CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20845

STATISTICAL REVIEW(S)

Statistical Review and Evaluation

NOV 4 1999

NDA: 20,845
Applicant: INO Therapeutics, Inc.
Drug Name: Inhaled Nitric Oxide (Nitric Oxide)
Indication: Persistent pulmonary hypertension of newborn (PPHN)
Document Reviewed: Vol. 9.1, 9.6, 9.7, 9.10, 9.11, 9.20, 9.21

The sponsor's resubmission of original NDA of Nitric Oxide (May 26, 1999) includes the results from a new randomized clinical trial, known as CINRGI. The original NDA was reviewed by FDA earlier but was withdrawn by the sponsor on September 16, 1997. This statistical review focuses on the sponsor's new study (CINRGI) in the resubmission.

1. Outline of CINRGI study

Design of study

Study CINRGI was a multicenter, placebo-controlled, double-blinded study and involved 212 full-term and near-full term neonates with echocardiographic or clinical evidence of pulmonary hypertension. The **objective** of the study was to assess the safety and efficacy of inhaled NO added to the conventional therapy for PPHN as compared to conventional therapy alone.

According to the sponsor, the patients in this study were receiving diluted treatment gas with endotracheal tube NO concentrations of 0 ppm (for placebo patients) or 5-20 ppm (NO patients) according to the randomization. The randomization was stratified by patient disease status (CDH, MAS, Pneumonia, PPHN, RDS, and other). Inhaled NO was started at 20ppm. For all patients the ventilator settings were held constant over the first 30 minutes of treatment. Weaning of the treatment gas was done by decreasing the percent of treatment gas. Neonates who had a PaO2 (arterial partial pressure of oxygen) larger or equal to 60 mmHg and a pH 7.35-7.55 after being in the study for 4 hours had the treatment gas concentration reduced to 5 ppm for the remainder of the treatment period. Treatment gas was continued at 5 ppm until the FiO2 was <0.7, the patient had received 96 hours of treatment, or the patient was 7 days old, whichever came first. The submitted data indicate that the gas treatment for the first patient started on 3/3/96 and for the last one on 12/18/98.

Endpoints / hypotheses / analyses / sample size

The **primary efficacy endpoint** was defined as the "need for ECMO" (NFE) in the original protocol (December, 1995). In the protocol, the criterion for treatment with ECMO was defined (See Appendix 1). The sponsor later (Amendment 1) clarified the meaning of "need for ECMO" as actual "use of ECMO" (UOE) rather than "met ECMO criteria" (MEC). For this reason, no detailed information on MEC was collected according to the sponsor (Appendix 2).

Table 1.1 Mean methemoglobin by treatment group

time	placebo (95% CI)	NO (95% CI)
Baseline	0.78 (0.66, 0.90)	0.79 (0.67, 0.91)
Hour 4	0.78 (0.63, 0.91)	1.34 (1.17, 1.51)
Hour 24	0.58 (0.44, 0.72)	0.92 (0.77, 1.07)

Follow-up duration / Patient withdrawal

No maximum follow-up duration was clearly specified for the primary variable, the use of ECMO. The conditions of exiting the trial were specified in the protocol (Appendix 4).

Sponsor's result

A total of 248 neonates were entered into the study. Of these, 36 were enrolled into the pilot study phase of the trial that was randomized but not blinded. A total of 212 patients were randomized in the blinded trial. Among them, 26 patients (with disease status as CDH or other) had an enrollment diagnosis of lung hypoplasia and were analyzed separately from the other patients. The efficacy population for this trial consisted of remaining 186 patients.

The two treatment groups seemed to be comparable with respect to demographic factors: age, gender, weight, Apgar score, and race. The two groups seemed to be comparable with respect to most baseline prognostic characteristics except for a few, including baseline airleak, arterial pressure, PaO2 (arterial partial pressure of oxygen), SaO2 (percent of oxygen saturation of the arterial blood), and OI (oxygen index). The imbalance with respect to these factors were generally in favor of NO group. For instance, a numerically higher mean value of OI (43.9) was observed in the placebo group as compared to that (35.0) in the NO group. The sponsor's explanation for the imbalances is that for some patients, the baseline oxygenation measurements were taken after the treatment started.

Table 1.2 Demographic and some baseline characteristics

characteristics	Placebo, n=89	NO, n=97	p-value
Mean age in hours	29.9	30.0	0.95
males	52 (58.4%)	44 (45.4%)	0.08
Gestational weeks (PE)*	38.8	39.2	0.20
white	44 (49.4)	40 (41.1)	0.30
Mean admission weight (kg)	3.3	3.4	0.24
Airleak syndrome (yes)	22 (24.7%)	11 (11.3%)	0.021
Arterial pressure (mmHg)	55.8	51.6	0.019
PaO_2 (mmHg)	54.3	77.6	0.007
SaO_2 (%)	84.1	89.6	0.018
OI (cm H_2O/mm H_g)	43.9	35.0	0.011

* by physical examination

The sponsor's analysis (Cohran-Mantel-Haenszel adjusting for underlying disease) based on the intent-to-treat patient population indicated a statistically significant group difference in use of ECMO (31/98 for NO and 50/88 for placebo, p=0.001). The

difference was still statistically significant when adjusted for baseline difference controlling by PaO2 or by OI categories (p=0.007 from both adjusted analyses). The difference in use of ECMO between the two treatment groups was not statistically significant in the 26 patients with lung hypoplasia (p=1.000).

Table 1.3 Number of use of ECMO by treatment

Population	PaO2 or OI	Placebo (%)	NO (%)	p-value
All 186 patients, ITT	Whole range	51/89 (57.3)	30/97 (30.9)	0.001
All 186 suj., adjusted for baseline PaO2 (mmHg) categories	unknown	4/7 (57.1)	4/8 (50.0)	0.007
	≤ 30	11/11 (100)	5/6 (83.3)	
	30 to ≤ 50	20/40 (50)	13/32 (40.6)	
	50 to≤70	12/19 (63.2)	4/25 (16.0)	
	70 to ≤ 100	2/6 (33.3)	3/13 (23.1)	
	>100	1/5 (20.0)	2/14 (14.3)	
All 186 suj., adjusted for baseline OI (cm H_2O/mmHg) categories	unknown	9/13 (69.3)	4/15 (26.7)	0.007
	≤ 30	7/20 (35.0)	9/43 (20.9)	
	30 to ≤ 40	9/20 (45.0)	4/15 (26.7)	
	40 to≤ 50	7/11 (63.6)	6/11 (54.5)	
	>50	12/24 (50.0)	8/14 (57.1)	
Patients with lung hypoplasia	Whole range	13/15 (86.7)	9/11 (81.8)	1.00

The sponsor compared the outcomes in oxygenation status between the two treatment groups and claimed a statistically significant difference between the groups with respect to several indicators of oxygenation (Tables 34-37, the sponsor's study report). However, the sponsor's analyses used, instead of an ITT patient population, only the information from the completers. These analyses might introduce a selection bias and thus not preferred.

The sponsor's analyses based on the 6-month or 12-month follow-up data showed no statistically significant difference in hospitalization. Six month death rates in the two groups were not statistically significantly different (5/89 for placebo and 4/97 for NO, p=0.738).

2. Reviewer's results and comments

This reviewer compared the numbers of use of ECMO between the two treatment groups using all 212 randomized or using only the 186 patients without lung hypoplasia (LH) at the time of enrollment (Table 2.1). There was a statistically significant difference in rate of use of ECMO between the two treatment groups. According to the sponsor, it was assumed in the protocol that all ECMO therapy would be captured by recording ECMO use during the initial hospitalization. The submitted data indicated that durations of patient hospitalization (calculated by this reviewer as the difference between the time of discharge to home and the time of the initiation of treatment gas) range from 6 days to several months. Majority of use of ECMO occurred within 6 days after an initiation of treatment gas except one case (7 days after the initiation of the treatment gas) and several cases with missing time of discharge to home (10 subjects in placebo and 3 subjects in the NO group). The patient follow-up seemed to be complete and comparable between the groups (Table 2.2).

Table 2.1 Comparison of incidence in use of ECMO

population	placebo	NO	p-value/not stratified*	p-value /stratified**
	# of ECMO / n (%)	# of ECMO / n (%)		
exclude LH	50/88 (56.8)	31/98 (31.6)	0.001	0.001
include LH	62/102 (60.8)	41/110 (37.3)	0.001	0.001

*X^2-test, ** Cohran-Mantel-Haenszel controlling by strata (underlying disease)

Table 2.2 time to discharge (day)

ECMO / treat / n	Quantiles				
	0%	25%	50%	75%	100%
No / pla. / 34	6	12	14	26	114
No / NO / 64	7	13.5	17	22.5	64
Yes / pla. / 44	10	17	21	30.5	54
Yes / NO / 27	11	19	26	32	46

The question now is whether or not the observed group difference in use of ECMO can be attributed to the effect of nitric oxide treatment. To answer it, this reviewer focused on the following issues.

(i) Unbfinding of treatment code

As explained before, there are sufficient grounds to suspect that the CINRGI study was largely unblinded. This is especially worrisome knowing that the primary endpoint, UOE (use of ECMO) can be subjective and initiation of ECMO depends on an investigator's judgement and discretion. It is possible for biases of the investigators to be introduced to the trial. For this study, extra cautions must be taken in examining and interpreting the trial results.

(ii) Potential bias due to delaying initiation of ECMO

To evaluate the effect of the NO treatment on use of ECMO, it is important to examine whether or not there was a delay of initiation of ECMO in the NO group as compared to placebo. The delay could be a result of more aggressive initiation of ECMO for placebo patients and/or more reluctant initiation of ECMO for the NO treated patients. If there was a delay in the NO group, the observed lower rate of use of ECMO in the NO group could be a direct consequence of the delay since with the delay of initiation of ECMO, a patient might pass the episode of need for ECMO and never needed ECMO again. In this case, one can not relate the effect of the nitric oxide treatment to the lowered rate of ECMO use in the NO group without knowing the causes of the delay. If there were causes unrelated to the effect of the NO treatment, the observed treatment effect would be confounded partially or completely with biases attributed to the delay from these causes.

A significant delay in initiation of ECMO in the NO group as compared to placebo was suggested by the data (Table 2.3a). The median duration from initiation of treatment gas to initiation of ECMO was 3.6 hours for placebo and 10.4 hours for the NO group. There

is a shift in distribution of the time to initiation of ECMO for the NO group as compared to the placebo group. As discussed above, such a delay can contribute to the observed lower ECMO rate in the NO group. A similar pattern can be seen among the patients who had OI>40 at baseline and received ECMO later. These patients might need ECMO urgently, but ECMO was initiated later in the NO group as compared to the placebo (Table 2.3b). In order to conclude a treatment effect of NO on use of ECMO, **one must demonstrate that the only cause of the delay was the effect of the nitric oxide treatment, or to demonstrate a negligible impact of the delay due to other treatment unrelated reasons.**

Table 2.3a Time to initiation of ECMO (hour)

Treatment / n	Quantiles				
	0%	25%	50%	75%	100%
Placebo / 50	1.0	2.6	3.6	9.5	100.2
NO / 31	2.1	3.3	10.4	18.8	150.8

p-value for the difference is 0.0149 (Wilcoxon two-sample test)

Table 2.3b Time to initiation of ECMO (hour) in patients with ECMO use and with baseline OI>40

Treatment / # of patients with > 40 OI at baseline	Quantiles				
	0%	25%	50%	75%	100%
Placebo / 25	1.0	2.4	3.0	6.9	85.5
NO /14	2.1	2.9	9.3	14.5	100.7

Many factors could cause the delay of initiation of ECMO in the NO group in CINRGI study. As discussed in (i), the investigators in this study were very likely to know patient treatment assignments, had ability to determine the time of initiation of ECMO, and might consciously or unconsciously delay initiation of ECMO for qualified patients in the NO group or accelerate initiation of ECMO for patients in placebo. If this was the case, the true effect of the NO treatment would be confounded with the bias introduced by the differentiatial initiation of ECMO between the two treatment groups. The delay of initiation of ECMO could also relate to a treatment effect. For instance, such a delay might be due to a patient's improvement in oxygenation. In this case, the observed lowered ECMO rate in the NO group could be viewed as a reflection of the effect of NO on oxygenation.

The CINRGI study does not provide sufficient information to answer the question regarding delay of initiation of ECMO in the NO group. For example, the sponsor did not collect information on the date and time when a patient met ECMO criteria at the first time (Appendix 2). This information is important to explore the nature and the cause of the delay, since MEC (met ECMO criteria) is more objective than use of ECMO.

To explore the difference in initiation of ECMO further indirectly, this reviewer compared the two treatment groups with respect to two important indicators of oxygenation: oxygenation index (OI) and PaO2. It was noted that there was an imbalance between the groups with respect to baseline OI and PaO2. In this reviewer's analysis, ITT patient population was used and the comparisons were made based on change from baseline, using LOCF imputation and t-test with unequal variances, rather than an

endpoint measurement. The following table summarizes the mean changes in OI and PaO2 during the 24 hour period.

Table 2.4 Analysis of change in OI and PaO2

OI			
Time	Placebo (n, Δ)	NO (n, Δ)	Nominal p-value*
Baseline	(n=75) 43.9	(n=83) 35.0	0.0119
30 min	(n=65) –4.4	(n=71) –11.8	0.0230
1 hour	(n=69) –4.9	(n=81) –12.0	0.0679
4 hours	(n=71) –4.9	(n=82) –13.3	0.0374
12 hours	(n=71) –8.1	(n=82) –14.9	0.0943
24 hours	(n=71) –8.8	(n=82) –15.1	0.1198
PaO2			
Time	Placebo (n, Δ)	NO (n, Δ)	Nominal p-value*
Baseline	(n=81) 54.3	(n=90) 77.6	0.0055
30 min	(n=75) 23.7	(n=80) 57.8	0.0093
1 hour	(n=78) 38.3	(n=89) 63.9	0.1023
4 hours	(n=79) 44.4	(n=89) 63.6	0.2422
12 hours	(n=79) 45.3	(n=89) 61.4	0.3005
24 hours	(n=79) 36.6	(n=89) 46.5	0.4874

* for group difference

The analyses fail to conclude that there is a sustained treatment effect of NO on oxygenation. Without any p-value adjustment for the multiple time points, the group differences in OI and PaO2 were significant only at Hour 0.5 (OI and PaO2) and Hour 4 (OI only). With Boferroni adjustment of p-values across the different time points, only the group difference in PaO2 at 30 minute was statistically significant. The results of the analysis might suggest that the NO treatment affected patient oxygenation more rapidly as compared to the conventional therapy used (as the background therapy) in placebo. The observed reduction in rate of use of ECMO in the NO group might be a consequence of this rapid effect.

(ii) Unbalanced baseline

As pointed out, there were significant imbalances in favor of the study drug with respect to baseline OI (p=0.011), PaO2 (p=0.007) and SaO2 (p=0.018). Fitting a logistic regression model with treatment and these baseline oxygenation indicators as covariates, a significant effect of baseline OI on ECMO rate was found (p=0.0001). The p-value for the treatment effect on ECMO rate adjusted for the baseline OI is p=0.0443, a large increase from p=0.001 without baseline adjustment. The lack-of-fit test did not indicate a misspecified model. When the patients' disease status (the strata) was included in the model, the p-value for the treatment effect was 0.0460. Two similar analyses were done using the –2 hour and –4 hour OI measurement to impute missing baseline OI. The resulting p-values were 0.0147 (w/o strata in the model) and 0.0158 (with the strata in the model).

Logistic regression adjusted for baseline OI

Analyses	Placebo # of ECMO / n (%)	NO # of ECMO / n (%)	Odds ratio NO vs. control	p-value
W/O strata,	50/88 (56.8)	31/98 (31.6)	0.492	0.0443
With strata	50/88 (56.8)	31/98 (31.6)	0.492	0.0460
W/O strata; -2, -4 hr OI in bsl	50/88 (56.8)	31/98 (31.6)	0.443	0.0147
With strata; -2, -4 hr OI in bsl	50/88 (56.8)	31/98 (31.6)	0.444	0.0158

The reviewer and sponsor's analyses suggest a difference in the rate of use of ECMO between the two groups even adjusted for the imbalance of baseline OI.

3. Reviewer's Conclusion

Study CINRGI was to assess the efficacy and safety of inhaled NO added to the conventional therapy as compared to the conventional therapy alone in patients with PPHN. A beneficial effect of the NO treatment was supposed to be indicated by a reduction in rate of use of ECMO (the primary endpoint).

The protocol or protocol amendments of Study CINRGI did not clearly address many important issues like endpoint definition, blinding procedure, patient follow-up period, time of randomization and study termination. Since the protocol is a general guidance for the trial, a negative impact of the ambiguity in the protocol on the conduct of the study can be anticipated.

The analyses of use of ECMO in this study indicate a group difference with respect to rate of use of ECMO. The p-values for such a difference range from 0.001 to 0.0460, depending on whether or not there is an adjustment for unbalanced baseline prognostic factors and the methods for the adjustment. To conclude a treatment effect based on the observed group difference, several aspects of the trial must be carefully examined.

The important issues identified in this review include (i) possibly unblinding treatment codes and (ii) potential bias due to the difference in time of initiation of ECMO between the two treatment groups. The submitted data suggest a significant delay in initiation of ECMO in the NO group as compared to placebo. Since the observed lower rate of use of ECMO in the NO group could be solely due to such a delay and the delay could be due to many causes, relevant or irrelevant to the NO intervention, it is very difficult to relate the observed group difference in rate of use of ECMO to the effect of the NO treatment in a clear-cut manner, knowing the potential unblinding in this trial and the possibility for an investigator to hold the initiation of ECMO temporarily for a patient. To conclude that there is a beneficial effect of the NO treatment on use of ECMO, one must show that the reduction in ECMO use in the NO group was solely attributed to the effect of the nitric oxide treatment. Unfortunately, the current database does not provide sufficient information for such a conclusion.

Although it is impossible for this reviewer to conclude the effect of the nitric oxide treatment on lowering rate of ECMO use, some analyses of oxygenation seemed to

suggest that the NO treatment yield a more rapid effect on oxygenation as compared to the conventional therapy. This might further suggest that the observed lower ECMO rate in the NO group as compared to placebo be a consequence of the rapid effect of the nitric oxide on oxygenation. Again, the observation about the possible relationship between the ECMO use and the improvement in oxygenation, by no means, is conclusive since the ambiguity of the efficacy outcome for the primary endpoint, and the post-hoc nature and the multiplicity involved for the secondary endpoints for oxygenation.

/S/

Lu Cui
Ph.D., Mathematical Statistician
11/3/99

* This reviewer has discussed the issues related to this NDA with Dr. Douglas Throckmorton.

Concur: Dr. H.M. James Hung /S/ 11/3/99

Dr. George Chi /S/ 11/4/99

cc:
NDA #20,845 Nitric Oxide
HFD-110
HFD-110 / Dr. Lipicky
HFD-110 / Dr. Stockbridge
HFD-110 / Dr. Throckmorton
HFD-110 / Ms. McDonald
HFD-344 / Dr. Barton
HFD-710 / Dr. Chi
HFD-710 / Dr. Mahjoob
HFD-710 / Dr. Hung
HFD-710 / Dr. Cui
HFD-710 / Chron.

lcui/20,845/d:\cui\review\nitric\rpt2.doc/5945302

Appendix 1 (ECMO Criteria)

APPENDIX A - Criteria for ECMO

1. Weight >2000 g
2. Gestational age ≥34 weeks
3. Mechanical ventilation for <10 days.
4. Reversible lung disease
5. No congenital heart, neurological, or multisystem disease (exceptions include small ASD/VSD lesions)
6. No intracranial hemorrhage >grade 1.
7. No severe coagulopathy
8. No severe asphyxia (10 minute Apgar 0-2 or initial base excess greater than -20)
9. Failure of maximal medical management (alkalosis, pressor support, vasodilator trial)
10. Plus one of the following:
    a. Oxygenation Index (MAP x FiO_2 x 100/PaO_2) >40 for 3 of 5 blood gases at least 1/2 hour apart
    b. PaO_2 <40 for 4 hours despite maximal medical management
    c. PaO_2 <35 for 1 hour despite maximal medical management
    d. Progressive hemodynamic deterioration unresponsive to maximal medical management (colloid infusions, catecholamine drugs, etc.)

Appendix 2
(The sponsor's response)

T BY:

INO Therapeutics, Inc.
An AGA Healthcare Company

facsimile transmittal sheet / fax number (908) 238-6633

To: Dr. Lu Cui	
From: Richard N. Williams, Ph.D.,	Tel: 908-238-6622
Company: FDA	Date: August 5, 1999
Fax number: 301-594-5494	No. of pages including cover: Two
Phone number: 301-594-5302	cc:
Re: Written Confirmation Regarding ECMO Criteria	
notes/Comments:	

NDA 20-845

Nitric Oxide

Dear Dr. Lu Cui,

As per your request, please find attached the written explanation of why we did not collect information on the temporal aspects of patients meeting ECMO criteria.

Please let me know if this answers your question, or if you require additional explanation.

Regards,

RW

Richard Williams, Ph.D.

The primary endpoint for the CINRGI Trial is the number of patients receiving ECMO.[1] The primary endpoint in the original version of the protocol was worded as "the need for ECMO."[2] The assumption of the author and Principal Investigators was that if a patient received ECMO, the patient needed it. It was understood by all involved in the trial, including investigators and INO Therapeutics, that the intention of the endpoint was the actual receipt of ECMO. However, in the review of the NINOS Trial in Ohmeda's (now INO Therapeutics, Inc.) original NDA submission (#20-845), a distinction was made between meeting ECMO criteria and the actual receipt of ECMO. In order to avoid similar confusion with the CINRGI Trial, the protocol was amended, changing the wording of the primary endpoint to "**treatment with ECMO.**"[3] The Analytic Plan issued to the FDA by Reese Clark, MD in December 1998 also specifically states the primary endpoint of the trial as the "**number of patients who receive ECMO.**"

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[1] NDA #20-845,. Volume 9.6, page 1452
[2] NDA #20-845,. Volume 9.7, page 1791
[3] NDA #20-845,. Volume 9.7, pages 1825,1844

Appendix 3
(Sample data collecting sheet)

Appendix 4
(Exiting criteria)

The patient will be exited from the study if:

1. The patient meets criteria for treatment with ECMO or is placed on ECMO.

   a. Oxygenation Index (MAP x FiO_2 x 100/PaO_2) >40 on 3 of 5 blood gases drawn 30 minutes apart

   b. PaO_2 <40 for 2 hours despite maximal medical management

   c. PaO_2 <35 for 1 hour despite maximal medical management

   d. Progressive hemodynamic deterioration (mean blood pressure <35) unresponsive to maximal medical management (colloid infusions, vasopressors, etc).

OR

2. The patient has an inadequate response to treatment gas or fails to tolerate being weaned from the study gas

   a. PaO_2 <60 on FiO_2 of 1.0 and optimized ventilator settings after 24 hours of treatment. Maximum dose =20 ppm for first 24 hours in study.

   b. PaO_2 <60 on FiO_2 of 1.0 and optimized ventilator settings 24-96 hours of treatment. Target dose =5 ppm during 24-96 hours in the study.

   c. Failure to tolerate a decrease in the treatment gas to 5 ppm after 24 hours (i.e., PaO_2 <60 on FiO_2 of 1.0 and optimized ventilator settings after 24 hours of treatment. Maximum dose =20 ppm for 0-24 hours in the study). See details in NO inhalation protocol - 7C

   d. Failure to tolerate discontinuation of treatment gas after 96 hours (i.e., PaO_2 <60 on FiO_2 of 1.0 and optimized ventilator settings after 96 hours of treatment. Target dose =5 ppm for 24 to 96 hours in the study). See details in NO inhalation protocol - 7C

OR

3. Initiation of the treatment gas is associated with a sustained deterioration in the patients gas exchange that is due to initiation of the treatment gas.

   a. PaO_2 decreases by more than 10 torr

   b. PaO2 falls below 40

c. The oxygen saturation decreases by more than 5%

d. The oxygen saturation falls below 88%

OR

4. Toxicity develops (Methemoglobin > 4%, NO_2 > 5 ppm)

5. Parents withdraw consent
6. The patient is discovered to have met a patient exclusion criteria. (e.g., diagnosis of congenital heart disease, or to have a lethal congenital anomaly)