiological parameters after 30 minutes of study gas

The INO-01/ -02 database is the only database that gives specific information regarding the durability of the physiological effects of I-NO. Because of subject withdrawal as the trial progressed, the interpretation of this data must necessarily be guarded. As an example, the table below shows the extent of the data on vital signs available for up times to 120 hours of study-gas administration.

Table 6.0.3.12.3a.1 Data available for the vital signs of individual subjects at various times after exposure to study gas from INO-01/ -02 trial.

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	I-NO Combined
Baseline	41	41	36	35	112
0.5 hours	41	41	36	35	112
12 hours	26	29	25	26	70
24 hours	23	24	19	12	55
48 hours	17	17	15	7	39
72 hours	15	15	11	4	30
96 hours	11	10	7	3	20
120 hours	9	8	3	2	13

a. Data from NDA, volume 2.18, Table T-8, T-9, and T-10, as well as volume 2.23 and the electronic dataset.

1. Effect of I-NO on oxygenation beyond 30 minutes

The sponsor proposed to examine the change in time-weighted OI (TWOI) as a part of one secondary endpoint in this trial, as a measure of the durability of the change in OI. This was defined as the area under the curve of the OI vs time for the first 24 hours or until discontinuation, divided by the number of hours of exposure to the study gas. In this calculation, the a 'negative' TWOI indicates a clinical improvement. From Table 6.0.3.12.2c.5, there was a significant difference between control and I-NO groups with regard to improvement in OI from baseline to 30 minutes. As can be seen from the table, there was also a significant difference between the TWOI of the control and the I-NO groups. Individual responses, however, were quite variable.

Table 6.0.3.12.3a.2 Effects of I-NO on TWOI beyond 30 minutes[a] in the INO-01/ -02 trial, as defined by the TWOI.

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 study gas, from NDA volume 2.17, Table 23.

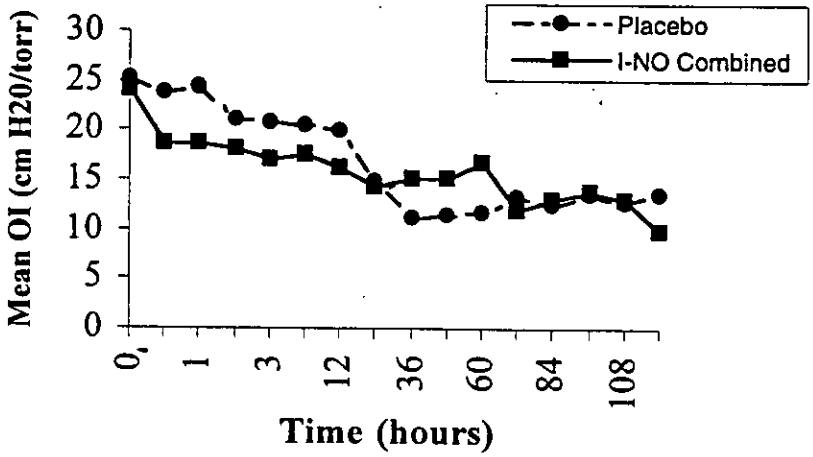
b. Repeated t test comparisons with control group were significant (<0.05) for the 5 and 80 ppm group, as well as the pooled I-NO group(shaded boxes above).

6.0.3.12.3a Effects of I-NO on physiological parameters after 30 minutes of study gas (cont)

1. Effect of I-NO on oxygenation beyond 30 minutes (cont)

The figure below compares the trend of OI for the control (N_2) and combined I-NO groups over time. Data for points beyond 24 hours are limited by the small number of subjects remaining and the data is shown until 120 hours, at which time fewer than 10 subjects remain in each of the 4 groups (9 placebo, 8 at 5 ppm, 3 at 20 ppm, and 2 at 80 ppm).

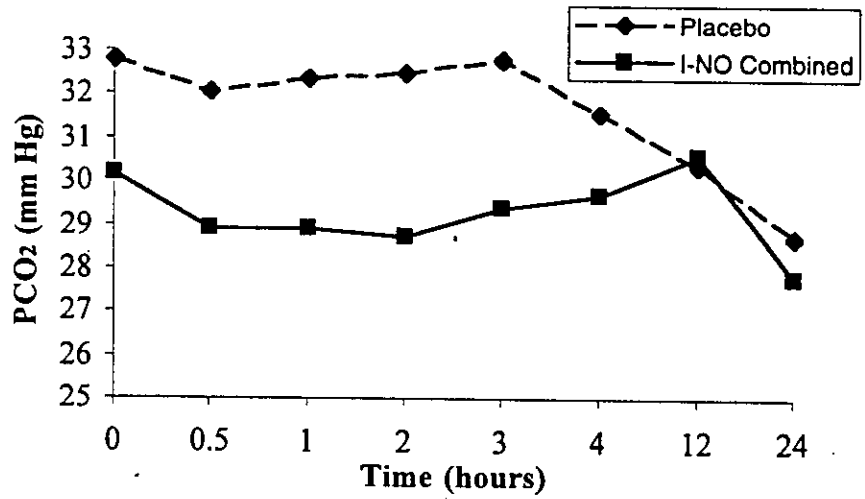
Figure 6.0.3.12.3a.3 Time vs. OI



2. Effect of I-NO on pCO_2 beyond 30 minutes

A safety concern is the reported effect of I-NO to increase NO_2 levels, which can impair CO_2 diffusion and cause increased pCO_2 levels. The figure below shows the mean pCO_2 for the placebo and I-NO subjects over the first 24 hours. The mean baseline pCO_2 in the I-NO group was numerically, but not significantly, lower than the control baseline. No trend towards CO_2 retention was seen overall.

Figure 6.0.3.14.9 Time vs. PCO_2

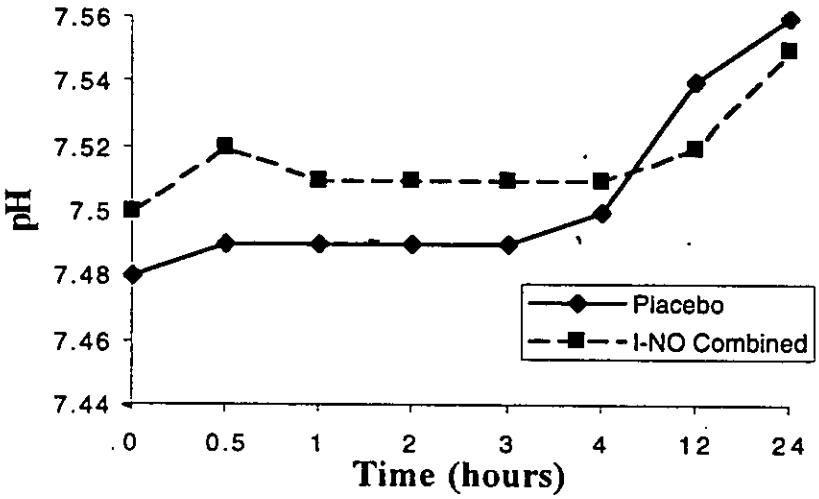


6.0.3.12.3a Effects of I-NO on physiological parameters after 30 minutes of study gas (cont)

3. Effect of I-NO on pH beyond 30 minutes

A similar analysis for changes in pH is complicated by the use of alkalinization as part of standard care for these infants. Examination of the acute effects of I-NO above showed no difference between control and I-NO with regards to pH.

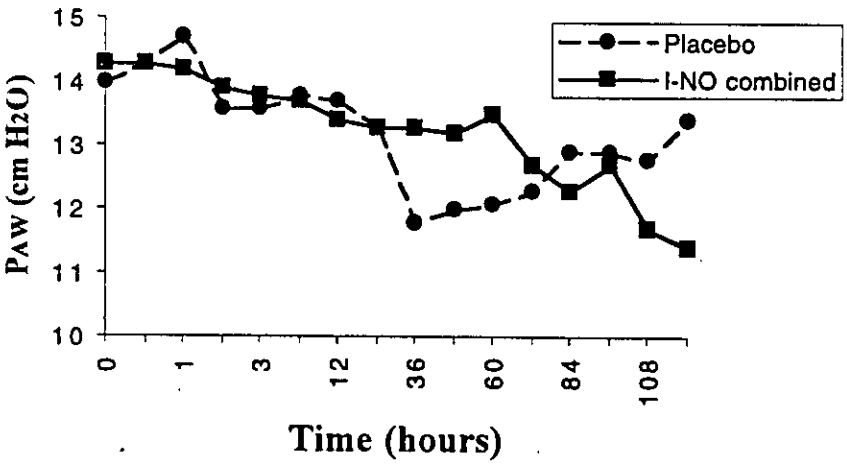
Figure 6.0.3.14.10 Time vs. pH



4. Effect of I-NO on mean airway pressures beyond 30 minutes

Another long-term change which might occur if I-NO speeds recovery from pulmonary injury is a more rapid decline in Mean Airway Pressure (P_{AW}), coincident with clinical improvement. The figure below shows the mean (±sd) values for the PAW during the hospitalization. Once again, the data beyond 24 hours has broad confidence limits due to the small numbers of subjects. In both groups there was a gradual trend towards improved P_{AW}, but no discernible effect of I-NO to accelerate this process. When the three doses of I-NO were looked at separately, there was, again, no effect to accelerate the decline in P_{AW} (data not shown).

Figure 6.0.3.14.11 Time vs. Mean Airway Pressure (PAW)

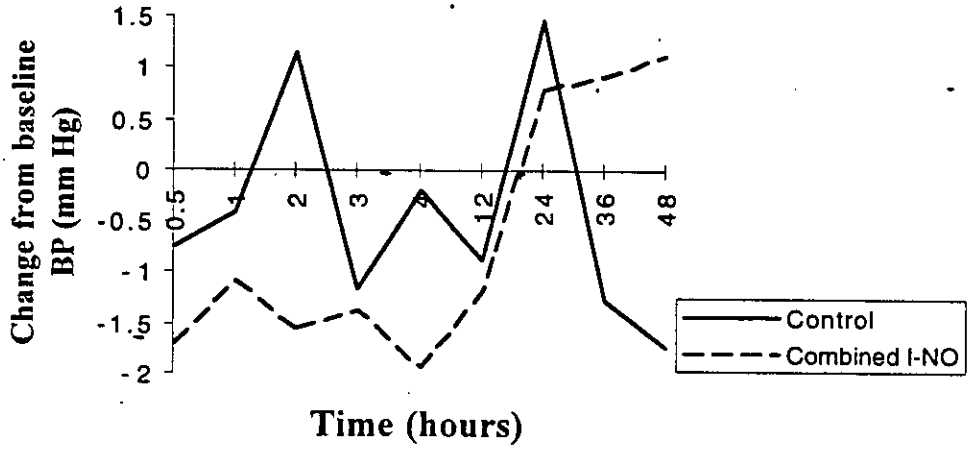


6.0.3.12.3a Effects of I-NO on physiological parameters after 30 minutes of study gas (cont)

5. Effect of I-NO on blood pressure beyond 30 minutes

No effect of I-NO on mean systemic blood pressure was detected during the chronic exposure to I-NO. The graphs below shows the mean change from baseline for the control and the pooled I-NO groups over the first 24 hours of exposure to study gas.

Figure 6.0.3.12.3a.7 Time vs. Change in Mean BP



6.0.3.12.3b Dose response effect of I-NO on acute oxygenation

Another analysis is to look at the percentage of subjects who improved their PaO_2 acutely after exposure to study gas. Shown are data from all subjects with PaO_2 values at baseline and 30 minutes. The table looks at the % of subjects in each group who had a 'complete response' as it was defined in the NINOS trial for oxygenation: an increase of >20 mm Hg in PaO_2 after 30 minutes. These data suggest a dose-response curve for the effect of I-NO, on oxygenation, and suggest that 80 ppm may have a greater acute effect on oxygenation than lower doses of I-NO.

Table 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.

6.0.3.12.3c Time to weaning

An indirect way to look at a benefit of I-NO is to ask if those subjects who were ultimately weaned from therapy did so faster in the I-NO group. The table below compares those subjects who ultimately met the criteria for weaning in the INO-01/ -02 study (see Section 6.0.3.8). There was a trend towards less time spent on study gas, and less time until weaning criteria are met in the I-NO groups compared with control, although no group was statistically different.

Table 6.0.3.12.3c.1Treatment gas exposure for patients who met weaning criteria[b].

Variable	Control	I-NO 5 ppm	20 ppm	80 ppm	Pooled I-NO
	n=18	n=22	n=19	n=10	n=51
Duration of study gas until weaning criteria met (hours)[a]	107±70	95±66	72±49	65±56	81±59
Duration of weaning (hours)	10.3±9	16.0±13	10.2±8	20.6±24	14.7±15
Total duration of study gas (hours)	118±69	111±65	82±47	86±56	96±58

a. weaning parameters are all of the following: FiO_2 <0.60; P_{AW} <10 cm H_2O; and postductal PaO2 ≥60 mm Hg, shown as mean±s.d.
b. None of the differences were statistically significant (p>0.05).

6.0.3. 13 Safety Results for the INO-01/ -02 trial

The safety results for deaths, drop-outs, and serious adverse events will be combined with the three other trials into a single safety review, which can be found in Section 8.0. Several specific safety parameters were defined prospectively, and these will be discussed below.

6.0.3.13.1 Comparison of defined safety parameters up to 28 days

The table below summarizes the results of the specified safety parameters measured at the end of hospitalization or 28 days. There were no significant differences between control and I-NO groups for any of the endpoints.

Table 6.0.3.13.1.1 Comparison of the rates of specific safety parameters from INO-01/ -02.[a] Note that not all subjects have data for a given parameter.

Changes in safety endpoints	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO	p value[i]
Duration of ECMO	103±32 (n=14)	118±106 n=9	103±96 (n=9)	183±189 (n=6)	129±63 (n=24)	
Time to ECMO	22±15 (n=14)	51±25 (n=10)	37±31 (n=9)	36±16 (n=6)	42±24 (n=25)	
Peak methemoglobin level at any time	0.7±0.42	0.89±0.88	1.16±0.69	5.77±2.8	2.57±2.8	
Peak NO_2 level at any time	0.59±0.8	0.53±.73	0.48±.62	2.6±1.2		
Incidence of seizures	7/41 (17%)	5/40 (12%)	10/35 (28%)	7/37 (19%)	22/112 (20%)	0.35
Incidence of air leak syndrome[f]	13/41 (32%)	9/41 (22%)	11/33 (33%)	10/36 (28%)	30/110 (27%)	0.78
Incidence of bronchopulmonary dysplasia[g]	5/40 (13%)	7/39 (18%)	3/32 (9%)	3/34 (9%)	13/105 (12%)	0.62
Subjects requiring O_2 at 28 days	6/41 (15%)	9/41 (22%)	3/33 (9%)	6/36 17%)	18/110 (16%)	0.51
Subjects with reactive airways disease at 28 days	1/40 (3%)	3/38 (8%)	1/30 (3%)	1/34 (3%)	5/102 (5%)	0.62
Incidence of sensorineural hearing loss[h]	5/36 (14%)	3/38 (8%)	6/29 (21%)	7/31 (23%)	16/98 (16%)	0.32
Intracranial abnormalities detected by ultrasound, CT or MRI scan						
Abnormality on cranial ultrasound[b]	4/28 (14%)	3/27 (11%)	3/23 (13%)	2/21 (10%)	7/71 (10%)	0.96
Intracranial hemorrhage or infarct detected by ultrasound[c]	1/28 (4%)	0/27 (0%)	1/23 (4%)	0/21 (0%)	1/71 (2%)	NA
Abnormality on CT or MRI scan of head[d]	9/18 (50%)	2/15 (13%)	8/19 (42%)	4/11 (36%)	14/45 (31%)	0.16
Interventricular hemorrhage	2/18 (11%)	0/15 (0%)	0/23 (0%)	0/11 (0%)	0/45 (0%)	NA
Periventricular hemorrhage	0/18 (0%)	0/15 (0%)	1/23 (5%)	1/11 (9%)	2/45 (4%)	NA
Intracranial hemorrhage[e]	1/18 (6%)				2/45 (4%)	NA
Periventricular leukomalacia	0/18 (0%)	0/15 (0%)	1/23 (5%)	1/11 (9%)	2/45 (4%)	NA
Extensive cytotoxic edema	0/18 (0%)	0/15 (0%)	0/23 (0%)	1/11 (9%)(	1/45 (2%)	NA
Subdural hematoma	0/18 (0%)	0/15 (0%)	1/23 (5%)	0/11 (0%)	1/45 (2%	NA

a. The sponsor identified the changes in methemoglobin and NO_2 levels, along with overall adverse events, as the most important markers.

b. Only those infants who had a normal cranial ultrasound at the start of the trial and an ultrasound at the end of the trial are included.

c. Category includes one subject with suspected white matter hemorrhage, one grade one germinal matrix hemorrhage, and one infarct, detected by ultrasound. Only subjects with normal baseline ultrasound were included.

d. Abnormalities detected at any time during the hospitalization. No baseline scans are available in most cases, making it difficult to date the onset of the abnormality.

e. Category includes parietal lobe, posterior fossa and frontal lobe hemorrhages.

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.

h. Sensorineural hearing loss was detected using brain stem auditory evoked responses (BAER).

i. p value calculated using chi-square test. NA indicates endpoints with too few events for statistical analysis.

6.0.3.13.2 Comments on specific safety parameters

In the following sections, specific safety outcomes from the INO-01/ -02 trial will be examined.

6.0.3.13.2a Nitrogen Dioxide levels

Mean NO_2 levels in the INO-01/ -02 trial.

In the INO-01/ -02 trial, the average NO_2 level was >1% at any time only in the 80 ppm I-NO group. The large majority of subjects in the I-NO group had peak NO_2 concentrations ≤3.0 ppm. Note, however, that the peak NO_2 concentration for the 80 ppm group was significantly higher overall, and 7/9 subjects who had NO_2 levels >3 at any time during the trial were in the 80 ppm group.

Table 6.0.3.13.2a.1 Peak NO_2 levels in ppm from the INO-01/ -02 trial.

Changes in safety endpoints	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm
Mean peak NO_2 level at any time	0.59±0.8	0.53±.73	0.48±.62	2.6±1.2
Peak NO_2 level at any time (ppm)				
0.0 - 1.0	32/42 (76%)	34/41 (83%)	30/35 (86%)	0/37 (0%)
1.1 - 3.0	8/42 (19%)	6/41 (15%)	4/35 (11%)	29/37 (78%)
3.0- 5.0	2/42 (5%)	1/41 (1%)	1/35 (1%)	4/37 (11%)
5.1 - 7.0	0/41 (0%)	0/41 (0%)	0/35 (0%)	3/37 (8%)
7.1 to 10	0/41 (0%)	0/41 (0%)	0/35 (0%)	0 (0%)

Individual elevations in NO_2 in the INO-01/ -02 trial.

Control Group

There were 5 individuals in the control group who had NO_2 levels of >2.0 ppm during the trial. Three of these individuals had levels which were >2.0 for at least two consecutive periods (30-60 minutes apart).

1. Subject #01-03004 had an NO_2 level of 2.0 at 30 and 60 minutes of study gas, did not respond and was withdrawn. The subject received ECMO, but did not die, and was discharged with no evidence of chronic lung disease.

2. Subject #01-07007, a Caucasian female, had an NO_2 level which ranged between 1 and 3 on control gas for the duration of therapy (30 minutes to 48 hours). She had no major adverse events (ECMO, chronic lung disease, death).

3. Subject #02-11001, a Caucasian female, had an NO_2 level which was 0.0 for the majority of the exposure to study gas (148 hours). During the period from 112 to 124 hours the NO_2 level was 2.0 to 3.0 (4 measurements), and measured 3.0 at hour 124. The infant was discharged home without receiving ECMO, and with no chronic pulmonary disease.

I-NO 5 ppm Group

There was one individual, an African-American female in the I-NO 5 ppm group (#02-04003), who had NO_2 =3.0 for 3 consecutive measurements after 8-12 hours of study gas. She had no major adverse events (ECMO, chronic lung disease, death).

I-NO 80 ppm Group

In the INO-01/ -02 trial, three subjects who received I-NO, 80 ppm, developed NO_2 levels > 5ppm.

1. Subject #01-04005 had NO_2 levels of 6.2 and 5.9 after 1 and 3 hours. His I-NO was tapered to 40 ppm and his NO_2 levels fell. His maximum methemoglobin level was 2.5 %. He did not require ECMO, and was discharged with a seizure disorder but not chronic lung disease.

2. Subject #01-06006 had an NO_2 level of 5.0 after 1 hour. Her NO_2 levels fell to 2.8 ppm without adjustment of I-NO concentration. However, her methemoglobin levels continued to rise (7.4 % after 8 hours), and I-NO was tapered off. She developed a series of pneumothoraces and ultimately died after receiving ECMO.

3. Subject #02-15006 had an NO_2 level of 6.2 after 30 minutes. The repeat value was 1.4 and no change in I-NO concentration was necessary. The infant was discharged home without receiving ECMO, and with no chronic pulmonary disease.

6.0.1.13.2b Methemoglobin concentrations

Mean methemoglobin levels in the INO-01/ -02 trial.

The average methemoglobin level was >1% at any time only in the 80 ppm I-NO group. After 12 hours of exposure to 80 ppm I-NO, the mean methemoglobin level peaked at 5.08±2.32%.

The average time to after starting I-NO to the development of elevated methemoglobin was 10.4±9.5 hours overall, but only 3 subjects developed elevated methemoglobin levels >10 hours after starting the I-NO. This is reflected in the median time to peak methemoglobin level, which was 8 hours. The I-NO was discontinued in four of the subjects and reduced and ultimately discontinued in the other 9 subjects.

In the INO-01/ -02 trial, the average peak methemoglobin level was significantly higher in the 80 ppm I-NO group. After 12 hours of exposure to 80 ppm I-NO, the mean methemoglobin level peaked at 5.08±2.32%.

Table 6.0.1.13.2b.1 Peak methemoglobin levels from the INO-01/ -02 trial (not all subjects had data available).

Changes in safety endpoints	Control	I-NO 5 ppm	20 ppm	80 ppm	Combined I-NO
Peak methemoglobin level at any time	0.7±0.42	0.89±0.88	1.16±0.69	5.77±2.8	2.57±2.8
Peak methemoglobin level at any time					
0.0 - 1.0%	31/40 (78%)	28/40 (70%)	18/36 (50%)	0/37 (0%)	46/113 (41%)
1.1 - 2.0	9/40 (22%)	11/40 (28%)	17/36 (47%)	2/37 (5%)	30/113 (26%)
2.1 - 30	0/40 (0%)	0/40 (0%)	0/36 (0%)	4/37 (11%)	4/113 (4%)
3.1 - 5.0	0/40 (0%)	0/40 (0%)	1/36 (2%)	9/37 (24%)	10/113 (9%)
5.1 to 10	0/40 (0%)	1/40 (2%)	0/36 (0%)	18/37 (49%)	19/113 (17%)
>10.0	0/40 (0%)	0/40 (0%)	0/36 (0%)	2/37 (5%)	2/113 (2%)

Individual elevations in methemoglobin concentration in the INO-01/ -02 trial.

In the INO-01/ -02 trial, thirteen subjects, all in the I-NO 80 ppm group, were 'treatment failures' as the result of methemoglobin levels >7%. This represents 35% of the 80 ppm group. A listing of these infants can be found in the table below, including outcome data for ECMO, ventilation therapies, neurologic disorders, and chronic lung disease (CLD). For those infants who dropped from study as a result of elevated methemoglobin levels, their narratives are in section 8.1.3.2.1 below. Of the infants identified with increased methemoglobin concentrations, two infants received ECMO, and one of those infants died. None of the infants had chronic lung disease (CLD). The infant that died (subject 01-06006) required discontinuation of I-NO due to elevated methemoglobin levels, and died several days after discontinuation. Overall, no short-term adverse outcomes were identified that can be linked to the elevated methemoglobin levels. Specifically, no link between elevated methemoglobin concentrations and chronic pulmonary disease is apparent.

6.0.1.13.2b Methemoglobin concentrations (cont)

Individual elevations in methemoglobin concentration in the INO-01/ -02 trial (cont)

Table 6.0.1.13.2b.2 Subjects from the INO-01/ -02 trial who had elevated methemoglobin levels.

Study Group	Subject #	Peak methemoglobin	Time to Peak	Outcome
I-NO 80 ppm				
	01-01005	7.2%	40	No ECMO Discharged without CLD
	01-03003	7.3%	16	No ECMO Discharged without CLD
	01-03016	8.4%	4	No ECMO Discharged without CLD
	01-03029	11.9%	8	No ECMO Discharged without CLD
	01-04005	7.4%	8	No ECMO Discharged without CLD, with seizure disorder
	01-05005	7.6%	12	No ECMO Discharged without CLD (some F/U data missing)
	01-06003	8.4%	8	No ECMO Received HFOV Discharged without CLD (some F/U data missing)
	01-06006	7.3%	9	Died No ECMO
	01-11004	8.4%	8	No ECMO Discharged without CLD with seizure disorder
	01-17004	9.3%	8	No ECMO Discharged without CLD
	02-04004	9.5%	4	No ECMO Discharged without CLD
	02-04006	10.8%	4	Received ECMO Discharged without CLD
	02-07003	7.8%	8	No ECMO Discharged without CLD

a. Data comes from review of individual case report forms, NDA volumes 3.4-3.10 and from electronic datasets.

6.0.1.13.2c Patient deaths in the INO-01/ -02 trial

The tables below list the deaths that occurred in the INO-01/ -02 trial. Narratives of the individual deaths are found in section 8.2 of the Safety Summary.

Table 6.0.1.13.2c.1 Deaths in the I-NO groups from the INO-01/ -02[a].

Trial	Subject	Received ECMO?	Time of Death (days)	Description[b]
Control				
	02-15005	No	5	PPHN Multiple chromosomal anomalies Withdrawal of support
I-NO				
	01-11012	No	21	Possible sepsis Progressive hypoxia Recurrent pneumothoraces Withdrawal of support
	01-17003	Yes	8	Meconium aspiration, Pseudomonas sepsis
	01-1006	No	5	Sepsis, Hypotension, bradycardia
	01-03023	No	11	Meconium aspiration, Seizures, gross neurologic deficits
	01-03025	No	6	PPHN Renal failure, encephalopathy, Pneumothorax
	01-11005	No	3	Meconium aspiration Pneumothorax, cardiopulmonary arrest
	01-03026	Yes	17	PPHN, alveolar capillary dysplasia
	01-06006	No	17	Perinatal hypoxia, PPHN, acidosis, recurrent pneumothoraces
	01-11011	No	3	Idiopathic PPHN, Recurrent pneumothoraces & and pneumopericardium

a. From section 8.1.1.
b. Summarized from narratives in section 8.1.1.

In the INO-01/ -02 trial, subjects were re-evaluated after 1 year to ascertain vital status, as well as to assess the incidence of other adverse events (discussed below). The table below summarizes the data on the subjects with available data.

Table 6.0.1.13.2c.2 Deaths from the INO-01/ -02 1 year follow-up data[a,c].

Trial	Subject	Received ECMO?	Time of Death (days)	Description[b,c]
INO-01/ -02 Control				
	02-14004	No	90	PPHN, SIDS death after discharge
I-NO 20 ppm				
	01-11015	No	32	Idiopathic PPHN, pneumothoraces, sepsis

a. Any death after one month.
b. Description of individual deaths is derived from company-provided narrative. Where possible, individual case report forms were scrutinized by this reviewer as well. This was done for all deaths in the INO-01/ -02 trial.
c. Full narrative summaries of each death are included below in section 8.1.1.

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

In the INO-01/ -02 trial, surviving infants were to have a follow-up assessment after 1 year. Several safety endpoints were to be assessed, including: vital status; medical history (including hospitalizations); neurologic and physical development; and audiology. The first table shows the extent of follow-up for the various tests. It is important to note that 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 6.0.1.13.3.1 Follow-up for infants in INO-01/ -02 at 1 month and 1 year of age[a].

	Control	I-NO 20 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
1 Month Follow-up					
Initial # of subjects	41	41	36	37	114
# Died	1	2	5	3	10
# Lost to Follow-up	0	0	1	0	1
Survived	40	39	30	34	103
12 Month Follow-up					
Initial # of subjects	40	39	30	34	103
# Died	1	0	0	0	0
# Lost to Follow-up	3	4	1	3	8
Survived	36	35	29	31	95
Overall					
Initial # of subjects	41	41	36	37	114
# Died	2 (5%)	2 (5%)	5 (14%)	3 (8%)	10 (9%)
# Lost to Follow-up	3 (7%)	4 (10%)	2 (6%)	3 (8%)	9 (8%)
Survived	36 (87%)	35 (85%)	29 (80%)	31 (84%)	95 (83%)

a. Data from Amendment to NDA, volume 6.1, Table 2.

Table 6.0.3.13.3.2 Vital status at 1 year of age for infants for infants with known follow-up in INO-01/ -02.

	Control	I-NO 20 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	41	41	36	37	114
Alive	36 (95%)	35 (95%)	29 (85%)	31 (91%)	95 (90.5%)
Dead	2 (5.3%)	2 (5.4%)	5 (14.7%)	3 (8.8%)	10 (9.5%)
p value[a]		0.978	0.180	0.556	0.419

The next tables show the incidence of the individual adverse events measured at the 1 year follow-up visit.

Mental development

Table 6.0.3.13.3.3 Mental development at 1 year of age for infants for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 20 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
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

6.0.1.13.3 Long-term safety results of the INO-01/ -02 trial, conducted at 12 months of age (cont)

Psychomotor development

Table 6.0.3.13.3.4 Psychomotor development at 1 year of age for infants for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
Accelerated development	2 (6%)	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

Audiology testing

Table 6.0.3.13.3.5 Results of audiology testing at 1 year of age for infants for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
None	22 (61%)	22 (63%)	19 (66%)	18 (58%)	59 (62%)
Mild	7 (19%)	2 (6%)	4 (14%)	3 (10%)	9 (10%)
Major	0 (0%)	1 (3%)	0 (0%)	1 (3%)	2 (2%)
Missing	7 (19%)	10 (29%)	6 (21%)	9 (29%)	25 (26%)

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

Neurologic testing

Table 6.0.3.13.3.6 Results of neurologic testing at 1 year of age for infants for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
None	28 (78%)	30 (86%)	20 (69%)	22 (71%)	72 (76%)
Mild	3 (8%)	1 (3%)	2 (7%)	1 (3%)	4 (4%)
Major	4 (11%)	3 (9%)	5 (17%)	5 (16%)	13 (14%)
Missing	1 (3%)	1 (3%)	2 (7%)	3 (10%)	6 (6%)

a. Examining physicians were asked to characterized neurologic abnormalities as none, mild or major.

Incidence of cerebral palsy

Table 6.0.3.13.3.7 Incidence of cerebral palsy at 1 year of age for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
None	33 (92%)	34 (97%)	24(83%)	25 (81%)	83 (87%)
Present	2 (6%)	0 (0%)	4 (14%)	3 (10%)	7 (7%)
Missing	1 (3%)	1 (3%)	1 (3%)	3 (10%)	5 (5%)

a. Examining physicians were asked to characterized neurologic abnormalities as none, mild or major.

6.0.1.13.3 Long-term safety results of the INO-01/ -02 trial, conducted at 12 months of age (cont)

Incidence of pulmonary abnormalities

At the 1 year follow-up visit, the family members were asked if the following pulmonary problems existed: home oxygen therapy; asthma; bronchiolitis; bronchitis; pneumonia; upper respiratory infection with severe cough; and smoking in household. Shaded areas represent problems whose rates differed between the control and I-NO groups.

Table 6.0.3.13.3.8 Respiratory system abnormalities at 1 year of age for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 20 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Original # of subjects	36	35	29	31	95
Home O_2 therapy	0/36 (0%)	8/35 (23%)	1/29 (3%)	3/31 (10%)	12/95 (13%)
Asthma	5/36 (14%)	6/35 (17%)	3/29 (10%)	3/31 (10%)	12/95 (13%)
Bronchiolitis	5/36 (14%)	7/35 (20%)	4/29 (14%)	1/31 (3%)	12/95 (13%)
Bronchitis	2/36 (6%)	3/35 (9%)	3/29 (10%)	2/31 (6%)	8/95 (8%)
Pneumonia	2/36 (6%)	2/35 (6%)	2/29 (7%)	3/31 (10%)	7/95 (7%)
Upper respiratory tract infection	10/36 (28%)	2/35 (6%)	2/29 (7%)	4/35 (11%)	8/95 (8%)

a. From individual case report forms for long-term follow-up, Volumes 7.1 through 7.12.

All but one of the infants who used supplemental O_2 after discharge was listed as using O_2 at the time of discharge. The table below records the use of supplemental O_2 at the time of discharge in the INO-01/ -02 trial, and the available follow-up for those infants after one year. The table illustrates that for the control infants, 4 of the control infants using O_2 at time of discharge were missing data at one year follow-up.

Table 6.0.3.13.3.9 Details of infants requiring supplemental O_2 at time of discharge and at 1 year of age for infants with known follow-up in INO-01/ -02[a].

Infants requiring supplemental O_2	Control	Missing Data[b]
At time of discharge		
Control	6/41 (15%)	
I-NO 5 ppm	9/41 (22%)	
I-NO 20 ppm	3/33 (9%)	
I-NO 80 ppm	6/36 (17%)	
Combined I-NO	18/110 (16%)	
At 1 year follow-up of same subjects		
Control	0/2 (0%)	4
I-NO 5 ppm	8/9 (89%)	1
I-NO 20 ppm	1/3 (33%)	0
I-NO 80 ppm	3/6 (50%)	1
Combined I-NO		

a. From individual case report forms for long-term follow-up, Volumes 7.1 through 7.12.
b. Missing data in the form of case report form for the 1 year follow-up examination, including details of the respiratory system review.

6.0.3.14 INO-01/ -02 Efficacy Summary

Study Design

This was a multi-center, double-blind, randomized, placebo-controlled trial designed to study the clinical response to 3 different doses of I-NO in neonates with documented PPHN.

Eligible subjects were randomized to one of four treatment groups: placebo (N_2); I-NO 5, 20 or 80 ppm. Randomized subjects received treatment until the I-NO was successfully weaned, the subject was withdrawn for treatment failure, or the maximum dose of 14 days of I-NO was reached.

Treatment failures were maintained on conventional therapy for PPHN after discontinuation of the study gas, including ECMO or 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.

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.

Two sets of investigators were used during the trial to maintain blinding.

6.0.3.14 INO-01/ -02 Efficacy Summary (cont)

Study Design (cont)

Overall, the trial was well-designed to determine the safe and effective dose I-NO in hypoxic respiratory failure in the neonate with PPHN. One difficulty encountered in the trial was in locating subjects who met the strict criteria for entry. The exclusion of infants who had previously received surfactant or high-frequency ventilation excluded 21% of the infants screened (see table 6.0.3.12.2a.1). The absence of echocardiographic evidence of PPHN also excluded 19% of the infants screened. The other major trial, the NINOS, instead required only that an echocardiogram be obtained, but allowed infants in the trial without pulmonary hypertension (see section 6.0.1). The NINOS trial also did not exclude infants with prior therapies, including surfactant or high-frequency ventilation. The result was that the INO-01/ -02 trial enrolled only 12% of the infants screened, while the NINOS enrolled >50% of the screened infants (from personal conversation with Dr. Ehrenkranz, Principle Investigator of the NINOS trial). The INO-01/ -02 trial also enrolled a population with a significantly lower OI, suggesting a less acutely ill population. This difference in the populations of the two trials is critically important when attempting to generalize the finding of the INO-01/ -02 trial to the entire population of neonates with hypoxic respiratory failure.

Primary and Secondary Endpoints

Primary Endpoints

1. The incidence of death, ECMO, bronchopulmonary dysplasia or abnormal neurologic sequelae (called PPHN major sequelae).

This primary endpoint of the INO-01/ -02 trial focused on the clinical consequences of hypoxic respiratory failure, including the occurrence of death or long-term pulmonary or neurologic dysfunction. In addition, the initiation of ECMO was included as one of the four primary 'PPHN endpoints'. This should be compared with the NINOS trial, where the primary endpoint was the incidence of death and/or the initiation of ECMO, not including any long-term adverse effects.

2. The incidence of death before 120 days and/or initiation of ECMO.

After the results of the NINOS trial were published, the sponsor added a 'primary analysis' comparing the incidence of death before 120 days and/or initiation of ECMO, following the results of the NINOS trial.

Secondary Endpoints

1. Physiologic response to I-NO, measured by change in OI and time-weighted OI (TWOI).
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).

The secondary endpoints in the INO-01/ -02 trial, likewise, were attempts to measure the clinical benefit of I-NO administration, in addition to assessing it's physiological effects. If I-NO is beneficial, its effects should be detectable in the form of improved clinical outcomes, in addition to decreased rates of ECMO use.

Long-term Follow-up Endpoints (measured at 1 year follow-up examination)

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

Finally, the INO-01/ -02 trial examined the clinical state of the surviving infants after 1 year, in order to gain information about the long-term consequences of exposure to I-NO in the peri-natal period. The sponsor also collected information on the clinical history of each survivor during the period after hospitalization.

Number of subjects/ randomization

A total of 320 subjects were planned for enrollment, 80 subjects in each of 4 categories: placebo (N_2); 5 ppm I-NO; 20 ppm I-NO; and 80 ppm I-NO. From 1282 subjects screened, a total of 155 were randomized (12% of the screened subjects), of which 69 successfully completed study therapy and were weaned off the treatment gas. Table 6.0.3.6.1 details the enrollment in each of the sites. San Diego Children's Hospital and the Children's Hospital of Oklahoma together accounted for 28% of all patients enrolled.

The critical issue for this trial was that it was discontinued prior to full enrollment due to difficulties with accrual after the publication of the NINOS results. Ultimately, INO-01/ -02 was able to enroll between 45% (20 ppm group) and 51% (control and 5 ppm group) of the planned number of subjects. This limited the studies ability to detect significant differences between control and I-NO subjects.

For the long-term follow-up, not all subjects had data collected. For data regarding the vital status of subjects, 7% of control and 8% of I-NO subjects were missing. For other testing, as many as 29% of the infants were missing data (for audiology testing in the I-NO 5 and 80 ppm group, see table 6.0.3.13.3.5). Other tests were missing intermediate numbers of subjects. Missing subjects cannot be inferred to be very well and so not with any established follow-up, since for some infants even vital status (alive or dead) is not known.

6.0.3.14 INO-01/ -02 Efficacy Summary (cont)

Inclusion/ Exclusion Criteria

As discussed above, the exclusion of subjects who received prior surfactant and high frequency ventilation (for >6 hours or within 2 hours of study entry) is an important difference between this trial and the NINOS trial. The effect of this was to limit the available population for entry into the trial. It also served to limit the trial to subjects who were not so acutely ill that the attending physician felt constrained to start maximal medical therapy (including surfactant and/or high-frequency ventilation) prior to submitting the infant for entry into the INO-01/ -02 trial.

Dosage/ Administration

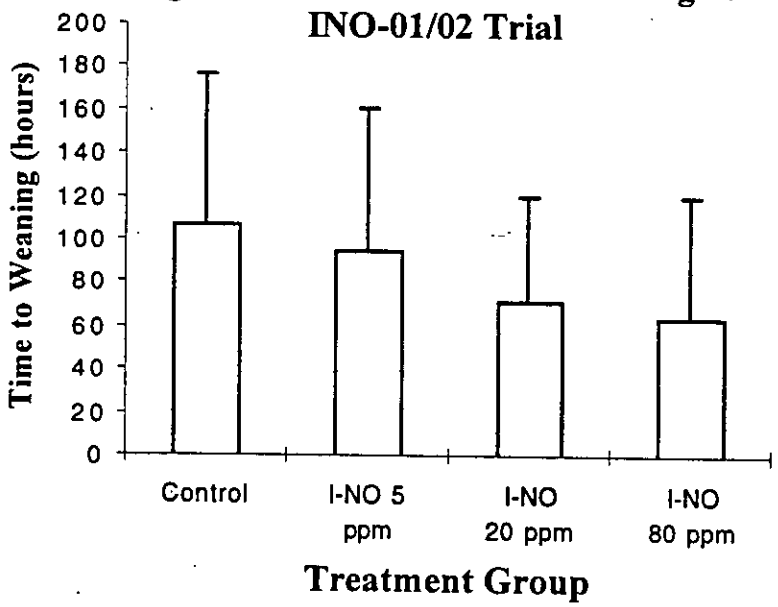
The INO-01/ -02 was the only submitted trial that examined separately the effects of more than one dose of I-NO versus a control in blinded fashion. The device used to administer I-NO was similar to that used in the other trials.

Like the INOSG trial, the INO-01/ -02 trial used N_2 as the control gas. In contrast, the NINOS trial used O_2 as the control gas.

Duration/ Adjustment of Therapy

The INO-01/ -02 trial measured the time that study gas was administered for those subjects who were ultimately weaned successfully. A decrease in this time would be inferred to reflect a more rapid improvement in pulmonary status, allowing for removal of study gas. In table 6.0.3.12.3c.1 above, the time to successful weaning, and the total duration of study gas were compared for the four study groups. The time to successful weaning is shown in the figure below. There appears to be a trend towards less time spent on until weaning criteria are met in the I-NO groups compared with control, although no group was statistically different.

Figure 6.0.3.14.1 Time to Weaning in INO-01/02 Trial



Statistical Considerations

Stopping the INO-01/ -02 trial early obviously complicates interpretation of its results. The sponsor argues that the failure of the trial to demonstrate a positive effect of I-NO is due to the loss of power that follows the decreased numbers of subjects.

Dr. Nuri had the following comment about the statistical review of this study.

1) The original protocol suggested that the sample size should be 96 infants to have an 80% likelihood of detecting a 50% reduction in the mean OI and PaO_2. This sample estimation was based on a Monte Carlo simulation for the Cochran-Mantel-Haenszel test assuming the placebo rates as 8% for death, 20% for abnormal neurological development; 28% for ECMO; and 2% for BPD. The corresponding rates for the I-NO group would then be: 5%; 10%; 14%; and 1% for the 5, 20, 80 and combined I-NO groups respectively. With the lowered number of subjects entered into the trial, the test used for analysis (Cochran-Mantel-Haenszel) would not have enough power to detect at least a reduction of OI of 50% compared to placebo.

6.0.3.14 INO-01/ -02 Efficacy Summary (cont)

Statistical Considerations (cont)

2) There is also the possibility that using the Monte Carlo simulation might result in and underestimation of the number of subjects required. This would mean that even if the trial had enrolled all of the proposed 320 subjects there might still have been insufficient power to detect a significant difference between the two groups.

A comparison of the proposed placebo rates and the rates actually seen in the trial is below, which suggests that the Monte Carlo was based on projected placebo rates which, if anything, underestimated the actual placebo rate. This suggests that the estimated sample size would have been adequate to detect a change in the OI of 50%.

Table 6.0.13.4.2 Comparison of the estimated incidence and the observed placebo incidence rates of the PPHN major sequelae in the INO-01/ -02 trial.

Endpoint	Proposed placebo rate	Actual placebo rate
ECMO	28%	34%
Death	8%	2.4%
Neurological abnormalities	20%	26%
Bronchopulmonary dysplasia	2%	13%

The use of change in OI to predict the number of subjects, however, has its own problems. An effect on this surrogate (change in OI), must be able to predict a benefit on the primary endpoint (PPHN major sequelae). That is, the sponsor assumed that if the OI was beneficially affected by I-NO, a beneficial effect of I-NO on the primary endpoint would also be seen. While such a linkage is attractive, there is no proof for it. If such a linkage fails, the estimates for the number of subjects may well also be incorrect.

Patient Demographics & Baseline Characteristics

Demographics and baseline characteristics were discussed in section 6.0.3.12.1, with data presented in tables 6.0.3.12.1.1 through 6.0.3.12.1.4. Overall, the control and I-NO groups were well-matched with regard to baseline hemodynamic, pulmonary and cranial ultrasound findings. There was an important difference in the underlying disease states responsible for the respiratory failure in the two groups, however. Significantly more subjects in the I-NO group had idiopathic PPHN. The consequences of this are not absolutely known, although a dogma suggested in the literature is that infants with 'pure' PPHN, without other pulmonary damage due to sepsis or meconium aspiration, should respond 'better' to the I-NO. If this were true, the inclusion of significantly more subjects with idiopathic PPHN in the I-NO group should have slanted the results in favor of I-NO.

Disposition of Subjects

Of the 1282 subjects screened, 155 (12%) were accepted into the INO-01/ -02 trial. Of these, 18 of the 41 control subjects (44%), and 51/114 (45%) of the I-NO group completed the trial. The other subjects were discontinued due to: 1) treatment failure [23/41 controls 56%) and 43/114 (38%) in the I-NO group]; 2) other reasons [8/41 control subjects (20%) and 19/114 (17%) in the I-NO group]; and 3) both treatment failure and other [1/41 controls (2%) and 13/114 (11%) in the I-NO group]. The most important reason other than failure to improve on treatment gas that led to subject withdrawal was elevated methemoglobin levels, which occurred only in the I-NO 80 ppm group (13 subjects).

Protocol violations and deviations

Table 6.0.3.12.2b.1 lists the protocol violations submitted by the sponsor. None of the violations were serious enough to require re-analysis of the efficacy data.

6.0.3.14 INO-01/ -02 Efficacy Summary (cont)

Analysis of Primary and Secondary Efficacy Outcomes

Table 6.0.3.14.2 Results: Incidence of primary endpoints from INO-01/ -02[a].

Endpoint	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO	p value
Primary endpoint[b]	23/41 (56%)	18/36 (50%)	21/35 (60%)	13/33 (39%)	52/104 (50%)	0.34
Death (within 28 days)	1/41 (2%)	2/40 (5%)	4/36 (11%)	3/37 (8%)	9/113 (8%)	0.44
Initiation of ECMO	14/41 (34%)	10/41 (24%)	9/36 (25%)	6/37 (16%)	25/114 (22%)	0.34
Neurological sequelae	10/39 (26%)	5/34 (15%)	11/32 (34%)	7/31 (23%)	23/97 (24%)	0.35
Bronchopulmonary dysplasia	5/40 (13%)	9/38 (24%)	3/31 (10%)	3/34 (9%)	15/103 (15%)	0.23
Death or initiation of ECMO[c]	16/41 (39%)	11/40 (28%)	14/36 (39%)	8/37 (22%)	33/113 (29%)	0.25

a. Data from NDA volume 2.17, pages 084708 to 085508, and electronic datasets.

b. Primary endpoint: incidence of one of the PPHN major sequelae: death; initiation of ECMO; acute neurologic abnormalities; or development of bronchopulmonary dysplasia. See primary endpoints above for definitions.

c. Primary endpoint from the NINOS trial, added to the protocol prior to the breaking of the blind: death before discharge or 120 days (whichever comes first) and/or the initiation of ECMO.

Primary Endpoint:

1. Comparison of the incidence of any one of the following events (called PPHN major sequelae) between control and I-NO groups: death; initiation of ECMO; evidence of abnormal neurological sequelae; and bronchopulmonary dysplasia.

Table 6.0.3.12.2c.3 shows the rate of occurrence of any one of the PPHN major sequelae according to treatment group. There was no significant difference between the control group and any of the I-NO groups was detected.

Similarly, the incidence of each of the PPHN major sequelae were compared in the control and I-NO groups. There was a trend towards a lower incidence rate for the initiation of ECMO in the I-NO group (34% vs. 22%, a 35% reduction). There was no trend towards reduction in the rate of either bronchopulmonary dysplasia (13% vs. 15%) or neurological sequelae (26 vs. 24%) in the I-NO group.

Within the group of subjects who went on to receive ECMO, no information regarding the reasons for initiating ECMO are available. We also do not have information regarding the clinical state of the infants when they started ECMO. The use of I-NO was associated with a longer time between entering the study and initiating ECMO. This could suggest that infants receiving I-NO avoided ECMO by a delay in their transfer to it, allowing for time for other therapies (surfactant, ventilation, alkalinization) to work. Once infants started ECMO, however, no difference in the time on ECMO was detected.

Table 6.0.3.14.3 Comparison of the rates of ECMO parameters from INO-01/ -02.[a]

Changes in safety endpoints	Control				Combined I-NO
Duration of ECMO	103±32 (n=14)	118±106 n=9	103±96 (n=9)	183±189 (n=6)	129±63 (n=24)
Time to ECMO	22±15 (n=14)	51±25 (n=10)	37±31 (n=9)	36±16 (n=6)	42±24 (n=25)

a. Data from electronic datasets.

2. Comparison of the incidence of death before 120 days and/or initiation of ECMO between control and I-NO groups.

Table 6.0.3.12.2c.3 shows the incidence rate for this endpoint according to treatment group. There was no significant difference between the control group and any of the I-NO groups. There was a trend towards a lower incidence rate of this endpoint in the I-NO group (39% vs. 22%, a 43% reduction from control rate).

There were excess deaths in the I-NO group when compared with the control group, both numerically and when expressed as a % of the total. This difference was not statistically significant (p=0.29 for control vs. combined I-NO group). Two other infants died before 120 days (the cut-off in the NINOS trial): one in placebo and one in 20 ppm I-NO group. If these two are factored in, the difference between control and the combined I-NO group is still not statistically significantly (p = 0.51). No additional infants for whom vital status information was available had died at the one-year follow-up.

6.0.3.14 INO-01/ -02 Efficacy Summary (cont)

Secondary Endpoints

1. Physiologic response to I-NO, measured by change in OI and time-weighted OI.

1a. Acute changes in PaO_2 and OI

Table 6.0.3.14.4 shows the acute effects of I-NO on oxygenation in the INO-01/ -02 trial. The data suggest that not only is there a significant acute effect of I-NO to increase PaO_2 and to decrease OI, but the effect may be dose-dependent between 5 and 80 ppm of I-NO.

Table 6.0.3.14.4 Acute effects of I-NO on measures of oxygenation[a].

'Exploratory' Variables	Control	I-NO 20 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Changes in clinical markers (from baseline to 30 minutes)[a]					
Mean OI	-1.3±7.7	-4.7±4.6	-4.3±9.6	-7.4±9.0	-5.5±8.7
Mean PaO_2	18.0±53	32.3±56	38.6±69	64.4±84	44.6±71
% increase in PaO_2 from baseline to 30 minutes	23.4%	31.8%	39.4%	50.3%	40.8%

a. Shown is the mean± s.d. of the change from baseline for each parameter, measured after 30 minutes. P values calculated using Student's t test. Data from NDA volume 2.18 Tables T-1 and T-7.

c. I-NO groups which differ from control significantly (<0.05) using Student t-test of group means are shaded.

Another analysis is to look at the percentage of subjects who improved their PaO_2 acutely after exposure to study gas, similar to the analysis performed in the NINOS trial. Shown is data from all subjects with PaO_2 values at baseline and 30 minutes. The table looks at the % of subjects in each group who had a 'complete response' as it was defined in the NINOS trial for oxygenation: an increase of >20 mmHg in PaO_2 after 30 minutes. These data also support a possible dose-response to I-NO, suggesting that 80 ppm may have a greater acute effect on oxygenation than lower doses of I-NO.

Table 6.0.3.14.5 (from table 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

a. p value determined using one-way ANOVA.

In conclusion, then, administration of I-NO was associated with an increase in PaO_2 and decrease in OI measured after 30 minutes which may be dose-dependent.

1b. Long-term changes in PaO_2 and OI.

The sponsor proposed to examine the change in time-weighted OI (TWOI) as a part of one secondary endpoint in this trial. This was defined as the area under the curve of the OI vs time for the first 24 hours or until discontinuation, divided by the number of hours of exposure to the study gas. In this calculation, the a 'negative' TWOI indicates a clinical improvement. From Table 6.0.3.12.2c.5, there was a significant difference between control and I-NO groups with regard to improvement in OI from baseline to 30 minutes. Another way to look at TWOI, then, is the durability of this response. As can be seen from the table, there was a significant difference between the TWOI of the control and the I-NO groups. Individual responses, however, were quite variable.

Table 6.0.3.14.6 Effects of I-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 but before subject discharge, from NDA volume 2.17, Table 23.

b. Repeated t test comparisons with control group were significant (<0.05) for the 5 and 80 ppm group, as well as the pooled I-NO group(shaded boxes above).

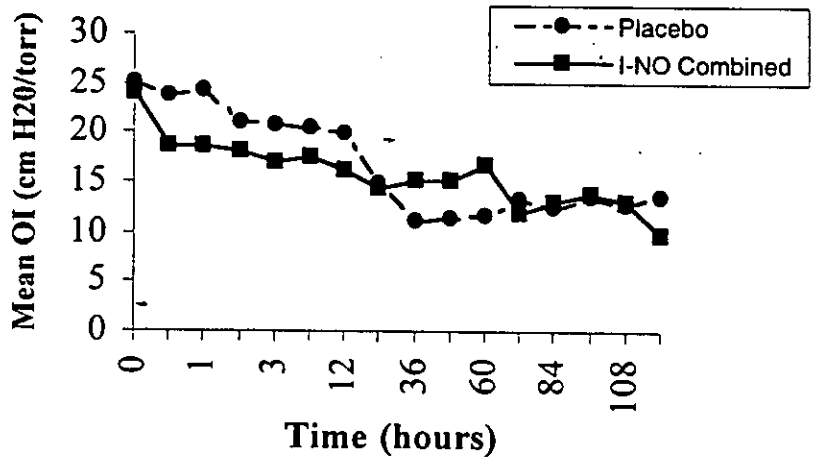
6.0.3.14 INO-01/ -02 Efficacy Summary (cont)

Secondary Endpoints (cont)

1b. Long-term changes in PaO_2 and OI (cont)

The figure below compares the trend of OI for the placebo (N_2) and combined I-NO groups over time. Data for points beyond 24 hours are limited by the small number of subjects remaining and the data is shown until 120 hours, at which time fewer than 10 subjects remain in each of the 4 groups (9 placebo, 8 at 5 ppm, 3 at 20 ppm, and 2 at 80 ppm).

Figure 6.0.3.12.3a.3 Time vs. OI



Overall, there was an acute significant effect of I-NO to decrease OI, which persisted during the length of I-NO administration (as measured by the TWOI). The difference between the control and I-NO OIs also dissipated with time.

2. Number of days requiring supplemental oxygen.

The time the infants required supplemental O_2 was 6 hours in the placebo group and 5 hours in the combined I-NO group (p = NS).

3. Number of days requiring mechanical ventilation.

The time the infants required mechanical ventilation was 8 days in the placebo group and 9 days in the combined I-NO group (p = NS).

4. Number of days in hospital.

The length of hospitalization was 26 days in the placebo group and 22.3 days in the combined I-NO group (p = 0.45).

Overall, no effect of I-NO on any clinical outcome included as a secondary measure (duration of supplemental O_2, mechanical ventilation, or hospitalization) was detected.

"Exploratory variables" (per the sponsor)/ acute physiological changes:

I-NO had a significant acute effect on measures of oxygenation, without having any detectable effect on pulmonary airway pressures or hemodynamics. Several of these parameters would be expected to change slowly; thus, no acute change would be expected. There was a significant improvement in the OI, from 24.0±9.2 to 18.7±11.5 in the combined I-NO group after 30 minutes (a 22% decrease). This is similar in magnitude to the 29% increase in FiO_2 (77 to 108 mm Hg) and a 7.6% decrease in the mean A-aDO_2 (573 to 529) which also occurred within the first 30 minutes of I-NO therapy. Other parameters of oxygenation also improve acutely (postductal O_2 saturation, mean PaO_2). There was no significant change in the mean PCO_2 or pH from baseline.

6.0.3.14 INO-01/ -02 Efficacy Summary (cont)

"Exploratory variables" (per the sponsor)/ acute physiological changes (cont)

Table 6.0.3.14.8 Results: comparison of acute changes in the specified 'exploratory variables' from INO-01/ -02 trial[a,c].

'Exploratory' Variables	Control	I-NO 20 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Changes in clinical markers (from baseline to 30 minutes)[a,b]					
Mean OI	-1.3±7.7	-4.7±4.6	-4.3±9.6	-7.4±9.0	-5.5±8.7
Mean PaO_2	18.0±53	32.3±56	38.6±69	128±64	108±45
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
Mean Arterial Pressure	-0.73±10.9	-2.39±9.69	-3.22±9.4	0.69±10.0	-1.7±9.8
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
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 O_2 gradient ($A\text{-}aDO_2$)	-19.5±56	-31.6±56	-39.6±68	-63.2±81	-44.3±70
Mean pCO_2	-0.75±5.5	-1.24±6.0	-1.31±3.6	-1.17±4.9	-1.24±4.9
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, measured after 30 minutes. P values calculated using Student's t test.

b. The long-term effects of I-NO on the clinical markers will be examined below.

c. I-NO groups which differ from control significantly (<0.05) using Student t-test are shaded.

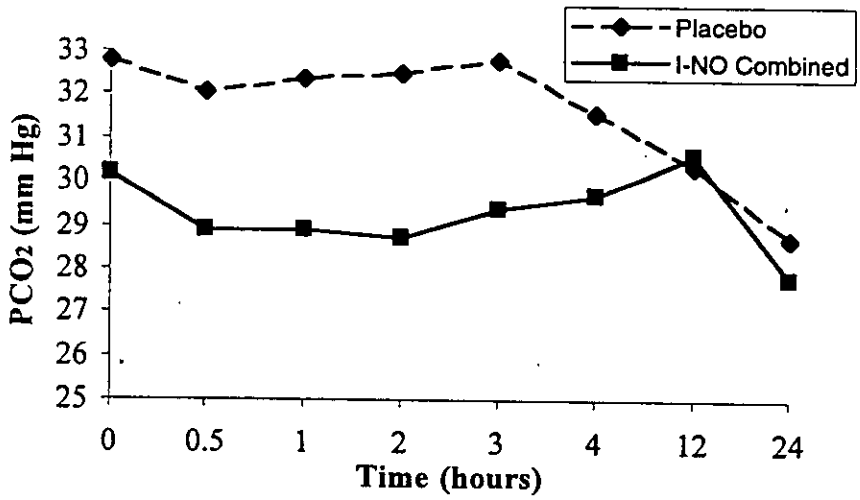
'Exploratory variables' (per the sponsor)/ chronic physiological changes

The sponsor also measured these 'exploratory' variables throughout the length of the trial, yielding information about the long-term effects of I-NO on these physiological markers. The importance of these measurements is two-fold. First, the durability of any acute beneficial effect is critical. Second, some of the long-term changes (i.e., pulmonary airway pressures, FiO_2), should fall in response to a clinically beneficial treatment. If I-NO has an effect beyond improving oxygenation, we should be able to measure changes in these parameters. Failure to detect such a change would support the notion that I-NO has no clinically relevant benefit.

1. Changes in pCO_2

A safety concern is the reported effect of I-NO to increase NO_2 levels, which can impair CO_2 diffusion and cause increased pCO_2 levels. The figure below shows the mean pCO_2 for the placebo and I-NO subjects over the first 24 hours. The mean baseline pCO_2 in the I-NO group was numerically, but not significantly, lower than the control baseline. No trend towards an effect of I-NO to reduce pCO_2 was seen.

Figure 6.0.3.14.9 Time vs. PCO_2



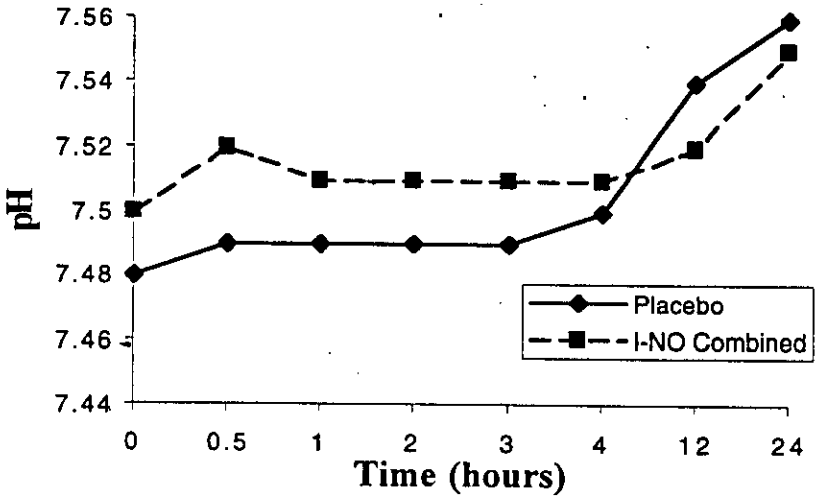
6.0.3.14 INO-01/ -02 Efficacy Summary (cont)

"Exploratory variables" (per the sponsor)/ chronic physiological changes (cont)

2. Changes in pH

A similar analysis for changes in pH is complicated by the use of alkalinization as part of standard care for these infants. Examination of the acute effects of I-NO above showed no difference between control and I-NO with regards to pH.

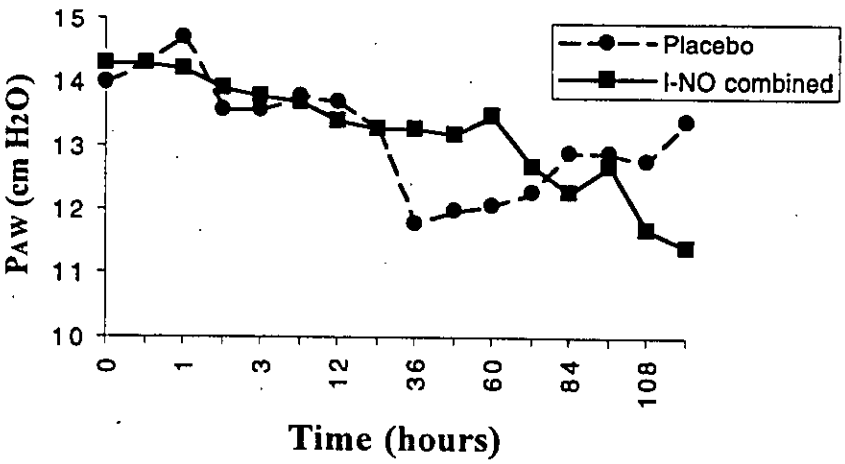
Figure 6.0.3.14.10 Time vs. pH



3. Changes in P_{AW}

Another long-term change which could be expected is a decrease in Mean Airway Pressure (P_{AW}) coincident with clinical improvement. If I-NO caused a more rapid recovery, a more rapid decline might be seen. The figure below shows the mean (±sd) values for the PAW during the hospitalization. In both groups there was a gradual trend towards improved P_{AW}, but no discernible effect of I-NO to accelerate this process. Once again, the data beyond 24 hours has broad confidence limits due to the small numbers of subjects.

Figure 6.0.3.14.11 Time vs. Mean Airway Pressure (PAW)



When the three doses of I-NO were looked at separately, there was, again, no effect to accelerate the decline in P_{AW} (data not shown).

6.0.3.14 INO-01/ -02 Efficacy Summary (cont)

"Exploratory variables" (per the sponsor)/ chronic physiological changes (cont)

With regard to the 'exploratory variables,' reflecting acute and chronic physiological effects, I-NO has an acute effect to increase PaO_2 and decrease OI. No acute effect on pH of pCO_2 was detected. During long-term follow-up, exposure to I-NO had a persistent beneficial effect on oxygenation, when compared with placebo, as measured by the TWOI. No acute or chronic effect of I-NO on pulmonary airway pressures was detected.

Overall efficacy summary

The database in the INO-01/ -02 trials is limited by the decreased sample size due to incomplete accrual. The effect of this is to decrease the power of the study to detect significant differences. Additionally, the reviewing statistician feels that performing the power calculation using the Monte-Carlo technique may underestimate the true number of subjects required. This means that even if the full proposed number of subjects had enrolled in the trial, the trial may have lacked sufficient power to detect the proposed difference in rates of events.

With regards to efficacy, this problem has been addressed first examining the efficacy results both statistically, as is normal for pivotal trials, and by looking at the trend of the data, comparing the overall effect of placebo and I-NO for the primary and secondary endpoints, as well as the PPHN sequelae that make up the primary endpoint (see table 6.0.3.12.2c.3). The data from the INO-01/ -02 trial will also be interpreted in the context of the other trials in the NDA in the overall efficacy summary (see section 7.0).

Finally, the data was examined for a dose-dependent effect of I-NO between 5 and 80 ppm I-NO for the primary and secondary efficacy endpoints. This dose-dependency was suggested for the rate of ECMO. The 'exploratory variables,' however, also suggest that such a dose-dependent effect of I-NO exists with regards to the acute changes in oxygenation. Since the INO-01/ -02 trial is the only trial submitted using more than one dose of I-NO in randomized fashion, this is the only data available to address the use of various doses of I-NO.

1. No significant effect of I-NO on the primary endpoint for the INO-01/ -02 trial was detected.

2. There was a trend towards a reduction in the rate of ECMO in infants receiving I-NO. This was associated with a longer period of treatment in the I-NO group before receiving ECMO, suggesting that part of the effect of I-NO may be to delay transfer to ECMO, allowing time for other effective therapies to work.

3. Administration of I-NO was associated with a numerical and percentage-wise increase in deaths in the INO-01/ -02 trial. Several of the subjects who died also had components of the Air Leak Syndrome as adverse events.

4. No significant effect of I-NO on the incidence of neurologic abnormalities or bronchopulmonary was detected. There was also no trend towards a beneficial effect of I-NO seen for either clinical endpoint.

5. No significant effect of I-NO administration on any clinical outcome included as a secondary measure (duration of supplemental O_2, mechanical ventilation, or hospitalization) was detected.

6. I-NO acutely increases PaO_2 in a dose-dependent between 5 and 80 ppm of I-NO in a significant percentage of subjects. Overall, 52% of the combined I-NO group and 20% of the control subjects had an increase of >20 mmHg in PaO_2 after 30 minutes (Table 6.0.3.12.3b.1).

7. I-NO acutely decreases OI and Arterial-alveolar O_2 gradient (A-aDO_2).

8. The effect to improve OI is durable for the duration of I-NO administration, as measured by TWOI.

9. I-NO had no significant acute or chronic effect on pCO_2 or pH. There was also no trend towards such an effect of I-NO seen for these measurements.

10. I-NO had no significant chronic effect on airway pressures. There was also no trend towards such an effect of I-NO seen for this measurement.

6.0.3.15 INO-01/ -02 Safety Summary

Study Design

The INO-01/ -02 trial collected information which was not collected during any of the other trials in the NDA. The INO-01/ -02 trial is the only source for unanticipated adverse events and adverse events associated with laboratory abnormalities (other than methemoglobin and NO_2) in the NDA. It is also the only trial to collect data serially on vital signs, the only trial to collect case report forms, and the only trial for which narratives of each death are available. The impact of this is to focus the overall safety review of the NDA largely on data collected in the INO-01/ -02 trial. Much of this data will be discussed in sections 8.2 and 8.3 below.

Safety Endpoint Measured in the INO-01/ -02 Trial

1. Methemoglobin levels.
2. Inhaled NO_2 concentrations
3. Adverse events
4. Routine lab chemistries and blood counts.

   Labs and hematology were collected at baseline and again within 12 hours of discontinuation of I-NO.

   The following laboratories were not collected as part of the submitted NDA database: electrolytes (Na^+, K^+, Cl^-, HCO_3^-); and urinalysis.
5. Hemodynamics (heart rate, blood pressure).

   Electrocardiograms were not collected as part of the NDA database.
6. Incidence of air leak (pneumothorax, pneumopericardium, pneumoperitoneum, pneumomediastinum, interstitial emphysema).
7. Incidence of bronchopulmonary dysplasia (BPD).
8. Requirement for supplementary O_2 at time of discharge or 28 days.
9. Incidence of seizures.
10. Surviving infants were to have a 1 year examination including the following safety assessments: vital status; medical history; neurologic assessment; audiology (using BAER); and a developmental test

Protocol Violations & Deviations

None of the protocol violations listed in table 6.0.3.12.2b.1 were related to safety.

Safety Outcomes not included in the Efficacy Summary (see table 6.0.3.15.1)

1. Methemoglobin levels.

In the INO-01/ -02 trial, the average peak methemoglobin level was significantly higher in the 80 ppm I-NO group. After 12 hours of exposure to 80 ppm I-NO, the mean methemoglobin level peaked at 5.08±2.32%. The increased methemoglobin levels developed after a mean of 8 hours in the I-NO 80 ppm group.

There was a clear association between the dose of I-NO and the risk of elevated methemoglobin levels, as was seen in table 8.1.6.2.1.3c.1.

Elevated methemoglobin levels led to the discontinuation of I-NO in four of the subjects. In 9 other subjects, the I-NO reduced and ultimately discontinued (listed in table 8.1.2.2c.1). No short-term adverse outcomes were identified that can be linked to the elevated methemoglobin levels, however. Of the infants identified with increased methemoglobin concentrations, two infants received ECMO, and one of those infants died. None of the infants had chronic lung disease (CLD) at the time of discharge. The infant that died (discussed in section 8.1) required discontinuation of I-NO due to elevated methemoglobin levels, and died several days after discontinuation.

2. Inhaled NO_2 concentrations.

In the INO-01/ -02 trial, the average peak NO_2 level was significantly higher in the 80 ppm I-NO group than any other group (see table 8.1.6.2.1.2c.1).

There was a clear association between the dose of I-NO and the risk of elevated methemoglobin levels. For instance, NO_2 levels in the 80 ppm group averaged 2.6±1.2 vs. 0.48±0.62 in the 20 ppm group.

Elevated NO_2 levels led to the discontinuation of one subject. One control and one I-NO subject with elevated NO_2 levels received ECMO, and one subject with elevated NO_2 levels died. The infant that died will be discussed further in section 8.1.

3. Adverse events.

All adverse events were identified by the individual investigators in INO-01/ -02.

The incidence rates for each adverse event identified will be analyzed in section 8.1 and included in the relevant body system in section 8.2.

6.0.3.15 INO-01/ -02 Safety Summary (cont)

4. Routine lab chemistries and blood counts.

Labs and hematology were collected at baseline and again within 12 hours of discontinuation of I-NO.

The following laboratories were not collected as part of the submitted NDA database: electrolytes (Na^+, K^+, Cl^-, HCO_3^-); and urinalysis.

The lab data will be analyzed in section 8.1 and included in the relevant body system in section 8.2.

5. Hemodynamics (heart rate, blood pressure).

Electrocardiograms were not collected as part of the NDA database.

The data on vital signs will be analyzed in section 8.2 and included in the relevant body system in section 8.1 and 8.2.

6. Incidence of Air Leak Syndrome (pneumothorax, pneumopericardium, pneumoperitoneum, pneumomediastinum, interstitial emphysema).

Adverse events related to pneumothoraces and Air Leak Syndrome (ALS) were collected in several ways in trial INO-01/ -02.

First, ALS was collected as a prospectively identified event. Each subject was reviewed by a data manager at the time of discharge, and any occurrence of ALS during the hospitalization identified. This data is presented in table 6.0.3.15.1, and no difference in the rate of ALS was detected between the control and I-NO groups.

Pneumothorax was also identified as an adverse event by individual investigators during the trial. These data are discussed further in section 8.1 and 8.2. Overall, an increased number of pneumothoraces were identified as adverse events in the I-NO group, as seen in the table below.

Table 6.0.3.15.1 (from table 8.1.5.4.2) Incidence of subjects with pneumothoraces identified by investigators as adverse events in INO-01/ -02.

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%)

The short-term clinical consequences of these adverse events are listed below.

Table 6.0.3.15.2 Listing of subjects with pneumothoraces identified as adverse events by investigators in the INO-01/ -02 trial[a].

Subject group	Duration of I-NO therapy	Outcome (28 days)
Control		
02-04001	204	Seizures, BPD
I-NO 5 ppm		
01-03024	2	No ECMO, HFOV/HFJV
01-03028	100	ECMO
01-05002	21	Seizures, ECMO
01-11012	10	Died
I-NO 20 ppm		
01-03025	113	Died
01-11005	70	Died
02-17001	98	
I-NO 80 ppm		
01-06006	144	Died
01-11011	62	Died
02-15003	10	
02-04004	6	

a. Data from electronic datasets.
b. Broncho-pulmonary dysplasia (BPD).
c. Reactive Airways Disease (RAD).

6.0.3.15 INO-01/ -02 Safety Summary (cont)

Of the subjects who died in the INO-01/ -02 trial, there were also five subjects who died having also had a pneumothorax. The only control subject who died did not have a pneumothorax.

Table 6.0.3.15.3 Deaths with air leak syndrome (ALS) adverse events in the INO-01/ -02 trial[a,b].

Subject	Duration of study gas (hrs)	Air Leak Syndrome?[b]	Refractory Hypoxemia	Support Withdrawn?	Notes
Control	No deaths with ALS				
I-NO 5 ppm					
01-11012	10	Yes	Yes	Yes	
I-NO 20 ppm					
01-11005	56	Yes	Yes	No	
01-11015	120	Yes	Yes	Yes	Died after 32 days First pneumothorax occurred Pre-I-NO
I-NO 80 ppm					
01-06006	144	Yes	Yes	No	Persistent methemoglobinemia
01-11011	60	Yes	No	No	Pneumopericardium

a. Data from section 8.1.1 and electronic datasets.
b. One other subject in the 20 ppm I-NO group, 01-11015, had a pneumothorax at birth, and died after 32 days.

This issue of a link between I-NO administration and ALS, including pneumothoraces, is discussed in section 8.2.7.2 below.

7. Incidence of bronchopulmonary dysplasia (BPD).

First, the occurrence of BPD was a prospectively identified event, and its occurrence was collected at the time of discharge. From table 6.0.3.15.1, no significant difference between control and I-NO groups was detected. Data on the occurrence of parts of the BPD syndrome were also collected in the trial, including the incidence of asthma and the need for supplemental O_2. Long-term data on the pulmonary health of subjects in the INO-01/ -03 was also collected, as detailed above. These issues are discussed in section 8.2.7.2.

8. Requirement for supplementary O_2 at time of discharge or 28 days.

Table 6.0.3.15.1 shows the incidence rates for infants requiring supplemental O_2 at time of discharge in the INO-01/ -02 trial. No significant difference between control and I-NO groups was detected.

There was, however, a significant difference in the number of infants who required O_2 after discharge, as shown in the table below.

Table 6.0.3.15.4 Respiratory system abnormalities at 1 year of age for infants with known follow-up in INO-01/ -02[a].

	Control	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Home O_2 therapy	0/36 (0%)	8/35 (23%)	1/29 (3%)	3/31 (10%)	12/95 (13%)

a. From individual case report forms for long-term follow-up, Volumes 7.1 through 7.12.
Overall, a higher number of subjects in the I-NO group required O_2 after discharge. This issue will be discussed in section 8.2.7.2.

9. Neurologic abnormalities up to time of discharge.

As summarized in table 6.0.3.15.1, the rate of neurologic abnormalities detected in the INO-01/ -02 database was small, limiting the detection of differences between the control and I-NO groups.

Overall, no differences in the rate of neurologic abnormalities between control and I-NO groups were detected.

10. Long-term follow-up endpoints (measured at 1 year follow-up examination):
    a. Incidence of hearing abnormalities.
    b. Incidence of developmental delay.

As shown in tables 6.0.3.13.3.3 to 6.0.3.13.3.7, no differences in the rates of any neurologic, developmental, or hearing abnormalities were identified in the available infants tested after one year.

6.0.3.15 INO-01/ -02 Safety Summary (cont)

Overall safety summary for INOSG

Some aspects of the safety database for INO-01/ -02 are to be discussed in sections 8.1 and 8.2, and have not been included here. This includes laboratory abnormalities, vital signs, deaths, and subject drop-outs.

Based on the available data, I-NO has no significant acute or chronic effect on hemodynamics. The data also suggest that subjects receiving I-NO 80 ppm are at significant risk for elevated methemoglobin levels and NO_2 levels.

Two of the safety issues which were identified as part of the INO-01/ -02 database have been discussed above: the increased number of deaths and the excess pulmonary toxicity in the I-NO group. These issues will be discussed later in section 8.2.7.2 as well.

No adverse effect of I-NO on either short- or long-term neurologic outcomes was identified in the INO-01/ -02 study.

6.0.3.16 01-02 INO-01/ -02 study reviewer's conclusions

Despite the incomplete patient accrual in the INO-01/ -02 trial, this is a critical trial for the overall NDA application, providing information not available from any other source. Due to the inclusion and exclusion criteria, only a small % of the screened subjects (12%) were eligible for enrollment. This population, largely, were those infants who were not so acutely sick that they required maximal, immediate intervention with all possible therapies (such as surfactant and HFOV/HFJV). Instead, the infants were able to be evaluated, including echocardiography, prior to enrollment. As a result, their average OI was substantially lower than that seen in the other trials (NINOS and INOSG).

The results of the INO-01/ -02 trial are consistent with those seen in the NINOS, despite the differences in baseline characteristics. The administration of I-NO improves oxygenation in a substantial fraction of subjects. I-NO administration was also associated with a decrease in the use of ECMO, as in the NINOS trial. No other clinical benefits were detected, however.

Several safety issues were identified in the INO-01/ -02 database, which will be investigated further in sections 8.1 and 8.2.

6.0.4.1 Ohmeda Protocol NO-03 (INO-01/ -02-03): Inhaled nitric oxide in the treatment of persistent pulmonary hypertension of the newborn (PPHN)

6.0.4.2 Sites of investigation and investigators

Table 6.0.4.2.1 Ohmeda INO-03 study sites and investigators.

Investigator	Site of Investigation
Michael Damask MD (Medical Monitor)	
Richard Straube MD (Medical Monitor)	
J. Kattwinkel MD	University of Virginia
G. Dudell MD	San Diego Children's Hospital
D. Davidson MD	Schneider Children's Hospital
K. C. Sekar MD	Children's Hospital of Oklahoma
E. M. Bifano MD	Crouse Irving Memorial Hospital
M. Cohen MD	Newark-Beth Israel Medical Center
D. Stevens	University of South Dakota

6.0.4.3.Background

Protocol NO-03 was finalized June 1996, with one amendment September 1996. The latter amendment changed the dosing from randomized doses of 5, 20 and 80 ppm to an open-label trial in which all subjects received 20 ppm I-NO.

Enrollment into NO-03 was halted after January 1997, because of extremely low patient accrual (after publication of the results of the NINOS trial).

6.0.4.4 Study Design

The primary objective of INO-03 was to extend the safety data on I-NO in infants with persistent pulmonary hypertension of the newborn (PPHN). A secondary objective was to collect further outcome data on a similar patient population as was enrolled in the INO-01/ INO-02 trial.

This was a multi-center, open-label trial on subjects with echocardiographic evidence of PPHN. Originally, subjects were to be randomized to 5, 20, or 80 ppm I-NO. The protocol was later amended to include only the 20 ppm dose.

6.0.4.5 Primary and Secondary Endpoints

The primary objective of the trial was to collect safety information.

Specific safety variables

1. Methemoglobin levels.
2. NO_2 concentrations.
3. Incidence of intraventricular hemorrhage and seizures.
4. Laboratory evaluations.
5. Incidence and relationship of all adverse events and specific adverse events to I-NO.

The trial also proposed to collect efficacy information, although this was not a primary aim of the trial.

Specific efficacy endpoints

1. Incidence of either death or ECMO.
2. The number of subjects in each group who had at least one major PPHN sequelae. Major PPHN sequelae were defined as:

a. Death;
b. Use of ECMO;
c. Development of bronchopulmonary dysplasia (BPD); or
d. Evidence of abnormal neurologic sequelae within 28 days of the study (intraventricular hemorrhage, brain infarct, or the presence of seizures).

6.0.4.6 Number of subjects/randomization

The initial protocol called for 240 subjects enrolled, 3 groups of 80 newborns receiving separate doses of I-NO: 5, 20 and 80 ppm. The amended protocol was for 80 subjects, all in the 20 ppm group. The amendment was necessitated by poor enrollment.

Details on the number of subjects screened are not available. The table below shows the number of subjects enrolled at each of the centers.

Table 6.0.4.6.1 Enrollment in INO-03 by site.

Site	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Total
Schneider Children's Hospital	0	1	0	1
San Diego Children's Hospital & Health Center	0	1	0	1
University of Virginia	2	0	1	3
Children's Hospital of Oklahoma	0	1	0	1
Crouse Irving Memorial Hospital	2	2	1	5
Newark-Beth Isarael Medical Center	0	1	0	1
University of South Dakota School of Medicine	0	2	0	2
Total	4	8	2	14

6.0.4.7 Inclusion/exclusion criteria

Inclusion Criteria for Primary Entry Into INO-03 Trial

1. Hypoxemia defined as a postductal PaO_2 between 40 and 100 mmHg on one determinations while breathing an FiO_2 =100%.
2. Mechanical ventilation with a $P_{AW} \geq$ 10 cm H_2O.
3. A diagnosis of pulmonary hypertension, including the following criteria:
   a. Evidence of a right to left or bidirectional cardiac shunt at the level of the patent ductus arteriosus or formen ovale.
   b. Pre- and Post-ductal O_2 saturation difference >10% (when the ductus can be visualized) or
   c. Right-to-left, or bidirectional shunting at the foramen ovale together with one of the following indicators of pulmonary hypertension:
      i. Moderate to severe tricuspid insufficiency jet with Doppler evidence of systolic pulmonary artery pressure ≥75% of systemic pressure.
      ii. Posterior systolic bowing of the intraventricular septum.
4. The infant must have an indwelling arterial line.
5. Term neonate: >37 weeks and a birth weight of greater than or equal to 2500 grams (>2000 grams if >39 weeks gestation).
6. Neonate <72 hours old.
7. Informed parental consent.

Exclusion Criteria for Primary Entry Into INO-03 Trial

1. Failure to meet any inclusion criterion.
2. Intraventricular hemorrhage (Grade 2-4) or subarachnoid hemorrhage.
3. Mean systemic arterial BP <35 mmHg after volume or vasopressor therapy.
4. Uncorrected polycythemia (central hematocrit >70%).
5. Cardiac lesions other than patent formen ovale, patent ductus arteriosus, or tricuspid regurgitation.
6. No evidence for pure left-to-right shunt.
7. Previous therapy with surfactant.
8. Use of jet or high frequency oscillatory ventilation (HFJV/ HFOV). More than 6 hours of HFOV at referring institution of any use of HFOV within 2 hours of treatment gas.
9. Clinical diagnosis of pulmonary hypoplasia, including congenital diaphragmatic hernia.
10. Phenotype consistent with a lethal chromosomal abnormality.
11. Intravenous vasodilator therapy (Tolazoline) beginning after inclusion criteria are met.
12. Uncontrollable coagulopathy and/or serious bleeding.
13. Enrollment in any investigational drug or other interventional study.

6.0.4.8 Dosage/Administration

Of the 14 subjects who were entered into the trial, 4 received 5 ppm, 8 received 20 ppm, and 2 received 80 ppm I-NO. While the initial subjects were blinded as to dose, the majority received open-label I-NO, 20 ppm.

The sponsor chose I-NO doses based on the available animal data as well as two small clinical studies(6, 12, 80). The upper limit of 80 ppm was chosen out of concern for the report of methemoglobin and NO_2 accumulation at higher doses in some subjects.

The treatment gases were administered via inhalation using an Ohmeda inhaled NO delivery device (Ohmeda MSD Inc.), connected to an Infant Star ventilator (Infrasonics) and a Fisher-Paykel MR-730 heated humidifier (Fisher-Paykel Healthcare). The inhaled NO was manufactured by BOC Specialty Gases from raw NO supplied manufactured by BOC Specialty Gases. To provide different doses of I-NO to subjects using the same delivery device and settings (for blinding purposes), multiple cylinder concentrations of NO were supplied to sites, where the gas was diluted 1:20 by the delivery device. Following approval of the amendment, all subjects received I-NO, 20 ppm, using the 800 ppm tank concentration which was diluted 1:40. To limit the formation of NO_2, the residence time of NO and O_2 together within the breathing circuit were minimized by keeping the flow rate of the gas >10 liters/minute. The exhaled gases were collected and discharged into the hospital vacuum system. Like the INO-01/ -02 trial, the INO-03 trials used N_2 as the control gas.

6.0.4.9 Duration/Adjustment of Therapy

Treatment gas was administered as soon as possible after a patient met all of the screening parameters. Once started, study gas was administered until any one of the following occurred:

1. the subject was discontinued from the study because they met 'treatment failure' criteria, defined as meeting any one of the following:.
   a. PaO_2 <40 mmHg at the beginning and end of a 30 minute period not due to a correctable mechanical problem;
   b. Mean systemic arterial pressure <35 mmHg after volume or vasopressor therapy;
   c. the subject died;
   d. the subject's methemoglobin >7% on two consecutive time points at least 30 minutes apart; or
   e. the subject's NO_2 level was persistently > 3 ppm for 30 minutes.
2. the subject had treatment gas prematurely discontinued for any of the following reasons:
   a. the parent(s) withdrew consent;
   b. the patient is found to have an exclusion criteria;
   c. the delivery or monitoring of I-NO malfunctions and cannot be corrected within 8 hours; or
   d. the principle investigator considered withdrawal to be in the best interest of the child.
3. the subject received 14 days of study gas.
4. the criteria for weaning of treatment gas are met, defined as all of the following:
   a. FiO_2 <0.60;
   b. P_{AW} <10 cm H_2O; and
   c. postductal PaO_2 ≥60 mmHg

If a subject met the criteria for weaning, or if the methemoglobin/ NO_2 levels were too high, the treatment gas was weaned in 20% decrement to 0%. Decreases occurred every 30 minutes to 4 hours, so long as the infant continued to meet criteria for weaning. In case of poor oxygenation, the gas could be increased up to 20%.

Elevated methemoglobin level was defined as >7% at two consecutive time points at least 30 minutes apart. Elevated NO_2 level was defined as > 3 ppm for 30 minutes.

6.0.4.10 Safety and Efficacy Endpoints Measured

1. Methemoglobin levels.
2. NO_2 concentrations.
3. Incidence of intraventricular hemorrhage and seizures.
4. Laboratory evaluations.
5. Incidence and relationship of all adverse events and specific adverse events to I-NO.

6.0.4.11 Statistical Considerations

Given the open-label nature of the trial, relative event rates between groups were to have been compared. No formal statistical analysis was planned.

6.0.4.12 Efficacy Outcomes from the INO-03 trial

6.0.4.12.1 Patient Demographics an Baseline characteristics

Table 6.0.4.12.1.1 Demographics of the 14 subjects enrolled in trial INO-03 at baseline.

Demographic Characteristic	Inhaled I-NO			Pooled I- NO
	5 ppm	20 ppm	80 ppm	
Total	4	8	2	14
Sex				
Male	4 (100%)	4 (50%)	1 (50%)	9 (64%)
Race				
White (%)	3 (75%)	4 (50%)	1 (50%)	8 (57%)
Black (%)	0 (0%)	3 (38%)	1 (50%)	4 (29%)
Hispanic (%)	0 (0%)	1 (12%)	0 (0%)	1 (7%)
Asian (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Other (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Age in hours	48±22	24±12	21±21	
Birth Weight (kg)	3.9±0.8	3.3±0.5	3.2±0.4	
Gestational age in weeks	39.8±1.5	39.6±1.9	38.5±2.1	
Mother's age in years	27.8±7.8	27.4±6	24±0	

a. Data shown is mean ± standard deviation.

The underlying disease responsible for the hypoxemic respiratory failure in the enrolled subjects is listed below.

Table 6.0.4.12.1.2 Underlying disease of the infants enrolled in INO-03.

Disease	Inhaled I-NO			Pooled I-NO
	5 ppm	20 ppm	80 ppm	
Meconium Aspiration (% of total)	1/4 (25%)	4/8 (50%)	1/2 (50%)	6/14 (43%)
Idiopathic PPHN	2/4 (50%)	1/8 (12%)	1/2 (50%)	4/14 (28%)
Sepsis/pneumonia	0/4 (0%)	1/8 (12%)	0/2 (0%)	1/14 (7%)
Repertory Distress Syndrome	1/4 (25%)	0/8 (0%)	0/2 (0%)	1/14 (7%)
Other	6 (15%)	2/8 (25%)	0/2 (0%)	2/14 (14%)

6.0.4.12.1 Patient Demographics an Baseline characteristics (cont)

Table 6.0.4.12.1.3 Baseline clinical characteristics of subjects enrolled in INO-03.

Clinical Characteristic	Inhaled I-NO			Pooled NO
	5 ppm	20 ppm	80 ppm	
Birth characteristics				
Cesarean section	2/4 (50%)	7/8 (88%)	2/2 (100%)	11/14 (78%)
Complicated delivery	2/4 (50%)	1/8(12%)	0/2 (0%)	3/14 (21%)
Apgar at 1 minute	8±0	4.8±3	4±4	
Apgar 5 minutes	9±0	7.3±2	6±3	
Apgar at 10 minutes	9±(NA)	7.3±3	7±(NA)	
Pulmonary status[a]				
Oxygenation Index (OI) cm H_2O/mmHg	22.3±5	25.8±8	18.7±4	
PaO_2	58±17	53±8	68±16	
Peak Inspiratory Pressure (cm H_2O)	31±8	32±6	34±6	
Positive End-Expiratory Pressure (cm H_2O)	3.8±0.5	4.5±1.1	3.5±0.7	
pH	7.46±0.09	7.48±0.08	7.32±0.01	
pCO_2	31±4	33±8	39±6	
Hemodynamic status[a]				
Diastolic BP (mmHg)	41±5	44±8	44±0.7	
Systolic BP (mmHg)	61±8	67±16	64±2	
Mean BP (mmHg)	50±7	55±14	52±3	
Heart Rate (beats per minute)	145±8	152±21	147±21	
Findings on Cranial Ultrasound				
Normal	3/4 (75%)	6/8 (75%)	2/2 (100%))	11/14 (78%)
Abnormal	1/41 (25%)	2/8 (25%)	0/2 (0%)	3/14 (22%)
Intraventricular hemorrhage	0/4 (0%)	1/8 (12%)	0/2 (25%)	2/14 (14%)
Other abnormal findings	1/4 (25%)	1/8 (12%)	0/2 (0%)	2/14 (14%)

a. Pulmonary, hemodynamic, and cranial ultrasound findings taken from the baseline values at time of randomization.

Table 6.0.4.12.1.4 Baseline echocardiographic findings in subjects enrolled in the INO-03 trial.

Clinical Characteristic	Inhaled I-NO			Pooled NO
	5 ppm	20 ppm	80 ppm	
Right to left or Bidirectional PDA shunt	4/4 (100%)	7/8 (88%)	2/2 (10%)	13/14 (88%)
Closed ductus arteriosus with other evidence of PPH[Na]	0/4 (0%)	1/8 (12%)	0/2 (0%)	1/14 (7%)

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.

6.0.4.12.1 Patient Demographics an Baseline characteristics (cont)

Table 6.0.4.12.1.5 Specific therapies used by subjects in the INO-03 trial prior to randomization.

Therapy	Inhaled I-NO			Pooled NO
	5 ppm	20 ppm	80 ppm	
Mechanical Ventilation	2/3 (66%)	5/8 (62%)	0/1 (0%)	7/12 (58%)
HFJV or HFOV	0/4 (0)%	1/8 (12%)	0/2 (0%)	1/14 (7%)
Resuscitation required	4/4 (100%)	8/8 (100%)	2/2 (100%)	14/14 (100%)

6.0.4.12.2 Disposition of subjects

The disposition of the 14 subjects successfully randomized into INO-03 is shown below in tabular form:

Table 6.0.4.12.2.1 Outcomes of subjects entered into the INO-03 trial.

	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Total # of subjects treated	4	8	2	14
Successful weaning of treatment gas within 14 days	4/4 (100%)	6/8 (75%)	0/2 (0%)	10/14 (71%)
Early discontinuation of therapy ('treatment failure')	0/4 (0%)	2/8 (25%)	2/2 (100%)	4/14 (29%)
Discontinuation due to cardiopulmonary instability	0/4 (0%)	2/8 (25%)	1/2 (50%)	3/13 (21%)
Discontinuation due to elevated methemoglobin	0/4 (0%)	0/8 (0%)	1/2 (50%)	1/14 (7%)
Discontinuation due to persistent elevation of NO_2	0/4 (0%)	0/8 (0%)	1/2 (50%)	1/14 (7%)

Table 6.0.4.12.2.2 Outcomes of subjects entered into the INO-03 trial.

	I-NO 5 ppm	I-NO 20 ppm	I-NO 80 ppm	Combined I-NO
Total # of subjects treated	4	8	2	14
Primary endpoint[a]	1/4 (25%)	5/7 (71%)	2/2 (100%)	8/14 (57%)
Death (within 28 days)	0/4 (0%)	0/7 (0%)	0/2 (0%)	0/14 (0%)
Initiation of ECMO	0/4 (0%)	2/8 (25%)	1/2 (25%)	3/14 (21%)
Neurological sequelae	0/4 (0%)	3/7 (43%)	0/2 (0%)	3/14 (21%)
Bronchopulmonary dysplasia	1/4 (25%)	2/7 (29%)	1/2 (50%)	4/14 (28%)
Death or initiation of ECMO[b]	0/4 (0%)	2/8 (25%)	1/2 (50%)	3/14 (21%)

a. Primary endpoint from the INO-01/ -02 trial: incidence of one of the PPHN major sequelae: death; initiation of ECMO; acute neurologic abnormalities; or development of bronchopulmonary dysplasia. See primary endpoints above for definitions.

b. Primary endpoint from the NINOS trial.

6.0.4.13 Safety Outcomes

6.0.4.13.1 Comparison of defined safety parameters up to 28 days

The primary safety concerns identified by the sponsor included the following:

1. Methemoglobin levels;
2. NO_2 concentrations;
3. Incidence of intraventricular hemorrhage and seizures;
4. Laboratory evaluations; and
5. Incidence and relationship of all adverse events and specific adverse events to I-NO.

1. Methemoglobinemia/ Elevated NO_2 levels

From table 6.0.4.13.1.1, one individual in the 80 ppm group developed increased methemoglobin levels. Subject 03-59003 developed methemoglobinemia (peak 5.7 ppm after 64 hours), and had the I-NO dose reduced. Methemoglobin levels fell, and the subject was weaned after 110 hours. The infant was discharged with both reactive airways disease and broncho-pulmonary dysplasia.