1 TITLE PAGE

CLINICAL STUDY PROTOCOL

INS-3: A Phase 3, Randomized, Double-Masked, Placebo-Controlled Study of the Efficacy and Safety of myo-Inositol 5% Injection to Increase Survival without Severe Retinopathy of Prematurity (Reduce-ROP) in Extremely Premature Infants

IND:	IND 70510
Investigational Product:	myo-Inositol 5% Injection
Date:	May 2, 2013
Development Phase:	3
Study Design:	Randomized, double-masked trial of myo-Inositol 5% Injection 80 mg/kg/day or placebo administered intravenously in divided doses every 12 hours to premature infants of $<28^{0/7}$ weeks gestation. Once enteral feeding is established, the same study drug dose and formulation will be converted to enteral administration. Study drug will be administered until the earliest of 34 weeks postmenstrual age, 10 weeks chronologic age, or discharge. ROP findings will be followed until final acute ROP status is reached, or 55 weeks postmenstrual age.
Investigators:	Investigator information is on file with the Sponsor at the *Eunice Kennedy Shriver* National Institute for Child Health and Human Development (NICHD). The currently active Inositol Subcommittee of the Neonatal Research Network (NRN) includes:

Dale Phelps, MD, Chair	Abhik Das, PhD
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The current Network Participating Centers and their Principal Investigators are listed on page 3 and in the MOP:

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This study will be conducted in compliance with the protocol, Good Clinical Practice and all other applicable regulatory requirements, including the archiving of essential documents.

1.1 Synopsis

NICHD	Protocol Number: INS-3
Name of Study Drug: myo-Inositol 5% Injection	Phase of Development: 3
Name of Active Ingredient: myo-Inositol	Date of Protocol Synopsis: May 2, 2013
Protocol Title: INS-3: A Phase 3, Randomized, Double-Masked, Placebo-Controlled Study of the Efficacy and Safety of myo-Inositol 5% Injection to Increase Survival without Severe Retinopathy of Prematurity in Extremely Premature Infants (Reduce-ROP)	
Objective(s): To determine the efficacy and safety of myo-Inositol 5% Injection compared to placebo for increasing the probability of survival without severe Retinopathy of Prematurity in premature infants <28 weeks' gestation followed through the determination of final/acute ROP status or 55 weeks PMA.	
Investigator(s): Dale L. Phelps, MD on behalf of the Inositol Subcommittee of the *Eunice Kennedy Shriver* National Institute for Child Health and Development (NICHD) Neonatal Research Network (NRN)	
Study Site(s): Details on file with NICHD: 18 NICHD NRN Centers (approximately 44 sites) in the United States Case Western Reserve University, PI: Michele C. Walsh, MD Children's Mercy Hospital, PI: William Truog, MD Cincinnati Children's Hospital Medical Center, University of Cincinnati, PI: Kurt Schibler, MD Duke University, PI: Ronald Goldberg, MD Emory University, PI: Barbara J. Stoll, MD Indiana University, PI: Brenda Poindexter, MD Research Institute at Nationwide Children's Hospital, PI: Leif Nelin, MD Stanford University, PI: Krisa Van Meurs, MD University of Alabama, PI: Waldemar A. Carlo, MD University of California, Los Angeles, PI: Uday Devaskar, MD University of Iowa, PI: Edward F. Bell, MD University of New Mexico, PI: Kristi L. Watterberg, MD University of Pennsylvania, PI: Barbara Schmidt, MD University of Rochester, PI: Carl T. D'Angio, MD University of Texas at Houston, PI: Kathleen A. Kennedy, MD University of Texas at Southwestern, PI: Pablo J. Sanchez, MD Wayne State University, PI: Seetha Shankaran, MD Women and Infant's Hospital of Rode Island, Brown University, PI: Abbot Laptook, MD	
Study Population: Premature infants of $<28^{0/7}$ weeks' gestation.	
Number of Subjects to be Enrolled: Approximately 1760 infants	

Methodology: This is a randomized, double-masked, placebo-controlled study designed to determine the effectiveness of myo-Inositol 5% Injection to increase survival without severe ROP among premature infants <$28^{0/7}$ weeks' gestation. Infants will be randomized in a 1:1 allocation, stratified by center and by 2 gestational age (GA) risk strata (<26 and 26-27 weeks) to receive either myo-Inositol 5% Injection or placebo. Assessments performed during the study include usual newborn intensive care procedures including repeat eye examinations until acute ROP status is final (which often extends after discharge) up to 55 weeks PMA, measurements of growth and the collection of clinical diagnoses throughout hospitalization to evaluate other common morbidities of extreme preterm birth. Adverse events will be recorded from treatment initiation until 7 days after the last dose of study drug, and concurrent medications will be recorded from 24 hours prior to randomization until 7 days after the last dose of study drug, or until discharge or transfer if sooner. Longer-term data will be collected using the separate NRN Follow-up Program Protocol at 22-26 months corrected age, including growth, neurodevelopmental testing, overall health status, re-hospitalizations, surgeries and diagnoses including ophthalmologic diagnoses and treatments following discharge.

Diagnosis and Main Criteria for Inclusion/Exclusion:

Main Inclusion:
Premature <28 weeks' gestation, survival at least 12 hours, informed consent, enrollment by 72 hours of age.

Main Exclusion:
Major congenital anomalies, any congenital eye anomalies, evidence of intrauterine congenital infections or moribund condition.

Investigational Product:	myo-Inositol 5% Injection (isotonic, preservative-free, sterile 5% solution of myo-inositol in water containing 0.5gm sodium chloride per liter (8.55mM), pH 6.5-7.5
Dose:	80 mg inositol/kg/day administered in divided doses every 12 hours.
Mode of Administration:	Intravenous until full feedings are established, then enteral.
Reference Therapy:	5% glucose(dextrose) USP for intravenous infusion
Dose:	80 mg glucose/kg/day administered in divided doses every 12 hours.
Mode of Administration:	Intravenous until full feedings are established and then enteral.

Duration of Treatment: Study drug will be administered daily, starting within 12-72 hours of birth and continued until the earliest of 34 weeks postmenstrual age (PMA; PMA = GA at birth plus chronologic age in weeks), 10 weeks chronologic age, or the time of hospital transfer or discharge.

Criteria for Evaluation:

Efficacy: The primary outcome is survival without severe ROP through Acute/Final ROP status determination (favorable); versus development of severe ROP or death prior to reaching Acute/Final ROP status (unfavorable) or 55 weeks PMA. Severe ROP is defined as Type 1 or more severe ROP in either eye confirmed by a second evaluation. Secondary outcome variables include bronchopulmonary dysplasia (BPD, NICHD physiologic definition), BPD or death from BPD prior to 37 weeks PMA, all cause death, any ROP, Type 2 ROP through the time that Acute/Final ROP status is reached, and severe intraventricular hemorrhage (IVH).

Safety: Prospective monitoring of neonatal morbidities and adverse events.

Statistical Methods: Sample size calculations are based on the primary analysis of the single trial for scientific publication. However, if the results demonstrate a clear benefit, additional analyses will be conducted for Regulatory Purposes as recommended in the FDA Guidance for Industry as described below. This prioritization of analyses precludes the necessity of adjusting alpha for the two study purposes. A sample size of 1760 infants provides 90% power at an overall α level of 0.05 with one formal interim analysis for detecting a ≥ 0.07 absolute reduction in unfavorable ROP status prevalence (the estimated clinically significant reduction due to treatment). Randomization will be stratified by center and gestational age strata.
The single formal interim analysis of efficacy will be conducted after approximately 1000 infants have been enrolled and reached primary endpoint. The interim analysis will be conducted using the overall study population and the primary analytic approach for publication. Cut-off p-values for testing for efficacy at this interim analysis will be determined based on a Bonferroni-type correction for multiple comparisons with a nominal α of 0.0001 at the interim analysis and a nominal α of 0.0499 for the final analysis. Specifically, if the p-values are <0.0001 for the treatment comparison for the primary outcome and <0.001 for the treatment comparison for mortality alone at the interim analysis, then the DSMC can recommend stopping enrollment.

Efficacy: The primary hypothesis for publication will be tested using a score test of the treatment effect in the Poisson regression model where the null hypothesis of no treatment effect on survival without severe ROP is rejected at either the single interim or final analysis with a p-value consistent with an overall Type 1 error rate of 0.05 after a Bonferroni-type correction for multiple comparisons.
For regulatory submission purposes, the enrolled population will be administratively split into two pre-specified regulatory sub-studies of up to approximately 880 infants each. Each sub-study has 69-83% power to detect an outcome reduction of 0.08-0.09 in unfavorable ROP status prevalence using a 2-sided test with a type 1 error of 0.05. Sensitivity analyses for both the overall study as well as sub-studies will be conducted in order to test the robustness of the findings.

Safety: Safety outcomes that will be analyzed at the completion of the study include rates of adverse events including serious adverse events, deaths and related adverse events; rates of clinical outcomes; and concomitant medication use.
While the study is ongoing the NRN DSMC will monitor safety at a preplanned frequency, when approximately 25%, 50% and 75% of enrolled subjects have completed study therapy. At these reviews, the DSMC can decide to recommend suspending or stopping enrollment if a safety concern is identified. However, no formal analyses of efficacy data will be conducted as part of these safety monitoring activities and the DSMC will not be able to recommend stopping the study for efficacy.

1.2 List of Abbreviations and Definition of Terms

Abbreviations

AN	Abbott Nutrition Division, Abbott Laboratories and/or their designated successor company
BPD	Bronchopulmonary Dysplasia
CRF	Case Report Form
DMS	Data Management System
DSMC	Data Safety and Monitoring Committee
EDC	Electronic Data Collection
ETROP	Early Treatment for Retinopathy of Prematurity Study
FDA	Food and Drug Administration
GA	Gestational Age
GCP	Good Clinical Practice
GDB	Generic DataBase of the NICHD NRN
GI	Gastrointestinal
ICH	International Conference on Harmonization
ICROP	International Classification of ROP
IGF-1	Insulin-like Growth Factor-1
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	Intention To Treat (population)
IV	Intravenous
IVH	Intraventricular Hemorrhage
MDI	Mental Developmental Index
MOP	Manual of Procedures
NDI	NeuroDevelopmental Impairment
NEC	Necrotizing enterocolitis
NICHD	*Eunice Kennedy Shriver* National Institute for Child Health and Human Development
NICU	Neonatal Intensive Care Unit
NRN	Neonatal Research Network
NS	Not Significant
PDA	Patent Ductus Arteriosus
PDI	Psychomotor Developmental Index

PMA	Postmenstrual Age
PVL	Periventricular Leukomalacia
RCT	Randomized Controlled Trial
RD	Risk Difference
RDS	Respiratory Distress Syndrome
ROP	Retinopathy of Prematurity
RTI	Research Triangle Institute
SD	Standard Deviation
sROP	Severe Retinopathy of Prematurity
TPN	Total Parenteral Nutrition
VEGF	Vascular Endothelial Growth Factor
USP	United States Pharmacopoeia

Definition of Terms

Corrected Age	Age, in months, after an infant's full term due date
Postmenstrual Age (PMA)	GA at birth plus chronologic age, in weeks: relates to full term = 40 weeks
Severe ROP	meeting criteria for ROP intervention: that is, ETROP defined "Type 1" ROP, or ROP of worse severity

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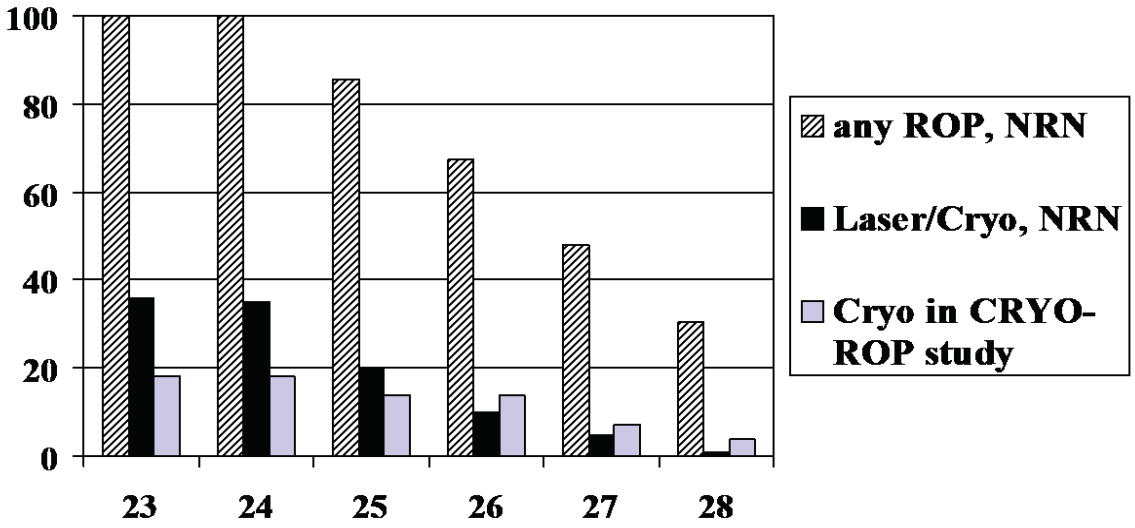
3 INTRODUCTION

3.1 Retinopathy of Prematurity

Retinopathy of prematurity (ROP) is a disorder of the developing retinal vasculature occurring in premature infants, that can progress to blindness. It occurs primarily in the lowest gestation infants and is a leading cause of blindness in the pediatric population both in developed, and increasingly in developing countries (Delport 2002, Aggarwal 2002, Gilbert 2008, Quinn 2010). The incidence of any ROP is inversely proportional to gestation, occurring in over 95% of 23- to 24-week infants, but only 30% of 28-week survivors (Figure 1). In the most recent large study, from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN), that determined the rate of severe ROP (receiving surgical peripheral retinal ablation to prevent possible retinal detachment), an average of 13% of the 24- to 27-week gestational age (GA) survivors developed severe ROP (SUPPORT 2010).

Figure 1: Rates of ROP in Survivors (NRN Unpublished Data: 2001 to 2003)



Interventions to prevent ROP have been a major research goal. Unfortunately the antioxidant vitamin E, d-penicillamine, and reduction in ambient light have not proven effective. Duration of oxygen exposure strongly correlates with ROP, and reasonable restriction of oxygen reduces severe ROP. However, if restriction is lowered to the bottom half of the accepted range, there is evidence of increased mortality (SUPPORT 2010). When severe ROP is detected by repeated indirect ophthalmology examinations, timely peripheral retinal ablation in severe cases can reduce progression to retinal detachment. However, even ROP that regresses (heals) spontaneously leads to increased rates of myopia, strabismus, amblyopia and acuity loss that is not correctable with lenses (Dobson 1994, CRYO-ROP 1996, CRYO-ROP 2001, Kushner 1982). Therefore, prevention of severe ROP, or even any ROP, must be the research goal for these infants.

3.2 Inositol and ROP

During research on inositol to improve surfactant insufficiency in preterm infants with respiratory distress syndrome (RDS), a decrease in the incidence of severe ROP, as well as acute and chronic lung disease, mortality and intracranial bleeding were all observed with inositol treatment (Hallman 1992/1990, Hallman 1986/1987; see Section 3.3.1). The effect on ROP was not expected, and it is possible that the inositol was sufficiently effective in reducing pulmonary morbidity that it lowered the risk for ROP as a secondary effect. However, polyphosphoinositides and inositol polyphosphates are essential in the chain of mediators leading to vascular growth, involving vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF-1) and growth hormone (Xia 1996, Romero 1993, Smith 1997, Tomic 1999). It is not known whether the lack of inositol or its derivatives may be rate limiting in the expression of VEGF or IGF-1, or in many other events that interfere with the retinal vascular development (Luttun 2002). Inositol increases cell growth in culture, endothelial cells in particular. Inositol may thus provide critical intermediates required for signal trafficking during the differentiation and growth of vascular cells. If inositol is permissive for sustaining the health of the endothelial cells of the growing retinal vasculature during its critical transition to extrauterine life, it may explain why infants with low inositol levels for age have more ROP (Friedman 2000).

The consistency of the findings across both of Hallman's trials and reduced ROP despite increased survival of sicker infants requires further investigation. Cochrane Library Meta-analyses published in 1997 and updated in 2003 and 2012 all concluded that: "Inositol supplementation results in statistically significant and clinically important reductions in important short-term adverse neonatal outcomes. A multi-center, randomized controlled trial (RCT) of appropriate size is justified to confirm these findings (Howlett 2003, 2012)."

3.2.1 Inositol in Human Infants

The myo-inositol isomer of inositol is the most abundant 6 carbon sugar alcohol in the body. Most inositol is found as the simple molecule, while small quantities are mono-, di-, tri- or tetraphosphate or pyrophosphate esters. A significant fraction is found as a component of the intracellular signalers, phosphoinositides. Phosphatidylinositol is a small but important component of surfactant, and inositol improves lung immaturity in animal models when supplemented before or after birth.

Regulation of inositol concentrations in serum and tissue is controlled by intake, endogenous synthesis, catabolism, intracellular transport, metabolism, and excretion. Inositol levels in utero are 2 to 100 times higher than in adults, and the levels decrease towards full term (Bry 1991, Carver 1997). High fetal serum levels are maintained by endogenous synthesis, low gestation-regulated inositol oxygenase (catabolism), and amniotico-entero-fetal recirculation of inositol that conserves the sugar that is lost to fetal urine (Hallman 1984, Bry 1991).

The fetus, infant, and adult synthesize 150-250 mg/kg/day of inositol endogenously, (Brown 2009) and it is amply available in the diet from fruits, vegetables, meat, grains and for the infant, human milk and supplemented formulas. Inositol is readily absorbed from the intestine, and despite high endogenous production, serum levels remain responsive to enteral supplementation in all ages tested (Holub 1986).

After birth, serum inositol levels fall when infants receive only intravenous fluids (which do not contain inositol), or enteral milk feeds that are not supplemented with inositol (Bromberger 1986, Carver 1997, Hallman 1992, Friedman 2000). Preterm infants fed human milk or an inositol-enriched formula maintain serum levels on the normally declining slope seen in utero with maturation (Bromberger 1986, Friedman 2000). During postnatal growth, serum inositol levels continue to decrease, despite high inositol intake, possibly due to the development in the renal cortex of the catabolic enzyme, inositol oxygenase. During the INS-2 study of inositol (see Section 3.3.2), it was observed that despite ongoing high inositol supplementation, plus increasing enteral formula/human milk intake, the amount of inositol excreted in the urine decreased over 6 weeks (as serum levels also fell), supporting the hypothesis that the decrease in urine inositol is due to maturation of the catabolic pathway and perhaps also reduced endogenous production (Phelps 2007, Abstract).

3.2.2 History of the Current Inositol Research

There is no FDA-approved IV inositol formulation to prevent or reduce the severity of ROP in premature infants. Given the favorable results observed in published trials of this compound and its strong safety profile, the NICHD NRN initiated a research program to determine if inositol supplementation administered soon after birth in extremely premature infants is safe and reduces the incidence of severe ROP, as well as other common morbidities seen in this population. The lack of an IV inositol formulation delayed the program; however, AN reviewed the proposal and committed to prepare and supply inositol for IV/enteral administration to the NICHD NRN for clinical trial use. The NICHD NRN and AN established a Clinical Trial Agreement to study the efficacy and safety of myo-Inositol 5% Injection in this population, with NICHD as the sponsor, and the NRN responsible for designing and conducting the studies using drug supplied by AN.

Two Phase 2 pharmacokinetic studies (INS-1 and INS-2) evaluating the single- and multiple-dose pharmacokinetics, safety, and dose-ranging of inositol administration in premature infants have been completed by the NICHD NRN. Results of these studies indicate that the treatment is safe and support the dose of 80 mg/kg/day selected for efficacy evaluation in the Phase 3 program.

3.3 Studies of Inositol in Premature Infants

Three randomized, controlled studies of inositol supplementation in premature infants were identified in the published literature. Results of the individual studies and a Cochrane meta-analysis are presented in Section 3.3.1. Results of the Phase 2 single-dose (INS-1) and multiple-dose (INS-2) pharmacokinetic studies sponsored by the NICHD NRN in support of the inositol clinical development program are described in Section 3.3.2.

3.3.1 Previous Studies of Inositol from the Published Literature

Hallman 1986/Hallman 1987

Hallman et al. reported results of a randomized, placebo-controlled study of inositol supplementation in 74 preterm infants (<2000 g birth weight) who required mechanical ventilation for treatment of RDS (Hallman 1986); additional biochemical studies of serum, urine,

and surfactant inositol levels were published in the subset of those who had only enteral administration, and had serum and urine levels collected (Hallman 1987). At 12-48 hours after birth, the infants were randomized to receive 10 days of inositol (enteral: 160 mg/kg/day divided into 4 doses given every 6 hours, or if unable to tolerate enteral administration, 120 mg/kg/day divided into 4 doses given IV every 6 hours) vs. placebo (5% glucose). The study was conducted in the pre-surfactant era and before the International Classification of ROP (ICROP 1984) was published, so that no in-hospital ROP examinations were performed. The study was designed to determine if inositol alleviated respiratory failure and increased survival free of bronchopulmonary dysplasia (BPD). No safety concerns were identified, and benefits were observed including a statistically significant greater proportion of inositol-treated infants who survived without BPD compared to those who were treated with placebo (see Table 1).

Table 1. Study Outcomes for Hallman et al. 1986

Number of infants	Inositol	Placebo	p-value
	37	37	
Gestational age, weeks, mean (SD)	29.5 (2.0)	29.5 (2.1)	
Birth weight, grams, mean (SD)	1276 (321)	1256 (387)	
Died (% of enrolled)	5 (13.5%)	10 (27.0%)	
BPD[a] (% of enrolled)	5 (13.5%)	11 (29.7%)	
Survival without BPD (% of enrolled)	28 (75.6%)	18 (48.6%)	<0.02
Retrolental Fibroplasia[b] (% of survivors)	1 (3.1%)	4 (14.8%)	
IVH: Grade III or IV (% of enrolled)	2 (5.4%)	5 (13.5%)	

[a] Oxygen at 28 days + x-ray
[b] Pre-ICROP, only a single eye exam was conducted at 9-13 months.

Hallman 1992/Hallman 1990

Hallman et al. conducted a second randomized, placebo-controlled study of inositol in 221 premature infants weighing <2000 g with RDS (Hallman 1992). At 4-12 hours after birth, infants were randomized to receive IV inositol 80 mg/kg/day or placebo for 5 days, with the regimen repeated on Days 10 and 20 if the infant remained on supplemental oxygen and had not established feeds. The study was designed to determine if inositol alleviated respiratory failure and increased survival without BPD. Approximately one-third of enrollees also participated in a surfactant trial and their randomization was stratified to ensure balance across the treatment groups. During this trial, 25 infants received <5 days of drug because they had established full enteral feeds of human milk before 5 days, 7 infants received <5 days of drug because they developed oliguria (n=4) or hyperglycemia (n=3) and study drug was stopped a day early (these occurred in both treatment groups). A total of 56 infants remained on oxygen at Day 10, but only 15 received a second course of treatment because 41 had established full enteral feeds.

Results showed that statistically significantly more inositol-treated infants survived without BPD (Table 2), and the rates of any ROP (13% versus 26%) and severe ROP needing intervention (0 versus 9%) and other morbidities were significantly lower in the inositol-treated infants. No safety concerns were seen, and 1 year follow-up showed no evidence of harm (Hallman 1992, Vaucher 1993).

Table 2. Study Outcomes for Hallman et al, 1992

	Inositol	Placebo	p-value	Comment
Number randomized (safety analysis)	119	114		<2000 g with RDS on ventilator
Number included in analysis of primary outcome	114	107		5 inositol, 4 placebo infants excluded[a]
Gestational age, wks mean (SD)	27.7(2.2)	27.9(2.0)		
Birth weight, grams mean (SD)	1098(332)	1104(348)		
Died	13 (11%)	26 (24%)	0.012	through 28 days
Pneumothorax	12 (10%)	23 (20%)	0.03	
BPD	20 (18%)	26 (24%)	0.22	Toce definition, Score 6 or more at 28 days (Toce 1984)
Survival without BPD	81 (71%)	55 (51%)	0.005	Toce definition, at 28 days
Any ROP	13/101 (13%)	21/81 (26%)	0.022	ICROP definition (ICROP 1984); % of 28-day survivors
ROP Stage 4 or worse	0	7/81 (9%)	0.012	ICROP definition; % of 28-day survivors
Cicatricial ROP at 1 year [n] none Grade I Grade II/III Grade IV	[96[b]] 88% 10% 2% 0	[73[b]] 73% 15% 4% 8%	0.009	Reese classification of cicatricial disease Wilcoxan-Mann-Whitney
IVH: Grade III or IV	15 (13%)	25 (22%)		
NEC	8 (7%)	7 (6%)		
Death after 28 days	4	7		
Neurological development of surviving infants at 1 year (age adjusted):				
Normal	76/96[b] (79%)	50/73[b] (68%)		
Minor handicap	11 (11%)	10 (14%)	NS	
Major handicap	9 (9%)	13 (18%)		

a Cases excluded from primary outcome analysis: death prior to receiving the randomized treatment (3 inositol and 4 placebo), lethal malformations (1 inositol and 1 placebo), or no RDS (1 inositol and 2 placebo).
b One infant in each treatment group was lost to follow-up.

Friedman 2000

Friedman et al. conducted a study of the relationship between oral supplemental inositol, serum inositol concentrations, and the development of ROP. He a) randomized 48 infants <1500 g recovering from RDS to enteral feeds of a low (43 mg/L) vs. high (450 mg/L) inositol-containing formula, b) followed an additional 17 infants who received fortified human milk feeds (average 128 mg/L), and c) followed 23 low inositol formula infants enrolled after the high inositol formula was no longer available. Ninety-three infants were enrolled and 88 who survived for eye examinations and had serum levels drawn were included in the data analysis. Age when feedings were begun was not reported. Infants who had been randomized to the low inositol formula were

reported separately from the non-randomized infants receiving the low inositol formula for clinical outcomes, but combined for ROP outcomes. Although there were no statistically significant differences in ROP outcomes, the incidence of severe ROP was lowest in the high inositol formula infants (Table 3). The results of a secondary post hoc analysis showed that infants with the lowest serum inositol levels at 0-3 days, and at >30 days had the highest rates of severe ROP.

Table 3. Study ROP Outcomes for Friedman et al. 2000

Treatment Group	Number of infants	>30-Day Serum Inositol (mg/L)	% with any ROP	ROP Stage 3 or 4
High inositol formula	24	46 ± 24	11 (46%)	1 (4%)
Fortified human milk	17	40 ± 27	8 (47%)	3 (17%)
Low inositol formula	47	36 ± 14	18 (38%)	10 (21%)

Cochrane Meta-Analysis

The Cochrane Reviews of studies involving inositol in the treatment of premature infants (Howlett 2003, 2012) judged only the 2 Hallman studies of sufficient quality adequate to combine in a meta-analysis. This showed statistically significant reductions in any stage ROP, Stage 4 ROP or ROP receiving surgery, death or BPD, death, and IVH Grades III or IV in inositol-treated infants (Table 4).

Table 4. Cochrane Meta-Analysis of Inositol for RDS in Preterm Infants

Outcome	Risk Difference(RD) and 95% Confidence Interval (CI) Comparing Inositol vs. Control
Any stage ROP	RD -0.082 [95% CI -0.159, -0.005]
Stage 4 ROP/ROP needing surgery	RD -0.078 [95% CI -0.128, -0.027]
BPD (on oxygen at 28 days)	RD -0.085 [95% CI -0.172, 0.003]
Death or BPD	RD -0.215 [95% CI -0.323, -0.107]
Death	RD -0.131 [95% CI -0.218, -0.043]
IVH Grade III or IV	RD -0.090 [95% CI -0.170, -0.010]

3.3.2 NICHD NRN-Sponsored Studies

3.3.2.1 INS-1 Study: Phase II Randomized, Double-Masked, Placebo Controlled, Safety and Pharmacokinetic Study of a Single Dose of Inositol in Premature Infants

The Phase 2 single-dose study (INS-1) has completed recruitment and analysis, and the manuscript has been submitted for publication.

The INS-1 Study evaluated the single-dose safety and pharmacokinetics of IV inositol in extremely preterm neonates (<30 weeks). Seventy-four infants of 23-29 weeks' gestation were randomized to receive a single IV dose of inositol at 0, 60 or 120 mg/kg. Blood and urine changes in concentration of inositol were measured and used to determine population pharmacokinetic parameters for these infants. The masked placebo group provided data on the endogenous processing of inositol, and permitted unbiased recording of potential side effects.

No differences in side effects or clinical events were observed. Comparison of the 2 inositol dose groups to the placebo group showed no differences in response during infusion of inositol (heart rate, respiratory rate, blood pressure, apnea events, flushing, etc.). Clinical events common among extremely premature infants were observed in this population at the expected rates, but there were no significant differences between the groups. A comparable population from the NRN is presented in Table 5 for comparison.

Table 5. Clinical Outcomes through Discharge or 120 Days (INS-1; Data Submitted for Publication)

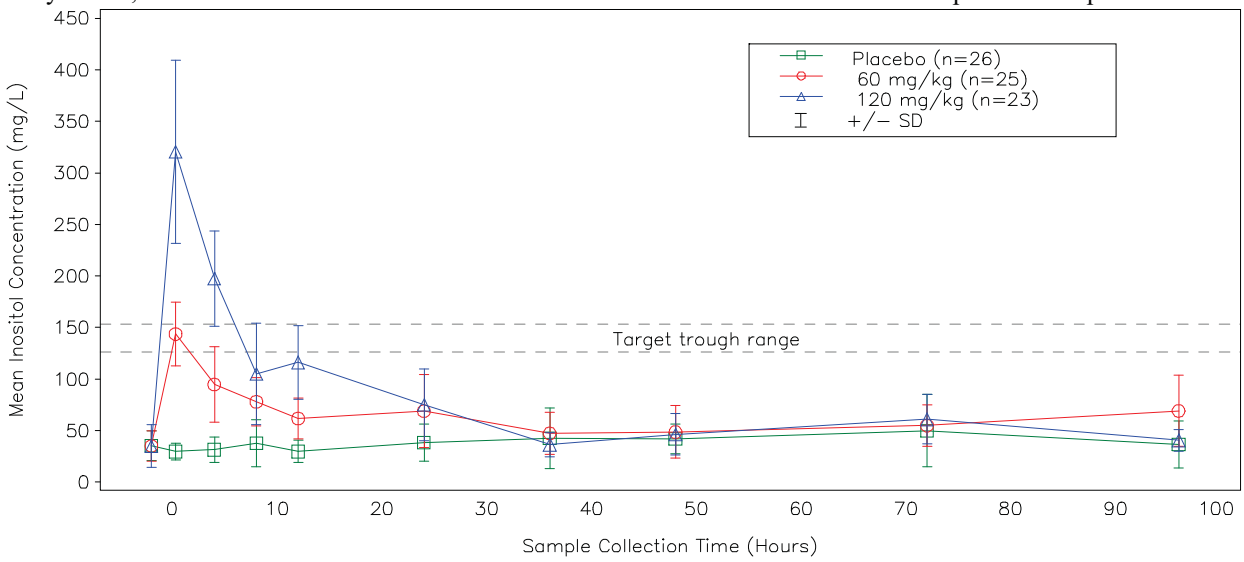
Characteristic	Placebo (N=25)	Inositol 60 mg/kg (N=25)	Inositol 120 mg/kg (N=24)	P-value	NRN Jan-Jun 2006[a] (N=963)
Gestational age, mean (SD) Birth weight, mean (SD)	26.48 (1.7) 981 (276)	26.48 (1.8) 990 (225)	26.9 (2.0) 989 (241)		
Died	3/25 (12%)	4/25 (16%)	3/24 (12%)	1.0	122/959 (13%)
BPD (O_2 at 36 weeks)	3/23 (13%)	6/21 (29%)	9/21 (43%)	0.09	277/832 (33.3%)
PDA	13/25 (52%)	7/25 (28%)	11/24 (46%)	0.20	405/963 (42%)
if PDA, indomethacin	7/13 (54%)	4/7 (57%)	9/11 (82%)	0.36	292/399 (73%)
if PDA, surgical ligation	2/13 (15%)	3/7 (43%)	4/11 (36%)	0.35	107/404 (26%)
IVH Grade III or IV	3/25 (12%)	5/24 (21%)	1/23 (4%)	0.22	155/936 (16.6%)
Sepsis, late onset	7/25 (28%)	7/25 (28%)	13/24 (54%)	0.09	277/946 (29%)
NEC	5/25 (20%)	2/25 (8%)	2/24 (8%)	0.49	94/963 (9.8%)
if NEC, surgery	2/5 (40%)	1/2 (50%)	1/2 (50%)	1.0	42/94 (44.7%)
Spontaneous GI perforation without NEC	0/25	2/25 (8%)	0/24	0.32	31/963 (3.2%)
Hearing Screening Test, Failed Both Ears	2/21 (10%)	0/17	1/19 (5%)	0.77	52/741 (7%)
ROP No Exam Favorable, Both Eyes Severe, 1 or Both Eyes Undetermined	 4/25 (16%) 4/25 (16%) 4/25 (16%) 13/25 (52%)	 5/25 (20%) 6/25 (24%) 2/25 (8%) 12/25 (48%)	 3/24 (12%) 8/24 (33%) 1/24 (4%) 12/24 (50%)	0.73	 165/955 (17.3%) 367/955 (38.4%) 47/955 (4.9%) 376/955 (39.4%)
ROP, if Status Known % Favorable	 4/8 (50%)	 6/8 (75%)	 8/9 (89%)	 0.21	 367/414 (88.7%)

[a] Subset of Generic Database infants: 23-29 weeks' GA, 600-1500 g birth weight, alive at 12 hours and no congenital anomalies.

Serum levels rose in a dose dependent manner and raw mean values are shown in Figure 2. A modified one-compartment population-pharmacokinetic model with an infusion period and linear elimination was developed that also allowed for the endogenous synthesis, metabolism and catabolism of inositol. The fitted model yielded estimated peak serum inositol levels that were dose-proportional with a typical infant having a volume of distribution of 511.5 mL/kg, a half-life of 5.22 hours and clearance of 67.9 mL/kg/hr. The estimated endogenous "concentration" of inositol was 39.3 mg/L.

Figure 2. Raw Mean Serum Inositol Concentrations

Study INS-1, Data Submitted for Publication. Collection times were clustered around the planned sample collection



times in order to obtain mean values.

Urine losses were high, especially over the first 24 hours, and dose-proportional. No diuresis occurred and no safety issues were identified at any dose.

Based on the results of the INS-1 study, the dosing plan for the multiple-dose study was modified to administer inositol every 12 hours to lower peak serum levels in order to reduce urine losses, and to study a wider range of doses.

3.3.2.2 INS-2 Study: Phase II Randomized, Double-Masked, Placebo-Controlled, Safety, Pharmacokinetic, and Dose-Ranging Study of Multiple Doses of Inositol in Premature Infants

The Phase 2 multiple-dose study (INS-2) has completed recruitment and initial analysis. These data are considered preliminary as the final manuscript and report are still under review.

The INS-2 Study evaluated the multiple-dose safety and pharmacokinetics of IV inositol in extremely preterm neonates (<30 weeks). One hundred twenty-two infants of 23-29 weeks' gestation were randomized to daily doses of 0, 10, 40 or 80 mg inositol/kg, divided into 2 doses and administered every 12 hours from enrollment (<72 hours of age) until the earliest of 34 weeks postmenstrual age (PMA), 10 weeks chronologic age, or the time of discharge from the hospital. Inositol or placebo was administered IV until enteral feedings were established, at which time the dose and same inositol formulation was administered enterally. Inositol concentrations in blood were measured within a sparse sampling population pharmacokinetic design over 70-90 days. Inositol was measured in 4 separate 24-hour urine collections over 6 weeks, and inositol in milk feedings was measured and used to help determine population pharmacokinetic parameters for these infants. The masked placebo group provided data on the endogenous processing of inositol, and permitted unbiased recording of potential side effects.

The specific types of adverse events reported during the study were generally similar among the inositol- and placebo-treated infants, with no statistically significant differences observed between the groups or 3 doses. A trend (p=0.0708) was observed for the incidence of infants who developed proteinuria, with a higher rate observed in the placebo group (9%) compared to the inositol groups combined (1%). Common clinical complications among extremely premature infants were observed in the study placebo group at the expected rates, and were similar or lower in the 3 inositol groups (Table 6). A comparable population from the NRN historical data is presented for reference.

Table 6 also shows the results for the primary outcome proposed for the Phase 3 program. Masked adjudication of subjects whose eye examinations did not reach final status was carried out as described in the statistical analysis plan. It is important to incorporate adjudication in a study of ROP because favorable outcomes are much more likely to have incomplete follow up than unfavorable outcomes when strict research definitions are used. (Unfavorable outcomes occur 90% of the time while infants are still in-patients, on average 4-6 weeks earlier than favorable endpoints.) These outcomes are shown for both the full enrolled population, and for the subgroup of infants 23-27 weeks', which is closer to the main trial target population of <28 weeks' gestation. The proposed primary outcome measure (severe ROP or death prior to final ROP determination) in the 23-27 weeks' target population occurred in 41% of the placebo group, 22% of the 10 mg/kg group, 36% of the 40 mg/kg group and 16% of the 80 mg/kg group. Infants who received the dose of 80 mg/kg/day selected for the main study had the lowest rate of severe ROP or death preceding determination of the ROP outcome (with or without adjudication of cases with incomplete follow up). No safety concerns were identified.

Serum inositol levels rose with the highest dose to the target range achieved in the Hallman studies during the first 2 weeks, and then, despite continued daily dosing, levels slowly fell so that by 6 weeks into treatment (a maximum of 10 weeks), levels were similar in the placebo and all 3 treated groups. Figure 3 shows the raw mean serum inositol levels from the subjects during the time they were receiving IV dosing, and Figure 4 shows the serum levels after subjects were changed to enteral dosing. There was no evidence of drug accumulation, and it is hypothesized that the infants modified endogenous production and catabolism to moderate the effects of the exogenous drug as they matured.

Due to the declining serum levels as the infants matured, it was not possible to estimate a population pharmacokinetic model from the serum levels collected after initiation of enteral administration. However, a multiple-dose version of the same modified one-compartment model used with the INS-1 study was compatible with the serum levels measured during IV administration. For a typical infant, the volume of distribution (676.7 mL/kg) and clearance (57.6 mL/kg/hr) were similar to those measured in INS-1. Estimates for the half-life (8.1 hr) and endogenous "concentration" (41.4 mg/L), were also comparable to the INS-1 estimates.

Table 6. Clinical Outcomes through Discharge or 120 Days (INS-2; Preliminary Unpublished Data)

Characteristic	Placebo	Inositol			P-value across groups	NRN 2008-2009[d]
		10 mg/kg/d	40 mg/kg/d	80 mg/kg/d		
	(N=35)	(N=29)	(N=30)	(N= 28)		(N=1415)
Gestational age, weeks, mean (SD)	26.5 (0.3)	26.6 (0.3)	26.7(0.3)	26.7 (0.4)		
Birth weight, grams, mean (SD)	884 (38)	897 (50)	939 (45)	920 (54)		
Died	6 (17%)	2 (7%)	6 (20%)	1 (4%)	0.165	162 (11%)
BPD (O2 @ 36 weeks) [a]	11 (38%)	7 (26%)	7 (30%)	8 (30%)	0.814	465 (38%)
Death from BPD before 37 weeks, or BPD	11 (38%)	8 (29%)	7 (30%)	8 (30%)	0.880	
PDA	13 (37%)	14 (48%)	14 (47%)	10 (36%)	0.677	615 (43%)
if PDA, indomethacin or ibuprofen	8 (77%)	9 (31%)	8 (27%)	8 (29%)	0.902	407 (29%)
If PDA, surgical ligation	3 (9%)	3 (10%)	1 (3%)	3 (11%)	0.745	152 (11%)
IVH grade any	13 (38%)	4 (14%)	10 (34%)	5 (18%)	0.078	405 (29%)
IVH Grade III or IV	10 (29%)	2 (7%)	6 (21%)	2 (7%)	0.052	233 (16%)
Sepsis, late onset	4 (11%)	6 (21%)	7 (23%)	5 (18%)	0.627	310 (22%)
NEC suspected or proven	5 (14%)	1 (3%)	4 (13%)	1 (4%)	0.281	125 (9%)
NEC requiring surgery	3 (9%)	0	2 (7%)	0	0.170	64 (5%)
Spontaneous GI perforation without NEC	2 (6%)	0	2 (7%)	1 (4%)	0.721	64 (5%)
Hearing screening test, failed in either ear	1 (4%)	1 (4%)	4 (20%)	3 (14%)	0.255	131 (12%)
ROP outcomes: *all subjects* (N) [b]	(35)	(29)	(30)	(28)		
Severe ROP in at least 1 eye	5/27 (19%)	3/26 (12%)	2/24 (8%)	2/23 (9%)	0.717	
Severe ROP in at least 1 eye or death before final/acute ROP status	11/33 (33%)	5/28 (18%)	8/30 (27%)	3/24 (13%)	0.274	
[c] Adjudicated: Severe ROP in at least 1 eye, or death prior to final/acute status.	11/35 (31%)	5/29 (17%)	8/30 (27%)	3/27 (11%)	0.227	
ROP outcomes: In Target population of 23-27 weeks (N)	(N=27)	(N=23)	(N=22)	(N=20)		
Severe ROP in at least 1 eye	5/19 (26%)	3/20 (15%)	2/16 (13%)	2/15 (13%)	0.762	
Severe ROP in at least 1 eye, or death before final/acute status.	11/25 (44%)	5/22 (23%)	8/22 (36%)	3/16 (19%)	0.288	
[c] Adjudicated: Severe ROP in at least 1 eye, or death prior to final/acute status	11/27 (41%)	5/23 (22%)	8/22 (36%)	3/19 (16%)	0.288	

[a] BPD was assessed at 36 weeks PMA, even if after discharge.

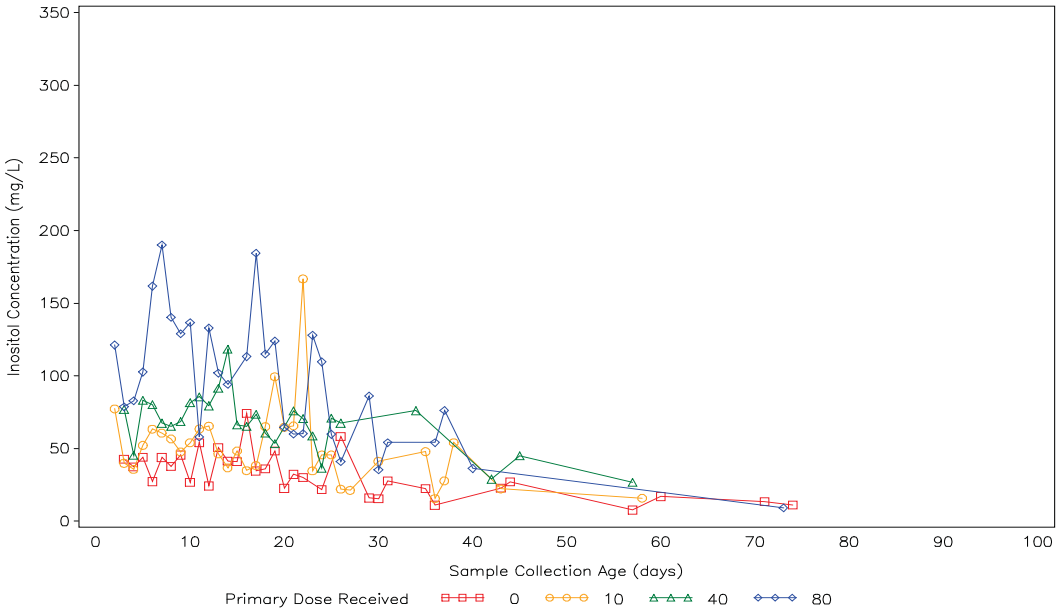
[b] ROP was followed until Acute/Final status, up to 55 weeks PMA, if necessary. For ROP outcomes, numerators and denominators are presented where denominators include only subjects for which the ROP outcome summarized could be determined.

[c] Outcomes if adjudication of incomplete ROP data resulted in a "most likely" conclusion

[d] A comparison subset of GDB infants 23-29 weeks' GA, alive at 12 hours, 600-1500 g. birth weight, and no congenital anomalies.

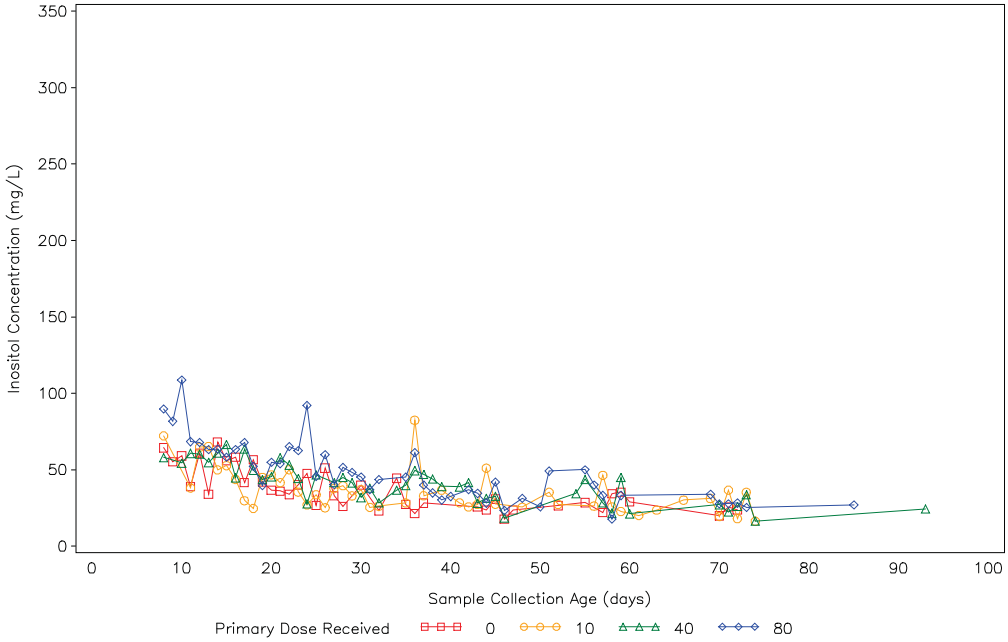
Urine levels of inositol excretion: During the baseline urine collection in Week 1, excretion in the placebo group was 33 mg/kg/day. The inositol-treated subjects showed elevated excretion in proportion to dose. Thereafter, 24-hour urine excretion fell in Weeks 2, 4 and 6 in all groups, despite continued daily administration of inositol at the same dose. We hypothesize that falling serum inositol levels are a combination of reduced endogenous production of inositol in the face of continued supplementation, and increased catabolism (the inositol oxidase enzyme increases in the renal cortex in the weeks after birth). Thus, the decreasing serum levels and urine excretion may reflect both postnatal maturation of the catabolism and decreased production.

Figure 3. Raw Mean Serum Inositol Concentrations Following IV Administration (INS-2 Study; Preliminary Unpublished Data)



Symbol (dose) legend for grayscale viewing: Square (0 mg/kg/day), Circle (10 mg/kg/day), Triangle (40mg/kg/day), and Diamond (80 mg inositol/kg/day)

Figure 4. Raw Mean Serum Inositol Concentrations Following Conversion to Enteral Administration (INS-2 Study; Preliminary Unpublished Data)



Legend Fig. 4: Symbol (dose) legend for grayscale viewing: Square (0 mg/kg/day), Circle (10mg/kg/day), Triangle (40mg/kd/day), and Diamond (80 mg inositol/kg/day)

4 STUDY OBJECTIVE

The primary objectives are:

- To determine the efficacy of myo-Inositol 5% Injection compared to placebo for increasing the probability of survival without severe Retinopathy of Prematurity in premature infants <28 weeks' gestation followed through the determination of acute/final ROP status or 55 weeks.

- To determine the safety of myo-Inositol 5% Injection compared to placebo in premature infants <28 weeks' gestation.

The secondary objective is to determine if myo-Inositol 5% Injection compared to placebo has an effect on other common morbidities of extreme premature birth.

5 INVESTIGATIONAL PLAN

5.1 Overall Study Design and Plan: Description

This is a Phase 3, randomized, double-masked, placebo-controlled study designed to determine the effectiveness of myo-Inositol 5% Injection to increase the incidence of survival without severe ROP through acute/final ROP determination up to 55 weeks PMA in premature infants $<28^{0/7}$ weeks' gestation. Approximately 1760 infants are to be enrolled at approximately 18 NICHD NRN Centers (approximately 44 sites) in the United States. Infants meeting the study selection criteria and for whom informed consent is obtained will be randomized to receive either 80 mg inositol/kg/day or placebo, administered in divided doses every 12 hours (40 mg/kg/dose). Study drug will be administered daily, starting within 12-72 hours of birth and continued until the earliest of 34 weeks PMA, 10 weeks chronologic age, or the time of hospital discharge or transfer. Inositol or placebo will be administered IV until enteral feedings reach 120ml/kg/day (or sooner if the infant is no longer receiving IV fluids), at which time the same dose and formulation will be administered enterally every 12 hours.

For publication purposes, the analysis of the primary efficacy outcome will consider the entire study population. In support of a new drug application (NDA) for use of myo-Inositol 5% Injection to increase survival without severe ROP through the determination of acute/final ROP status, the analysis of the primary efficacy outcome will be conducted for the entire study population and separately within pre-specified regulatory sub-studies created by administratively splitting infants enrolled at each study center into two sub-studies.

Assessments performed during the study include customary newborn intensive care procedures including repeat eye examinations until ROP status is final (which often extends after discharge), measurements of growth, cranial ultrasounds or other imaging per usual practice, and the collection of clinical diagnoses throughout hospitalization to evaluate other common morbidities of extreme preterm birth. Adverse events will be recorded from time of treatment initiation until 7 days after the last dose of study drug, and concurrent medications will be recorded from 24 hours prior to randomization until 7 days after the last dose of study drug or until discharge or transfer if sooner. Using the separate NICHD Follow-up protocol, longer term data will be

collected at 22-26 months corrected age, including growth, neurodevelopmental testing, overall health status, rehospitalizations, surgeries and diagnoses, including ophthalmic diagnoses and treatments since discharge.

5.2 Selection of Study Population

5.2.1 Inclusion Criteria

An infant will be eligible for study participation if he/she meets the following criteria:

1. Inborn or out born infants of either gender or any race with best obstetrical estimate of gestation <28 weeks ($27^{6/7}$ weeks and younger). Gestational age will be determined by best obstetrical estimate per NRN Generic Database (GDB) protocol (using the hierarchy of best obstetrical estimate using early ultrasound dating, maternal menstrual dating confirmed by examination, or Neonatal gestational age assessment by physical examination). The GA assigned will be carried forward for all study purposes.

2. Alive at 12 hours.

3. Age in hours up to 72 hours, although we will seek enrollment as early as feasible after consent and 12 hours.

4. Informed consent signed and dated by parent and/or guardian, which includes likelihood of completing follow-up ophthalmic examinations as an outpatient, and long-term follow-up.

5.2.2 Exclusion Criteria

1. An infant will be excluded from the study if he/she meets any of the following criteria:

2. Major congenital malformations

3. Congenital malformations of the eye identified prior to randomization.

4. Overt evidence of intrauterine congenital infections (“TORCH”) or life-threatening impairment of renal, hepatic, or cardiac function (considered moribund).

5.2.3 Concurrent Therapy

Systemic medication that the subject receives from 24 hours prior to randomization until earliest of discharge, transfer, or through 7 days after the final dose of study drug will be recorded along with date(s) of administration including start and stop dates. Inhaled medications and eye drop medications will be considered systemic and recorded. Non-systemic medications such as topical emollient creams or powders will not be recorded, nor will IV feedings of dextrose, amino acids, lipids, albumin, multivitamins, or infusions of normal saline or blood products. The MOP provides specific and more detailed instructions.

5.3 Efficacy and Safety Assessments/Variables

5.3.1 Specific Treatment Plan and Subject Management

Assessments of efficacy and safety performed during the study and during follow-up are presented in Table 7, Study Activities.

Table 7. Study Activities

Procedures	12-72[a] hours after birth	Study Day / Study Week	1	2-35 / 1-5	36-70 / 6-10	71-105 / 11-15	106-140 / 16-20	141-175 / 21-25	176-210 / 26-30	22-26 months corrected
Informed Consent	X									
Randomization [a]	X									
Cranial Ultrasound [b]				X	X					
Study Drug Administration [c]			X	X	X					
Ophthalmologic Examination [d]				(X)	X	X	X	(X)	(X)	
Infant Growth [e]				X	X	X	X	(X)	(X)	X
Concurrent Medications [f]	X		X	X	X	X				
Adverse Event Assessments [g]	X		X	X	X	X				
Clinical Diagnoses [h]	X		X	X	X	X	X	X	X	X
Follow-up Evaluation [i]										X

[a] With allowance for up to 72 hours; however, the goal is to enroll as soon a practicable after 12 hours
[b] Cranial ultrasounds will be performed at times consistent with clinical practice to evaluate for IVH and PVL. Otherwise they will not be mandated by the study
[c] Daily study drug administration will begin following consent and end at 34 completed weeks PMA, 10 weeks chronologic age, or at the time of discharge, whichever occurs first.
[d] Ophthalmologic examinations will begin at Week 31 PMA and continue every 1 to 3 weeks based on findings until Acute/Final ROP status is achieved.
[e] Weight is recorded weekly, head circumference is recorded every 2 weeks, and length is recorded every 4 weeks.
[f] Recorded from 24 hours prior to randomization until discharge, transfer, or 7 days after the last dose of study drug administration, whichever occurs first.
[g] Recorded from treatment initiation until 7 days after last dose of study drug
[h] Clinical diagnoses will be collected from the medical record throughout hospitalization and again at the follow-up visit.
[i] Follow-up evaluations and interval medical data will be collected at 22-26 months corrected age per the NRN Follow-up Protocol (described separately)

5.3.1.1 Study Procedures

5.3.1.1.1 Usual Preterm Infant Care

Physical assessment for congenital anomalies will be performed with newborn intensive care unit (NICU) admission procedures and recorded in the medical record. This information and communication with the NICU care team is used in determining eligibility for consent. Cranial ultrasounds or other imaging will be performed at times consistent with clinical practice to evaluate for intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL). Typically, the evaluation for IVH occurs early (Days 5-14), while the evaluation for PVL must occur later, after 4 weeks of age. The age at ultrasound and the worst grade on either side of the brain and extent of PVL as determined by the neonatal team will be recorded. (Papile 1978)

Clinical diagnoses will be collected from the medical record throughout hospitalization utilizing the definitions as described/defined in the NRN GDB Protocol and INS-3 MOP.

Infant growth will be monitored by collection of weight (weekly), head circumference (every 2 weeks), and length (every 4 weeks).

Ophthalmology evaluations for ROP are performed according to the jointly published guidelines from the American Academy of Ophthalmology, American Academy of Pediatrics, and American Association of Pediatric Ophthalmology and Strabismus (Guidelines 2013). Examinations are to begin when the infant reaches the later of 31 weeks PMA or 4-6 weeks chronologic age. Repeat examination intervals are scheduled based on the findings of the current examination and are usually at 2-3 weeks for infants who have no evidence of ROP but whose retinal vasculature remains immature, 1-2 weeks for those with early ROP (stages 1 or 2 in zone II or in zone III), and 1 week or less if the ROP is aggressive (any ROP in zone I, or stage 2-3 in zone II) but not meeting criteria for intervention.

5.3.1.1.2 Ophthalmologic Examinations

Although ophthalmic evaluation for ROP is usual care, special attention to these examinations is required because they are central to the primary outcome, and a confirming examination is required for severe ROP. Confirming exams are not universally conducted in usual care.

Type 1 ROP, as defined by the ETROP multicenter study (2003) is the current minimal degree of severity of ROP found to warrant intervention (laser, cryotherapy or other approaches to control accelerating retinal neovascularization). Moderate ROP that does not fully meet these criteria (now termed "Type 2" ROP) had better outcomes when left to its natural course (52% spontaneously resolved) or treated when/if it reached Type 1 criteria, as compared to when it was treated at the level of Type 2 ROP (ETROP 2011)(Repka 2011). Therefore since Type 2 ROP does not have the same prognostic clinical importance as Type 1 (except as a warning to watch closely), the criteria for warranting intervention (Type 1) was chosen as the adverse endpoint for this study.

Each center will have at least 1 study-certified ophthalmologist who is the Lead Ophthalmologist for that center. This physician is a co-investigator on the study, has read the protocol and serves as the spokesperson and coordinates communication with other ophthalmologists performing examinations or treating ROP in the NICU and as outpatients at that Center. All ophthalmologists participating in ROP examinations in the community (varies from 1 to several) will be informed about the INS-3 protocol, and the one to one relationship of our study practices to recommended practice including: timing of first and repeat examinations, recording of examination findings using the International Classification of ROP (ICROP 2006) conventions, and the criteria for intervention for ROP (Early Treatment for Retinopathy of Prematurity [ETROP] Type 1) (ETROP Study Group 2003) (2013 Guidelines for Perinatal Care)(2013 AAP/AAO/AAPOS Screening examination of premature infants for ROP).

The procedure that is study related, and may not be routine in every center, is the confirming examination when ROP determined to need intervention has been identified. In most of the centers, general or pediatric ophthalmologists perform the initial and repeat examinations. If an infant is close to needing intervention or needs intervention, a retinal surgeon is normally consulted to evaluate and perform the treatment, if (s)he agrees. This surgeon's evaluation will constitute the confirming examination, and in those centers, is the usual practice. For those centers where a single ophthalmologist performs both regular examinations and treatment of severe ROP, a second examination from an independent examiner is required, or if this proves impossible in particular circumstances, posterior pole fundus photographs are obtained to enable later independent confirmation of the findings. (Photographs will not require evaluation in real time in order to avoid delaying intervention which is time sensitive.)

Eye examinations will begin by Week 31 PMA and continue every 1/2 to 3 weeks as indicated by the clinical findings until each eye has reached the Acute/Final ROP status (up to a maximum of 55 weeks PMA for study purposes). Results of the examinations are recorded on the study CRF for each eye separately and include the lowest zone not fully vascularized, the highest stage of ROP in that lowest zone, the highest stage in any zone, the presence or absence of Pre-plus Disease or Plus Disease, and whether or not the criteria for ETROP Type 1 ROP requiring intervention have been met.

> **Type 1 ROP** is defined in each eye separately and is ROP meeting any of the following combination of findings:
>
> in Zone II: Plus Disease, with any Stage 2 or Stage 3 ROP;
>
> in Zone I: Plus Disease with any stage ROP;
>
> or Stage 3 ROP, even without Plus Disease.

The infant has reached **Acute/Final ROP status** when either of the following eye outcomes is observed: (Based on the 2013 AAP/AAO/AAPOS Guidelines for the Detection of ROP requiring treatment).

Favorable Acute/Final ROP Status: when both eyes are either:

Fully vascularized to the ora serrata on a single examination

or the retinal vessels have grown into Zone III, observed on two sequential examinations, showing only immature vessels, regressing ROP or Stage 1 or Stage 2 ROP.

or the vessels have unequivocally reached Zone III on one examination in an infant of 35 weeks PMA or older, if there has been no previous ROP in Zone I or Zone II, and this examination is not the only examination the infant has had.

or PMA has reached 50 weeks and there is no prethreshold or worse ROP present.

Prethreshold ROP =

in Zone II, Stage 3 ROP or

in Zone I, any stage ROP

Unfavorable Acute/Final ROP Status: when one or both eyes show any of the following findings/circumstances, confirmed by a second examiner (or documented by fundus photograph):

ETROP Type 1 ROP (see above)

ROP that has progressed to stages worse than ETROP Type 1 ROP, including classic CRYO-ROP threshold, or any degree of retinal detachment;

Note: MISSING OUTCOME: If an infant receives an intervention for ROP before meeting criteria for an Unfavorable outcome or does not have a confirming examination (or fundus photographs), the infant's outcome will be considered a missing outcome. Intervening prior to this point is a study protocol violation and will require completion of a Protocol violation form by the treating physician explaining the circumstances. Handling of missing and indeterminate outcomes for the publication and regulatory submission analyses are discussed in Section 8.1.4.

An infant who dies before reaching a favorable Acute/Final ROP status will be considered to have an unfavorable Acute/Final ROP outcome, since death is a competing outcome for severe ROP (Freemantle 2003).

For infants who reach an unfavorable ROP status, a confirmatory examination is the preferred approach for validating the outcome, (but if such an examination cannot be performed, fundus photography for later evaluation is required). Infants that receive intervention without a confirming examination (or photographs) will be categorized as having a missing outcome. The confirming examiner is normally the retinal surgeon who will perform the intervention, and must

perform and record an examination of the infant prior to intervention. If the confirming examiner does not agree with the first examiner, the second examiner records his/her findings and then the 2 physicians should discuss the case. If they do not agree, a repeat examination is scheduled within 1-7 days based on that discussion and the clinical risk. At that time, the process is repeated. Again, if the first examiner judges Type 1 ROP, a second examiner is to confirm the results. Commonly, such repeat examinations are conducted together with both examiners and consensus reached. If a confirming ophthalmologist is not available at a particular center, high-resolution posterior pole fundus photographs that will permit a later independent reading should be obtained prior to intervention.

As part of standard medical practice, premature infants are followed closely with regular ophthalmic examinations until reaching an ROP resolution (i.e., progression to needing intervention or regression/healing to maturity or unequivocally established as healing in Zone III). Typically, 95% of infants reach this endpoint by 44 weeks PMA, but this is often after discharge home, thus requiring continued outpatient follow-up. Based on large research databases, favorable status is reached by 44 weeks PMA in 95% of infants and by 48 weeks PMA in 99% of infants (Reynolds 2002). Unfavorable outcomes occur earlier, on average, but the 99th percentile is at 46 weeks PMA and the range extends from 31 to 54 weeks PMA.(Palmer 1991) Therefore, if at 55 weeks PMA, the Acute/Final ROP status has not been determined, the ROP follow-up will be discontinued for study purposes, although clinically indicated follow-up must be continued. Data on subsequent ophthalmic treatment and sequelae will be collected during follow-up at 22-26 months corrected age.

The Investigator at each study center or their designee will track examinations occurring after discharge or transfer, obtaining results from the outpatient examinations until Acute/Final ROP status is reached. The MOP provides detailed instructions on the recording of the ophthalmologic examinations, and use of the CRFs.

5.3.2 Efficacy Variables

5.3.2.1 Primary Variable

The primary outcome is survival without severe ROP through Acute/Final ROP status determination (favorable) versus development of severe ROP or death prior to reaching Acute/Final ROP status (unfavorable) followed up to 55 weeks PMA.

- Death in relation to the primary variable will be defined as from any cause before Acute/Final ROP status is determined.

- Favorable Acute/Final ROP status is defined in the above section and requires that no ROP, or only mild ROP has occurred in both eyes and that the eyes have matured beyond the risk of developing severe ROP.

- Unfavorable ROP is defined in the section above and requires that one or both eyes reach ROP severity warranting intervention.

5.3.2.2 Secondary Variables

- BPD: NICHD Physiologic Definition: Requiring oxygen to maintain an oxygen saturation of at least 90% at 36 weeks PMA (Walsh 2004).

- BPD (Physiologic Definition) or Death from BPD prior to 37 weeks PMA, with cause of death certified by the Center PI that BPD is the primary cause, or a significant co-contributing cause of death.

- All cause death: defined as death from any cause following randomization. The primary, underlying cause of death will be certified by the Principal Investigator at each center who may also list co-contributing causes.

- Any ROP: defined as ROP of any severity that is observed on at least 2 examinations in either eye through the time that Acute/Final ROP status is reached. ROP that is observed on only 1 of multiple eye examinations will not be considered "any ROP". An infant who has only 1 eye examination prior to death, and that examination shows any stage ROP, will be also be considered as having "any ROP".

- Type 2 ROP through the time that Acute/Final ROP status is reached: defined as 1 or both eyes reaching Type 2 ROP (ETROP 2003), but not Type 1. Type 2 ROP is defined as: (ETROP 2003).

  - in Zone II: Stage 3 ROP without Plus Disease, or

  - in Zone I: Stage 1 or 2 ROP without Plus Disease

- Severe IVH: IVH Grades 3 or 4 on either side of the brain, or extensive PVL. IVH will be classified as described by Papile (Papile 1978). PVL will be graded by the characteristics of the periventricular white matter on ultrasound or MRI done between 4 to approximately 6 weeks after birth per usual care (Barkivich 2000) and/or porencephalic cyst.

5.3.3 Safety Variables

The safety variables will be the occurrence of adverse events and serious adverse events, in addition to the clinical diagnoses outlined below. These safety variables are described in detail in the MOP and recorded prospectively on the adverse event CRF. The grading of severity is guided by a Neonatal Toxicity Table (see the INS-3-06 case report form and Toxicity Table in the MOP).

5.3.3.1 Additional Clinical Outcomes Monitored for Safety

Unless otherwise specified, data collection for other common complications of prematurity is through the time of NRN "status", i.e., the first occurring of: discharge home, death, transfer, or 120 days following birth. These include:

- Occurrence of necrotizing enterocolitis (NEC): Stage II or worse, whether treated (medically or surgically) and if the infant survived (modified Bell's classification [Walsh 1986]).
- Isolated gastrointestinal perforation judged not to be due to NEC.
- Late onset sepsis: culture positive septicemia/bacteremia (≥72 hours of age) treated with antibiotics for ≥ 5 days or died before treatment was completed.
- Occurrence of clinically significant patent ductus arteriosus (PDA), and if received intervention with prostaglandin inhibitors, and/or surgery. This does not include prophylactic Indomethacin or Ibuprofen administered without the diagnosis of clinically significant PDA.
- Seizures treated with an anticonvulsant for >72 hours
- Total days on parenteral nutrition (including amino acids and/or lipids)
- Days on oxygen, days on ventilator
- If hearing screening was performed, did the infant fail in one or both ears (defined as never passing a hearing screening in one or both ears)

5.3.3.2 Variables Collected At the Long-Term Follow-up at 22-26 Months Corrected Age

Data collection for other common complications of prematurity obtained during long-term follow-up evaluation at 22-26 months will be conducted using the separate NICHD-NRN Follow-up Protocol. These data include:

- Neurodevelopment at 22-26 months corrected age (i.e., 22-26 months past due date) using the Bayley Scales of Infant Development III.
- Vision loss as diagnosed by an ophthalmologist as legally blind, and subdivided into "ophthalmic origin", or "not ophthalmic origin" (i.e., cortical blindness is non-ophthalmic in origin and indicates that there is no retinal detachment or other abnormal fundus or ocular finding, except optic atrophy. Such cases will be considered central [neurologic] in origin.)
- Hearing loss requiring that hearing aids be prescribed.

- Cerebral palsy by severity category (absent/mild/moderate/severe).

- Overall health status per recall from the parent/guardian (including survival, re-hospitalizations, surgeries, ongoing medications, and chronic illnesses).

- An INS-2 specific additional data sheet will collect additional detail on ophthalmic re-hospitalization, surgeries, ongoing medications and chronic conditions (Subsequent ophthalmic surgery related to ROP, or other ophthalmic treatments, including glasses, contacts, scleral buckle, vitrectomy, cataract surgery, strabismus surgery, glaucoma treatment, etc.). This will form a final confirmation of the clinical findings and sequelae of ROP in each child following discharge. To facilitate collecting this information from outpatient ophthalmology visits (anticipated in all infants, and to involve serious medical issues in about 20%), explicit consent to obtain outpatient office records or hospital records will be included in the consent form.

5.4 Removal of Subjects from Therapy or Assessment

5.4.1 Discontinuation of Individual Subjects

Parents or legal guardians can elect to withdraw their infant(s) from the study at any time without compromise to the infants' care. Infants will be withdrawn from the study immediately if the Investigator believes it to be in the best interest of the subject. Infants who are prematurely discontinued from the study are not to be replaced, and their data will be included in the final analyses up to the point when or if parents withdraw permission to collect further data. Parents may withdraw an infant from the study treatment and also permit continued data collection (i.e., safety and outcome measures).

5.4.2 Discontinuation of Entire Study

NICHD may terminate this study prematurely, either in its entirety or at any study center, for reasonable cause by written notice to each center's Principal Investigator (or direct communication if urgent). Advance notice is not required if the study is stopped due to safety concerns. If NICHD terminates the study for safety reasons, NICHD will immediately notify the Investigators by telephone and subsequently provide written instructions for study termination.

5.5 Treatments

5.5.1 Treatments Administered

Within 12-72 hours of birth, all eligible subjects will begin treatment with 80 mg inositol/kg/day or placebo, according to individual randomization. Study drug will be administered daily and continue until the earliest of 34 weeks PMA, 10 weeks chronologic age, or the time of discharge. Study medication will be administered IV until enteral feeds reach 120ml/kg/day (or sooner if

the infant is no longer receiving IV fluids), at which time the same dose and formulation will be administered enterally.

Inositol: myo-Inositol 5% Injection is an isotonic, preservative-free, sterile 5% solution of myo-inositol in water containing 0.5 gm sodium chloride per liter (8.55mM), pH 6.5-7.5. It is administered via IV infusion using syringe pump over 15-30 minutes twice per day at 12-hour intervals at a dose of 80 mg inositol/kg/day (40 mg inositol/kg/dose), which is equivalent to 1.6 mL/kg/day (0.80 mL/kg/dose).

Placebo: 5% dextrose (5% glucose) in sterile water (D5W pyrogen and preservative free) United States Pharmacopoeia (USP) for IV infusion. Placebo will be administered in the same dose (80 mg glucose/kg/day divided in 2 doses administered every 12 hours) and dispensed in the same manner as the inositol.

Study drug should be infused into a vein rather than an artery because safety for arterial infusion has not yet been tested. It can always be given with a small saline flush preceding and following its infusion into a vein while other infusions are held during the study drug infusion. Administration through the port of a multi-lumen line is acceptable since there is no mixing of solutions until it reaches the blood stream. In addition, in vitro testing was conducted with commonly used continuous drug infusions (for this population) including various TPN solutions and the results show inositol to be physically compatible with these selected solutions. The study drug may be co-infused with these solutions. See the Investigator's Brochure for specific testing completed and a listing of compatible co-infusion solutions.

Conversion to Enteral Administration

Once the infant has advanced to established enteral feedings (120ml/kg/day, or sooner if IV fluids are discontinued), study drug will be administered enterally via feeding tube, or by usual oral medication methods if the infant no longer requires a feeding tube. The same dose and formulation compounded for IV administration will be used for enteral administration.

5.5.2 Identity of Investigational Product

Myo-Inositol 5% Injection will be provided by AN and supplied to each center by dedicated clinical supply services. Placebo (5% dextrose in water USP) will be provided by each individual center or site's pharmacy from commercial stock supply.

Myo-Inositol 5% Injection will be supplied to the centers in 5 mL single-use vials containing 3 mL of solution. Each vial will contain 150 mg inositol in 3 mL (50 mg/mL). The solution will be sterile, at neutral pH, pyrogen-free and preservative-free. Sodium chloride is also present in the solution; each daily dose (80 mg inositol/kg/day) will add 0.014 mEq/kg/day of sodium intake.

Each vial of myo-Inositol 5% Injection will be labeled with the manufacturer's name and address, study sponsor, protocol number, Investigational New Drug (IND) caution statement, drug identification including lot number and vial number, and storage conditions.

Unit doses of inositol and placebo will be prepared and dispensed from each site's pharmacy in delivery syringes labeled for the ordered mg/kg dose as "Inositol Study Drug YY.Y mg, 0.8 mL/kg, administered IV over 15-30 minutes, or given as an enteral slow bolus, if feeding." [Note: The exact format of labeling will be per the normal pharmacy procedures to minimize the chance of errors.]

Myo-Inositol 5% Injection must be stored at controlled room temperature (15-25°C). When loaded into delivery syringes under sterile conditions, the drug is stable for more than 72 hours when stored at 2-8°C, and more than 48 hours when stored at 25°C/60% relative humidity.

Investigational products are for investigational use only, and are to be used only within the context of this study. The study drug supplied for this study must be maintained under adequate security and stored under conditions specified on the label until dispensed for subject use, returned to NICHD or representative or destroyed per instructions from NICHD and according to local regulations.

5.5.3 Method of Assigning Subjects to Treatment Groups

All infants will be centrally randomized using a secure, password protected web-based randomization system within the RAVE™ DMS available 24 hours/7 days per week via any computer with high-speed internet access. Directions for the randomization system are presented in the MOP. Eligible infants will be assigned a unique randomization number that allocates them in a 1:1 ratio to receive inositol or placebo. To ensure balance between the treatment groups, randomization will be stratified by center and by 2 gestational age risk strata (<26 and 26-27 weeks).

Randomization will be performed at the overall study level. For regulatory analyses, the randomized study population will be administratively split within study center in a pre-determined manner to define the regulatory sub-study A and sub-Study B (see SAP).

5.5.4 Selection and Timing of Dose for Each Subject

Infants will receive either: 80 mg inositol/kg/day or placebo (80 mg glucose/kg/day), administered in divided doses every 12 hours (40 mg/kg/dose). Study drug will be administered daily and continued until the earliest of 34 completed weeks PMA, 10 weeks (70 days) chronologic age, or the time of discharge. Inositol or placebo will be administered IV until enteral feedings are established, at which time the same dose and formulation will be administered enterally every 12 hours. If an infant subsequently becomes ill and enteral feedings are discontinued, dosing will resume IV until they are able to sustain enteral feeds again (120ml/kg/day, or sooner if IV fluids have been discontinued).

Study drug doses will not be adjusted for estimated inositol enteral intake from formula or human milk.

If an infant is discharged from the participating center hospital (home or back-transfer), study drug will be stopped unless the infant is transferred to a center/site that is also participating in the INS-3 protocol (repeat consent may be needed).

5.5.4.1 Adjusting Dose for Growth

The per-kilogram doses of inositol and placebo will be based on the birth weight of the infant, until such time that the infant's weight exceeds the birth weight (usually 1-3 weeks). Thereafter, on a once-per-week basis, the daily dose will be recalculated for current weight based on the weight measured that week.

If an infant becomes too unstable to weigh, or in the event serious third space edema develops (as for instance may occur if an infant is septic), the clinically estimated 'dry weight' (weight preceding the event) of the infant should be used instead of current weight (which may differ by as much as double or more of the "dry weight"). This is the usual clinical practice in the NICU for calculating dosing for other drugs in these circumstances. This 'dry weight' is adjusted by the clinical team on a weekly basis allowing for underlying growth if good calories are being given. When the clinically evident edema has resolved, or the estimated "dry weight" is within 10% of the measured weight, the dose calculation will revert to being based on current weight.

5.5.4.2 Discontinuation of Dosing for Oliguria

Renal insufficiency represents a special case that sometimes occurs in premature infants. Because so much inositol is excreted in the urine in the initial weeks after birth, study drug administration will be discontinued if an infant becomes oliguric (urine output of 0.5 mL/kg/hour or less, over 24 hours), or if the serum creatinine rises to 1.8 mg/dL or higher during the study. If, however, an infant entered the study with an elevated creatinine due to maternal elevated creatinine (allowed up to 2.5 mg/dL) and the serum creatinine is falling, this would not meet the criteria for stopping drug.

If a recognized, reversible cause of oliguria is diagnosed (e.g., congestive heart failure from a PDA, status post indomethacin administration for PDA, sepsis with hypotension), and the infant fully recovers (urine output >1.0 mL/kg/hour over 24 hours for at least 3 days and creatinine <1.8 mg/dL and falling), study drug will be resumed at the previous dose. However, if the period of renal dysfunction lasts more than 14 days, study drug will not be resumed, even if the condition diagnosed is a recognized, reversible cause of oliguria. If a recognized cause of oliguria cannot be identified, even if the episode resolves, study drug will not be resumed.

5.5.5 Masking of Study Group (Blinding)

Inositol and placebo (dextrose/ glucose) are both clear, colorless, slightly sweet solutions that are identical in appearance and contain the same concentration of carbohydrate (50 mg/mL).

Each site's study-certified pharmacist will be responsible for tracking, dispensing, and documentation of study drug, as well as reporting directly to the Data Coordinating Center any protocol deviations/violations that would involve unmasking if other study personnel were to report them.

The site pharmacist will dispense study drug in unit dose delivery syringes in order to mask all clinicians, research personnel, and families to the investigational product administered. In the event of a serious adverse event that appears related to study drug, dosing may be stopped without unmasking. A MedWatch form will be completed and reported to NICHD, the Data Coordinating Center, and the local Institutional Review Board (IRB) per their procedures. The NICHD will determine if individual reporting to the FDA and AN is indicated. If events make it necessary, the pharmacy at each site will be able to unmask the study drug given to an individual infant; however, the pharmacist must contact the Center's Investigator, the overall study Principal Investigator, or the NICHD Program Scientist prior to releasing this information, and a protocol violation form will be completed. Contact information for these individuals is provided in the MOP.

5.5.6 Treatment Compliance

Each dose of study drug administered is to be documented including the date, time, actual dose of inositol/placebo received, route, and dosing weight. The Investigator or his/her designated and qualified representatives will administer study drug only to subjects enrolled in the study in accordance with the protocol. The study drug must not be used for reasons other than that described in the protocol.

5.5.7 Drug Accountability

Study drug will be shipped to each site's pharmacy. The study-certified pharmacist will verify that study drug supplies are received intact and in the correct amounts. It will be the responsibility of the site's study-certified pharmacist to maintain an accurate (running) inventory of all study drug dispensed and to perform and verify an overall accountability of the study drug throughout the study. All study drug doses must be inventoried, accounted for, and returned to NICHD or destroyed per instructions from NICHD and according to local regulations. Detail regarding the tracking and maintenance of study drug supply is provided in the MOP.

5.6 Discussion and Justification of Study Design

The multicenter, randomized, double-masked, placebo-controlled, parallel-group design chosen for this study is generally acknowledged as the "gold standard" for obtaining unbiased estimates of treatment differences.

The study population chosen for evaluation in this study consists of extremely premature infants (<28 weeks' gestation) who are at high risk for developing ROP. As the incidence of any ROP is inversely proportional to gestation, randomization has been stratified by 2 gestational age strata (<26 and 26-27 weeks), to ensure balance between the treatment groups.

The measurements to be performed in this study are standard for the care and outcome assessment for premature infants. The age of 55 weeks PMA selected for the latest date of evaluation of the primary ROP endpoint was chosen because >99% of acute/favorable outcomes have occurred by this age, and all except rare cases of severe ROP outcomes have developed by this age (Reynolds 2002).

The selection and timing of inositol dosing chosen for this trial was based on published studies as well as the NICHD-sponsored Phase 2, pharmacokinetic, dose-ranging study. Inositol administered at 80 mg/kg/day was the effective dose in the second and larger Hallman trial (Hallman 1992), and in the INS-2 pharmacokinetic study, this dose achieved the serum levels of 126-153 mg/L observed in the Hallman studies during the first 2 weeks of dosing. Administering 80 mg inositol/kg/day in divided doses at 12-hour intervals reduces peak serum levels and therefore reduces renal losses. Repeated dosing did not lead to increasing serum levels over 10 weeks.

The decision to continue treatment through 34 weeks PMA was based on the following rationale: a) Prevention: if ROP is going to develop, it normally is manifest by this time, b) Treatment: ROP that is going to be severe usually changes character and accelerates between 30 and 34 weeks PMA so that extending supplements to this point seemed to offer an advantage, c) Effect on serum levels: results from the repeated dosing INS-2 study of doses between 5 and 80 mg/kg/day show that, despite prolonged dosing, serum levels in all dose groups converge towards the placebo group by 6-10 weeks after birth, and d) Practical considerations: continued dosing after discharge (34-40 weeks PMA on average) becomes impractical.

6 ADVERSE EVENTS

Adverse events (AE) are monitored during the study to ensure timely detection of events that may affect the safety or continued participation of research subjects. In INS-3, this extremely high-risk and fragile population will each experience both expected and unexpected adverse events. Adverse events and their relationship to study, severity, time of experience, expectation, actions taken to resolve the event and final outcome will be recorded as documented in the medical record, or if reported by the NICU team even before documentation.

All adverse events will be followed to conclusion or recorded as ongoing if there will be no resolution. These adverse event rates will be part of reporting the final results of the study, and for the safety monitoring performed by the Data Safety and Monitoring Committee (DSMC).

6.1 Definitions

6.1.1 Adverse Event

An AE is defined as any untoward medical occurrence in a research subject temporally associated with the use of a drug in humans, whether or not considered drug related. Such an event can result from use of the drug as stipulated in the protocol or labeling, as well as from accidental overdose. Any worsening of a pre-existing condition or illness is considered an adverse event. Laboratory abnormalities and changes in vital signs are considered to be adverse events only if they meet protocol-specific criteria. These criteria are described in the Neonatal Toxicity Table and instructions for its use in the MOP.

6.1.2 Serious Adverse Event

If an AE meets any of the following criteria, it is to be reported to NICHD, the Data Coordinating Center of RTI, and the local IRB (per Center-specific IRB procedures) as a serious adverse event (SAE) within 24 hours of the Center being made aware of the event. See section 6.4 for these important reporting requirements.

(Note: Based on the premature infant population studied, the serious adverse event categories of events that result in congenital anomaly/birth defect or require intervention to prevent permanent impairment or damage (devices), or require hospitalization, do not apply when defining events for these infants, and are not listed.)

Death of Subject	An event that results in the death of a subject.
Life-Threatening	An event that, in the opinion of the Investigator, would have resulted in immediate fatality if medical intervention had not been taken. This does not include an event that would have been fatal if it had occurred in a more severe form.
Prolongation of Hospitalization	An event that prolongs the subject's hospital stay.
Results in persistent or significant disability/incapacity	An adverse event that results in a substantial disruption of the ability to conduct normal life functions, i.e., the adverse event resulted in a significant, persistent or permanent change, impairment, damage or disruption in the infants body function/structure, physical activities and/or quality of life.

Important Medical Event Requiring Medical or Surgical Intervention to Prevent Serious Outcome	An important medical event that may not be immediately life-threatening or result in death or hospitalization, but based on medical judgment may jeopardize the subject and may require medical or surgical intervention to prevent any of the outcomes listed above (i.e., death of subject, life-threatening, prolongation of hospitalization). An example of such events would be an allergic bronchospasm requiring intensive treatment.

6.1.3 Serious Adverse Events That Require Expedited Reporting

The NICHD will determine if individual serious adverse events a) meet the criteria for expedited reporting in accordance with 21CFR 312.32 IND safety reporting and b) if so, are promptly reported to the FDA and AN where indicated. See Section 6.4. Center PIs will determine if individual serious adverse events meet local IRB reporting criteria and report accordingly.

6.2 Characterizing Adverse Events

6.2.1 Severity/Intensity of Adverse Events

The Investigator will use the following general definitions to rate the severity/intensity of each adverse event. The NICHD Neonatal Toxicity Table is provided to guide the Investigators in judging severity/intensity in light of the NICU setting and the fragile condition of the extremely preterm infant (see MOP for the Neonatal Toxicity Table). The categories used in the CRF include: Mild, Moderate, Severe, Life Threatening and Death. Because of the nature of the study population, mild events will not be recorded as adverse events unless they are also unexpected.

Mild	The adverse event is transient, is very common in this population and easily tolerated by the subject.
Moderate	The adverse event causes the subject discomfort or interrupts the subject's usual function.
Severe	The adverse event causes considerable interference with the subject's usual function.
Life Threatening	The adverse event put the subject at substantial risk of dying at the time of the adverse event.
Death	Death was an outcome of the adverse event

6.2.2 Unexpected versus Expected Adverse Events

An unexpected adverse drug experience is defined as "[a]ny adverse drug experience, the specificity or severity of which is not consistent with the current Investigator's Brochure; or, if an Investigator's Brochure is not required or available, the specificity or severity of which is not

consistent with the risk information described in the general investigational plan or elsewhere in the current application," as amended (21 CFR 312.32(a)).

The Investigator will code each adverse event as to whether this is an adverse event that is expected in this population, or if the event was unexpected in this population, or unexpected at the particular time in the course of the infant's hospital course. The IB presents recent morbidity and mortality data on a large cohort of infants similar in GA to the infants who will enroll in the REDUCE-ROP (INS-3) study.

6.2.3 Relatedness of the Adverse Event to the Study Drug

The Investigator will use the following definitions to assess the relationship of the adverse event to the use of study drug: In addition, each adverse event will be coded as to whether it is related to study drug, or to a study procedure.

Definitely Related	An adverse event that is temporally and logically/medically directly related to administration of study drug or a study procedure.
Probably Related	An adverse event has a strong temporal relationship to study drug or recurs on re-challenge and another cause of event is unlikely or significantly less likely.
Possibly Related	An adverse event has a strong temporal relationship to the study drug and another cause of event is equally or less likely compared to the potential relationship to study drug.
Probably Not Related/Unlikely	An adverse event has little or no temporal relationship to the study drug and/or a more likely other cause of event exists.
Not Related/None	An adverse event is due to an underlying or concurrent illness or effect of another drug and is not related to the study drug (e.g., has no temporal relationship to study drug or has a much more likely other cause of event).

For causality assessments, events meeting the categories of definitely, probably, or possibly will be considered "associated." Events that are probably not or not related will be considered "not associated." In addition, when the Investigator has not reported a causality or deemed it not assessable, NICHD will consider the event associated.

6.2.4 Duration and Resolution of an Adverse Event

The adverse event recording form requires recording of the date of onset and resolution for each adverse event, (or documentation that it will not resolve) through 7 days after discontinuation of study drug. Serious adverse events and those events requiring IND safety reporting will be followed until resolution or determination that no resolution is possible.

6.3 Adverse Event Collection Period

All adverse events reported will be collected from the time of first study drug administration until 7 days following discontinuation of study drug. In addition, if an existing condition was present before study drug was started, but became substantially worse afterwards, it will also be reported as an adverse event. Serious adverse events and those events requiring IND safety reporting will be followed until resolution or determination that no resolution is possible.

6.4 Adverse Event Management and Reporting

All adverse events will be recorded on the study CRF and entered in the Data Management System (DMS). Timely mandatory reporting is required for adverse events that are:

1. Serious; and

2. Unexpected (unanticipated); and

3. Definitely, probably, or possibly related to the study drug.

Within 24 hours of discovering a serious adverse event (SAE), the study coordinator, in consultation with the Center's Investigator, will report these events to the NICHD, the Data Coordinating Center, and their own IRB per local requirements. A MEDWATCH form (FDA form 3500A) will be completed and submitted via FAX or e-mail within 24 hours of the Center being made aware of the SAE. Determination of whether the event may be unexpected and at least possibly related to study drug will be made by the clinical team in collaboration with the NICHD. If the serious adverse event meets criteria for expedited reporting, NICHD or their designee will notify the FDA and AN in accordance with set policy.

7 PROTOCOL DEVIATIONS

Compliance with the protocol will be monitored throughout the study. Any deviations or violations noted during conduct of the study are to be reported by the Center's Investigator or their designee. Due to the nature of the study, protocol deviations will need to be reported from both masked research personnel as well as unmasked pharmacy personnel separately. The MOP details the process for reporting protocol or pharmacy deviations or violations.

8 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

8.1 Statistical and Analytical Plans

8.1.1 Independent Data Safety and Monitoring Committee

The NRN DSMC is composed of experienced experts in neonatology, biostatistics, bioethics, and developmental pediatrics. In addition, specific consultants relevant to the protocol also

participate in the DSMC, which for this study includes pediatric ophthalmologists. The current composition of the DSMC is listed in the MOP.

The NICHD NRN DSMC will be responsible for monitoring study safety and study performance as well as reviewing the results of the planned formal interim analyses.

The DSMC will monitor safety at a preplanned frequency when approximately 25%, 50% and 75% enrolled subjects have completed study therapy. At these intervals, the DSMC will review tabular summaries of adverse events and study data to determine whether there are any safety concerns that may impact continuation of the trial, or evidence that study procedures should be changed or the trial should be halted, only for reasons relating to the safety of the study subjects or inadequate trial performance (e.g., poor recruitment of subjects).

Specifically when monitoring safety, the DSMC will monitor the following at minimum:

- Recruitment by center and overall study progress.
- Randomization: to ensure that balance of baseline characteristics is being achieved by randomization.
- Rates of adverse events including serious adverse events, study drug-related adverse events, and deaths.
- Rates of clinical outcomes.
- Additionally, one formal interim analysis of efficacy and futility will be conducted after approximately 1000 infants have been enrolled and reached primary endpoint (Section 8.1.5).

8.1.2 Data Sets Analyzed

Depending on type, each analysis will be conducted within one or more of the following analysis populations. For the overall study analyses for publication, each population will include all infants that meet the population definition. For the regulatory submission analyses conducted within regulatory sub-studies, each sub-study will have a set of analysis populations per the definitions below that only comprise the infants assigned to that regulatory sub-study.

The safety population will include all infants who were randomized and received at least 1 dose of study drug. The safety population will be used for all safety analyses and infants will be grouped according to actual treatment received.

The ITT population is the primary population for formal efficacy analyses. This population includes all subjects randomized. For these analyses, subjects will be analyzed as part of the study arm to which they are assigned by randomization, regardless of the actual therapy they received.

8.1.3 Demographic and Other Baseline Characteristics

Demographic and baseline characteristics are recorded using the NRN GDB Protocol and include GA (weeks), GA stratum, birth weight, head circumference and length, age at start of study drug, sex, race, ethnicity, out born status, use of antenatal steroids, chorioamnionitis, delivery by cesarean section, 1 and 5 minute Apgar scores, use of chest compressions or resuscitation drugs in the delivery room, temperature at 1 hour of age and early onset sepsis (<72 hours). In addition, maternal data are collected on maternal pregnancy complications, type of insurance and educational level.

8.1.4 Efficacy Analyses

For scientific publication, Robust Poisson regression adjusting for center and GA strata will be used to estimate adjusted relative risk (and associated 95% CI) for myo-Inositol 5% Injection vs. placebo. The primary hypothesis will be tested based on a score test of the treatment effect in the Poisson regression model where the null hypothesis of no treatment effect on survival without severe ROP is rejected at either the interim or final analysis with a p-value consistent with an overall Type 1 error rate of 0.05 and a Bonferroni-type correction for multiple comparisons. (Casella and Berger 2002) For this analysis, individuals with indeterminate primary outcome status (i.e. alive through 55 weeks PMA and did not meet final acute ROP status but have ROP assessment data available), will go through an endpoint adjudication process and the resulting adjudicated endpoints will be used in the analyses. Individuals with completely missing outcomes (i.e. no ROP follow-up or death information available) or for which an adjudicated endpoint cannot be determined will be excluded from the analyses.

The following analyses will be conducted for a regulatory submission, provided there is evidence of efficacy. As suggested by the FDA, the primary outcome will be treated as a three level ordinal endpoint where the unfavorable outcomes are assigned a value of -1, indeterminate outcomes are assigned a value of 0, and favorable outcomes are assigned a value of +1. No adjudicated outcomes will be used.

The outcomes will be displayed in the following table:

Table 8. Outcomes Display of all enrolled subjects for the Regulatory Analysis

Outcome Status	Placebo (N1)	Inositol (N2)	Difference
Favorable (1)	n11 (n11/N1)	n21 (n21/N2)	XX
Unfavorable (-1)	n12 (n12/N1)	n22 (n22/N2)	XX
Indeterminate (0)	n13 (n13/N1)	n23 (n23/N2)	XX
Missing (by multiple imputation)	n14 (n14/N1)	n24 (n24/N2)	XX

This endpoint will be analyzed using a 2-sided Mantel-Haenszel chi-square test using modified ridit scores for ordinal data controlling for strata defined by study center and gestational age with

the analyses being completed separately within each of the two regulatory sub-studies. Individuals with completely missing outcomes (i.e. no ROP follow-up or death information available) will be imputed using a multivariate technique for multiple imputation. Formal inference based on the regulatory analyses is conditional on the findings of the primary analysis for scientific publication demonstrating a benefit (i.e. null hypothesis rejected at either the interim or final analysis with a p-value consistent with an overall Type 1 error rate of 0.05 and Bonferroni-type correction for multiple comparisons). This prioritization of analyses precludes the necessity of adjusting alpha for the additional regulatory analyses.

Various sensitivity analyses for both publication and regulatory submission will be performed to assess the impact of missing/indeterminate outcomes and robustness of the finding across multiple clinically relevant subpopulations. For example, if the results of the primary publication analysis of this multi-center trial are positive, then sensitivity analyses of the primary outcome will be performed to examine internal consistency of the study results. Specifically, consistency of treatment effect across subsets defined by GA strata and center separately will be assessed using the robust Poisson regression model. For GA strata, an interaction between GA strata and treatment will be added to the model. A p-value for the interaction >0.2 will be indicative of no interaction effect. If heterogeneity of treatment effect is present, then the likelihood ratio test (Gail and Simon 1985) will be used to determine whether the interactions are qualitative in nature (i.e., a test of the null hypothesis that inositol is at least as good as placebo in every subset of subjects vs. the alternative hypothesis that a cross-over effect exists such that placebo outperforms inositol in at least 1 of the subset of subjects). This same approach will also be used to assess consistency of treatment effect across centers. However, to ensure adequate power for identifying qualitative interactions among centers, the consistency analyses will be conducted with centers clustered into 4 subgroups defined by baseline prevalence of unfavorable ROP status (high vs. low) and expected enrollment size (large vs. small).

The decision to include adjudicated outcomes in the publication analyses as well as in sensitivity analyses for the regulatory submission is based on past studies showing that unfavorable ROP outcomes occur earlier in the hospital course and these subjects are normally under intense follow up efforts for the failure of their ROP to begin to regress (heal). Infants who meet favorable outcomes often have an indolent course of ROP, not meeting criteria for a favorable outcome until weeks after being discharged home. In the weeks following discharge, families often are under duress with the new baby at home and miss appointments or move away from study centers. As such, infants with incomplete follow up are almost always improving towards a favorable outcome.

8.1.5 Interim Analyses

One formal interim analysis of efficacy and futility will be conducted after approximately 1000 infants have been enrolled and reached primary endpoint. Interim analyses will be conducted using the overall study population and the primary analytic approach for publication detailed in Section 8.1.4. Cut-off p-values for testing for efficacy at the interim analysis will be based on a Bonferroni-type correction for multiple comparisons with a nominal α of 0.0001 at the interim

analysis and a nominal α of 0.0499 for the final analysis (Casella and Berger 2002). Specifically, if the p-value for the treatment comparison for the primary outcome is < 0.0001 and in addition, if the separate comparison of mortality alone is significant at < 0.001 at the interim analysis, then the DSMC can recommend stopping enrollment. The DSMC can recommend stopping enrollment for futility if the conditional power for the primary test of treatment effect on survival without sROP is less than 0.3 at this time.

No formal analyses of efficacy data will be conducted as part of the safety monitoring activities described in 8.1.1 and the DSMC will not be able to recommend stopping the study for efficacy at these reviews. Therefore, no α will be spent for the safety reviews. Further detail is provided in the SAP.

8.1.6 Safety Analyses

All safety analyses will be performed using the safety population (i.e., as treated) unless otherwise specified. Rates of adverse events including serious adverse events, study drug-related adverse events, deaths, clinical outcomes and concomitant medication use will be evaluated. Descriptive p-values comparing the study arms will be provided for most safety outcomes and will be obtained using regression model-based analyses. The safety analyses will be repeated within each regulatory sub-study.

8.2 Determination of Sample Size

Sample size calculations are based on the primary analysis of the single trial intended for scientific publication; however considerations for available statistical power are also presented for the submission analyses with the regulatory sub-studies.

The underlying prevalence of unfavorable ROP status (meeting criteria for Type I ROP or worse or expiring before ROP acute/final status is reached) is assumed to be 0.30 (SUPPORT 2010). For the scientific publication analyses based on a 2-sided Mantel-Haenszel test of the primary hypothesis with α=0.05, a total sample size of 1672 infants (836 per group) provides a 90% power for detecting a treatment effect of ≥0.07 absolute reduction in unfavorable ROP status prevalence in the inositol arm compared to placebo.

It is also estimated that approximately 3-5% of the enrolled population may be lost to follow-up without sufficient information to obtain an adjudicated ROP endpoint. These subjects will be non-evaluable.

Accordingly, the final minimum required sample size to retain 90% power at an overall α level of 0.05 with one formal interim analysis for efficacy for detecting a ≥0.07 reduction in unfavorable ROP status prevalence is 1760 infants.

For the regulatory analyses, the study population will be administratively split in a pre-determined manner. To assess the power for the regulatory submission analyses within each sub-

study, simulations were conducted assuming that there will be 880 subjects within each sub-study, an underlying prevalence for sROP of 0.30, 5% subjects with completely missing outcome information and varying prevalence of indeterminate outcome and treatment effect (while the overall study is powered to test for minimally clinically significant treatment reduction, prior studies suggest a larger treatment effect may exist). If the true treatment effect is a 7% reduction in the myo-Inositol 5% Injection group, each sub-study analysis only has between a 56 to 62 percent chance of demonstrating a significant benefit for that sub-study. However, if the true treatment effect is an 8-9% reduction, the power for each sub-study analysis increases to between 69-83%.

Although the study is only powered to formally test one primary hypothesis of efficacy (treatment effect on survival without sROP), a variety of secondary outcomes will be assessed in an exploratory manner. Due to the exploratory nature of these secondary analyses, no additional adjustments for multiplicity outside of accounting for the interim analysis are planned. Given a sample size of 1760 (effective sample size of 1672 infants), the probabilities of detecting a potential treatment effect (i.e., 30% reduction or increase) for the overall study population in common clinical outcomes of secondary interest are summarized in Table 9.

Table 9. Probabilities of Detecting Potential Treatment Effect on Secondary Outcomes

	Underlying Prevalence	Absolute Effect for 30% Change	Probability of Detecting	
			Reduction	Increase
Death all cause	0.18	0.054	0.87	0.78
BPD physiologic definition	0.38	0.114	0.99	0.99
BPD or death from BPD before 37 weeks PMA	0.49	0.147	1.00	1.00
Any ROP	0.65	0.195	1.00	1.00
Type 2 ROP	0.25	0.075	0.96	0.92
Severe IVH (Grade III/IV or shunt or PVL)	0.20	0.060	0.90	0.83

9 ETHICS

9.1 Institutional Review Board (IRB)

Good Clinical Practice (GCP) requires that the clinical protocol, any protocol amendments, the Investigator's Brochure, the informed consent, all other forms of subject information related to the study and any other necessary documents be reviewed by an IRB. These documents are maintained on the Research Triangle Institute NRN website, available to study personnel by login and password.

The IRB at each center/site will review the ethical, scientific and medical appropriateness of the study before it is conducted. IRB approval of the protocol, informed consent and subject information, as relevant, will be obtained prior to the authorization of drug shipment to a study site.

If there is a change in the protocol, it is documented with a technical memo sent to each of the study centers, and a copy is kept on the website together with the amended, dated protocol. Such an amendment to the protocol is coordinated centrally by the Data Coordinating Center of Research Triangle Institute, and will require IRB approval prior to implementation of any changes made to the study design. The Investigator at each center will be required to submit, maintain and archive study essential documents according to International Conference on Harmonization (ICH) GCP.

Any serious adverse events that meet the reporting criteria, as dictated by the protocol and local IRB regulations, will be reported to NICHD, Research Triangle Institute, and to the local IRB per their procedures. During the study, the Investigator should promptly provide written reports to the IRB of any changes that affect the conduct of the study and/or increase the risk to subjects. Written documentation of the submission to the IRB should also be provided to NICHD.

9.2 Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, ICH guidelines, applicable regulations and guidelines governing clinical study conduct and the ethical principles that have their origin in the Declaration of Helsinki. Responsibilities of the Investigator are specified in the MOP.

9.3 Subject Information and Consent

The Investigator or his/her representative will explain the nature of the study to the infant's parent(s)s or legal guardian, and answer all questions regarding this study. Prior to any study-related procedures being performed on the infant, the informed consent statement will be reviewed, signed and dated by the parent(s) or legal guardian, the person who administered the informed consent, and any other signatories according to local requirements. A copy of the informed consent form will be given to the parent(s) or legal guardian and retained as per clinical center practice. An entry must also be made in the infant's dated source documents to confirm that informed consent was obtained.

10 SOURCE DOCUMENTS AND CASE REPORT FORM COMPLETION

10.1 Source Documents

Source documents are defined as original documents, data and records. This may include hospital records (including electronic medical records), clinical and office charts, laboratory data/information, pharmacy dispensing and other records, recorded data from automated instruments, photographs and/or x-rays. Data collected during this study must be recorded on the appropriate source documents; that is, the data can be found on the appropriate source documents.

The exception to this is when a study related judgment is made by the investigators and recorded on a CRF, but is not appropriate to be entered in the medical record. In many cases, a research note can be entered in the medical record and this is recommended whenever possible. For example: if the study coordinator obtains a length and head circumference measurement according to the study protocol training, and records it on the CRF, it would be appropriate to also place a research note in the medical record with those measurements, or give them to a primary provider (house officer, bedside nurse, nurse practitioner or attending physician) to record in the medical record in their daily note.

The Investigator(s)/center(s)/site(s) will permit study-related monitoring, audits, IRB review, and regulatory inspection(s), providing direct access to source data documents (including electronic medical records).

10.2 Case Report Form Completion

All data will be collected by appropriately trained research personnel trained in the responsible conduct of research involving human subjects. All data will be kept secured and confidential, with access limited only to research personnel and appropriate auditing bodies (NICHD, FDA, IRB, AN, etc.) Data will be collected at the center and transmitted to the Data Coordinating Center of Research Triangle Institute.

The data collected on the GDB CRFs provide much of the safety information for this study. Additional data forms are used for enrollment and randomization, drug dosing, serial ophthalmic examinations with documentation of confirming examinations, etc. These forms have been developed and tested throughout the INS-2 inositol dose-ranging pharmacokinetic study. These also include adverse event recording, so that compliance with NRN, National Institutes of Health, local IRB and FDA reporting requirements for serious adverse events will be assured. The MedWatch forms will be used for reporting serious adverse events.

During the Follow-up Phase, data regarding ophthalmic complications and subsequent surgery since the time of primary ophthalmic endpoints will be obtained at the 22-26 month evaluation.

Prior to study start, a training session will be conducted for the Center staff. Ophthalmologists are familiar with the ICROP used in typical nursery ROP examinations. Those unfamiliar with the ICROP will require certification through the BOOST II web-based ROP certification process in addition to conducting examinations with experienced ophthalmologists. All ophthalmologists will be provided with instruction on how the CRFs are to be completed by the coordinators, so that they will understand what data are essential for completion of the forms. Emphasis will be placed on systematic use of the ICROP and the importance of confirming examinations for infants that meet criteria for severe ROP.

Electronic data collection system: The electronic data collection system (EDC) and integration with case report form/work sheets and training is described in the Manual of Procedures.

11 DATA QUALITY ASSURANCE

Prior to the initiation of the study, a training meeting will be held with study personnel, the Investigators, and appropriate Center personnel. This meeting will include a detailed discussion of the protocol, performance of study procedures, and CRF data collection.

As data are entered into the EDC, computer logic checks will automatically check for such items as missing, potentially incorrect or inconsistent study data. If such data are identified, the system will automatically query the Center for clarification or correction of the data. Additionally manual queries will be added by the coordinating center staff as needed based on missing, potentially incorrect or inconsistent study data identified via centralized data reviews or center monitoring visits.

12 USE OF INFORMATION

The information developed during the conduct of this clinical study is considered confidential and will be used by NICHD to test the hypothesis and publish the outcomes of the study.

Subject to the restrictions of the NICHD-AN Clinical Trial Agreement (TTB Ref# 00569-04; year 2005), AN will have access to and review of Private Identifiable Information in Raw Data only for on-site quality auditing. AN will receive Private Identifiable Information only if necessary for purposes of satisfying FDA or other health authorities' reporting requirements, and for internal research purposes directly related to obtaining regulatory approval of Inositol. AN is prohibited from access, review, receipt, or use of such information for other purposes. All other Raw Data in NIH's Possession & Control will be made fully available to AN for their own analysis and for application to the FDA, and will be publically released by NICHD only in accordance with applicable federal regulations and guidelines.

Summary Data shall be the property of the NICHD NRN and shall not be released to the public without prior review by NICHD. AN retains the right to access the Raw Data and Summary Data for regulatory purposes and to use the Raw Data and Summary Data for any regulatory filings it deems necessary or appropriate.

Individuals participating in the study conduct will contribute to publications and analysis only through the policies and procedures of the NICHD NRN.

The Investigator at each center will maintain a confidential subject identification code list of all subjects enrolled in the study (by name and subject number). The code list will not be transmitted to RTI or to AN, but will be made available as needed during audits and/or site visits.

13 COMPLETION OF THE STUDY

The Investigator will conduct the study in compliance with the protocol, MOP, and Policies and Procedures of the NICHD NRN. The Inositol Subcommittee and Research Triangle Institute will prepare the data analyses and publications following conclusion of the study.

Each center's Investigator will provide interim annual reports and a final report to their IRB following conclusion of the study, and maintain research files in secured storage for audit per the NRN policies.

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