COVER

PROTOCOL including STATISTICAL ANALYSIS PLAN

TITLE: Synergistic Pharmacologic Intervention for Prevention of ROP (SPIPROP STUDY)

NCT NUMBER**:** NCT02344225

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Title: **Synergistic Pharmacologic Intervention for Prevention of ROP (SPIPROP STUDY)**

Protocol Number: **1 U54HD071594**

Study Drugs: **Ibuprofen IV & Ketorolac (Acuvail) Ophthalmic Solution**

IND Number: **122395**

Phase: **II b**

Sponsor: **NIH-NICHD**

Principal Investigator: **Jacob V. Aranda MD, PhD, FRCPC, FAAP**

SUNY Downstate Medical Center

Division of Neonatology, Dept. of Pediatrics

450 Clarkson Ave, Box 49

Brooklyn, NY 11203

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1.0 INTRODUCTORY STATEMENT AND INVESTIGATIONAL PLAN

Retinopathy of prematurity (ROP), the most common cause of blindness in children, is a developmental retinal vascular disorder characterized by abnormal growth of retinal blood vessels in the incompletely vascularized retina of extremely low gestational age neonates (ELGANs), ≤28 weeks (w); ≤1250 grams (g). In 2010, Brooklyn, NY had 170 of 209 (81.3%) neonates born < 1,000 grams developed ROP (all stages), with 12/209 (5.7%) progressing to Stage 3+ treated with Laser or Avastin, leading to significant impairment of vision and a lifetime of darkness. The incidence and severity of ROP increase with decreasing birth weight and gestational age. The multicenter Cryotherapy for Retinopathy of Prematurity study indicated that the incidence of ROP increased from approximately 40% in infants with birth weights 1101 to 1200 g to more than 90% in those with birth weights 501 to 600 g. ROP increased from approximately 35% in infants with gestational age of 31 weeks to more than 95% in those with gestational age of 24 weeks. Over 90% percent of neonates born <25 weeks gestation have been reported to develop ROP with over 70% who developed proliferative disease (stage ± 3) and over 60% treated with retinal ablation. Impaired visual acuity (< 0.33) was found in 32.6% of boys and 9.2% of girls, who were blind living in darkness or had severe visual handicaps. In New York, 27.3% (1839 of 6745) of infants with birth weight <1200 g developed ROP. The incidence of any ROP was 68% among infants of <1251 g with a higher rate in Brooklyn, NY. Although the overall incidence of ROP has decreased, it still remains high in ELGANs at some centers, with threshold ROP occurring in 5% of ELGANs in the US, with as much as 30% of them becoming blind. Although milder ROP (Stage 1 & 2) regress, visual problems requiring early use of corrective lenses are common. The proven risk factors for the development of ROP are immature retinal vasculature, hyperoxia, and circulatory and respiratory instability. Other factors include extremely low birthweight (ELBW), early gestation, patent ductus arteriosus (PDA), intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD)/chronic lung disease (CLD), necrotizing enterocolitis (NEC), and sepsis. Because ROP continues to be a major problem, and due to its multi-factorial nature, a better understanding of the underlying mechanisms is vital for its prevention. Current methods for treatment and prevention are highly invasive leading to increased pain and distress in these micropremies. The search for new, effective, and targeted pharmacotherapies warrants a high priority. Our proposed studies will investigate whether targeted, early, locally applied non-steroidal anti-inflammatory drugs (NSAIDs) such as Ketorolac (Acuvail) or systemic Ibuprofen potentiates Caffeine in preventing the cascade of events leading to severe ROP and a lifetime of blindness and darkness

1.1 Rationale:

Caffeine and NSAIDs have already been shown to decrease the risk of severe ROP in ELGANs. Here we propose a novel approach of combining topical ketorolac (Acuvail) or systemic Ibuprofen (Neoprofen) with systemic caffeine citrate (Cafcit) to optimize their efficacy for prevention of ROP. The overarching goal of this proposal is to investigate the synergistic effects of NSAIDs potentiated with systemic Caffeine on the incidence and severity of ROP. Our specific aims are

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three-fold: 1) To establish the synergistic effect of local ophthalmic NSIADs and systemic caffeine as optimal therapies for the attenuation and/or prevention of severe ROP. We hypothesize that ocular Ketotolac or systemic Ibuprofen potentiated with systemic Caffeine will prevent or diminish the severity of ROP. We will evaluate the safety, tolerability, and efficacy of early postnatal local ophthalmic NSIADs for prevention of severe ROP in ELGANs and determine the pharmacodymanics and pharmacogenomics of ocular NSAIDs potentiated with caffeine for prevention of ROP; 2) To identify a "critical" number of arterial oxygen desaturations as a key risk factor for severe ROP. We hypothesize that there is a "critical" number of daily arterial oxygen desaturations experienced by ELGANs during the first two weeks of life that is a key risk factor for severe ROP. We will further define the role of biomarkers of angiogenesis such as vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-I and proteases such as matrix metalloproteinases (MMPs); as well as biomarkers of oxidative stress and lipid peroxidation such as 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) in ROP and correlate the levels with the number of arterial oxygen desaturations. MMPs also cleave Notch/Dll4, which acts as a regulator of VEGF signaling; and 3) To determine whether infants at risk for severe ROP are haploinsufficient for the delta-like ligand 4 (Dll4). We hypothesize that ELGANs at risk for severe ROP will have different pattern of gene expression specifically related to the Notch signaling pathway, as has been previously shown in animal models. We will examine cord blood, cord tissue, and placental tissue to compare the gene profile of VEGF and Notch signaling pathways among infants who develop severe ROP and those who do not, and determine whether NSAIDs and/or Caffeine will confer protective benefits on Notch/Dll4 signaling and prevent the development of severe ROP.

1.2 Number of Patients:

Total = 120 (40 per arm)

1.3 Ibuprofen Risks:

Ibuprofen has been used to prevent or treat PDA with similar efficacy and a more favorable safety profile than Indomethacin. Ibuprofen has been shown to have milder effects on cerebral, renal and mesenteric blood flow. However, prophylactic (within 48 hours of life) and early treatment (median 6 days) with Ibuprofen in infants at risk for PDA is associated with an increased risk of spontaneous intestinal perforation (SIP). More over a recent Cochrane Review of 27 clinical trials found that the risk of developing NEC was reduced for Ibuprofen, the duration of ventilatory support was reduced with Ibuprofen, the risk of NEC was reduced, there was less evidence of transient renal insufficiency in infants who received ibuprofen. The authors concluded that Ibuprofen is as effective as Indomethacin in closing a PDA and reduces the risk of NEC and transient renal insufficiency. Given the reduction in NEC ibuprofen currently appears to be the drug of choice. Too few patients have been enrolled in studies assessing the effectiveness of a high dose of ibuprofen versus the standard dose and early versus expectant administration of ibuprofen to make recommendations. See drug package insert (Appendix III).

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1.4 Ketorolac Risks:

Ketorolac is a (NSAID) derived from Indomethacin. Its mechanism of action is blocking the synthesis of prostanoids by inhibiting the cyclooxygenase (COX) enzymes in arachidonic acid (AA) metabolism. The reported adverse effects are linked predominantly to their inhibitory action of platelet aggregation with hemorrhage being the principal adverse reaction. Topically administered Ketorolac decreases prostaglandin concentration in aqueous humor without modifying the intraocular pressure. Ophthalmic Ketorolac has not been shown to be quantifiable in plasma. Ketorolac ophthalmic solution is usually used in older adults with retinal disorders. It is used to diminish the cystoid macular edema that complicates the surgery of cataracts suggesting drug effects on the inner retina. Other adverse effects of ophthalmic Ketorolac are occasional episodes of discomfort, ocular burning, corneal lesions and corneal haziness. Corneal toxic lesions have been reported with NSAIDs such as nepafenac and case reports of reversible toxic keratolysis in adults with prolonged ketorolac treatment. The use of ketorolac ophthalmic solution in pediatrics is frequent as an analgesic in corneal abrasions, and in allergic and post surgical conjunctivitis. The FDA recognizes its indication for allergic conjunctivitis, ocular pain, post surgical ocular inflammation, ocular pruritus and photophobia. See drug package insert (Appendix IV).

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2.0 PROTOCOL SYNOPSIS

Protocol Title	**S**ynergistic **P**harmacologic **I**ntervention for **P**revention of **ROP** (SPIPROP Study)
Sponsor:	NICHD
Product	IV Ibuprofen, Ketorolac (Acuvail) ophthalmic solution
Objectives:	• To evaluate the safety, tolerability, and efficacy of early postnatal local ophthalmic NSIADs potentiated with caffeine for prevention of severe ROP in ELGANs. Our hypothesis is that ocular ketorolac or systemic ibuprofen potentiated with systemic caffeine will prevent or diminish the severity of ROP. • To determine the number of daily arterial oxygen desaturations that is critical for the development of severe ROP, correlate this number with the levels of serum VEGF, IGF-I, 8-iso-$PGF_{2\alpha}$, and MMPs, Our hypothesis is that there is a "critical" number of daily arterial oxygen saturations experienced by extremely low gestational age neonates during the first two weeks of life that is a key risk factor for severe ROP. • To determine whether infants at risk for severe ROP are haploinsufficient for the Dll4. Our hypothesis is that extremely low gestational age neonates at risk for severe ROP will have a different pattern of gene expression specifically related to the Notch signaling pathway, as has been shown in animal models.
Study Design:	Multi-center, Phase 2 open label, randomized, prospective, pharmacokinetic and safety study of for the prevention and treatment of ROP in extremely low gestational age neonates.
Study Population:	• All infants <28 weeks • All infants <1250 grams • Requiring oxygen therapy and/or ventilator support within the first 2 days of life • All infants requiring Caffeine Citrate for Apnea of Prematurity as per standard of care
Number of Infants:	120 infants (n=40 per arm)
Number of Centers:	5
Treatment:	• **Group 1 (n=40):** Caffeine citrate IV (20 mg/kg loading dose followed by 5 mg/kg/day maintenance dose) + placebo saline IV (1 ml/kg

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	loading dose followed by 0.25 ml/kg) for 5 days + sterile normal saline (one drop 2 times per day, every 12 hours) for 14 days. • **Group 2 (n=40):** Caffeine citrate IV (20 mg/kg loading dose followed by 5 mg/kg/day maintenance dose) + Ibuprofen IV (10 mg/kg loading dose followed by 2.5 mg/kg/day) for 5 days + sterile normal saline (one drop 2 times per day, every 12 hours) for 14 days. • **Group 3 (n=40):** Caffeine citrate IV (20 mg/kg loading dose followed by 5 mg/kg/day maintenance dose) + placebo saline IV (1 ml/kg loading dose followed by 0.25 ml/kg) for 5 days + Ketorolac ophthalmic solution (one drop 2 times per day every 12 hours) for 14 days. **The doses of Caffeine Citrate are as per standard of care for Apnea of Prematurity.**
Treatment Duration	Drug or placebo administration will be initiated between 0 to 72 hours of life and continue until 5 to 8 days of life (Ibuprofen), or 14 to 16 days of life, depending on time of initiation of the drug or placebo. Study drug/placebo will be discontinued in infants with any serious adverse event (SAE). Monitoring for adverse events will occur until death or discharge from the neonatal intensive care unit (NICU).
Specimen Collection	1) Mixed Cord blood: Mixed cord blood approximately 1.0 to 3.0 cc per patient 2) Cord tissue: 6 samples per patient (approximately 5 mm thick, 200 mg) 3) Placental tissue: 7 samples per patient (approximately 5 mm thick, 200 mg) from maternal-fetal interface, fetal side, and intervillous space. 4) Blood samples: Total 5 for this study (0, 1, 7, 14, and 21 days) approximately 0.3 mL each 5) Urine samples: Total 5 for this study (0, 1, 7, 14, and 21 days) approximately 1.0 to 3.0 mL each 6) Mixed cord blood, cord tissue and placental tissue will be used to assess for haploinsufficiency of Notch/Dll4. Patients can still be enrolled in the study if cord and placental samples cannot be obtained at the time of delivery.
Safety:	The protocol will rely on three mechanisms for safety: 1) The data safety monitory board (DSMB) 2) Adverse event (AE) and SAE reporting 3) The active, daily, real time oversight of the clinical staff

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	Neonates will be clinically monitored and ophthalmologic exams performed as per the standard of care. Safety assessments will include but are not limited to: the physical examination; routine laboratory values; renal function; hematologic parameters; urine output; gastrointestinal function (bleeding) & NEC; daily examination by the neonatal staff of the cornea for haziness and lesions; and progression of ROP if any by the Ophthalmologists. All SAEs will be closely monitored throughout the course of the study.
Statistical Consideration:	The primary objective of this study is to assess the safety and potential efficacy of caffeine plus NSAIDs to prevent ROP. We will enroll 40 evaluable neonates for each group (total n=120). Evaluable subjects are those who fulfill inclusion and exclusion criteria and survived for 36 weeks postconceptional (PCA) age eye examination. The estimated rate of ROP (all stages) for neonates <1250 grams in the Brooklyn Queens Neonatal Network (BQNN) is 58% and severe (Stage 3 plus) is 5.7%. We hypothesize that early synergistic intervention is safe and will decrease the incidence of ROP in the combination therapy treated patients by 50%. Assuming an ROP incidence of 57% (all stages), a total of 120 evaluable neonates or 40 infants in each group would be needed to have a statistical power of 80% to detect a 50% relative reduction in the risk of ROP at an alpha =0.05 beta =0.20. This is a small, adequately powered phase II, safety, efficacy, pharmacodynamic trial which will generate requisite data needed for the design and implementation of a conclusive randomized phase 3 trials. Randomization will be done using sealed envelopes at each site in blocks of 6.
Inclusion Criteria	Neonates at high risk for ROP as outlined by the American Academy of Pediatrics, Section on Ophthalmology; American Association for Pediatric Ophthalmology and Strabismus; and American Academy of Ophthalmology will be enrolled. Inclusion criteria are: 1) all infants with a birth weight of less than 1250 grams; 2) all infants with a gestational age of 28 weeks or less; 3) all infants who required oxygen therapy and/or ventilator support within the first 2 days of life; and 4) All patients on Caffeine Citrate for Apnea of Prematurity as per standard of care.

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Exclusion Criteria	Exclusion criteria are: 1) Infants with major congenital anomalies, congenital sepsis, chromosome abnormalities (incl. duct-dependent cardiac anomalies). 2) Fused eyelids 3) Maternal antenatal NSAID exposure < 72 hours before delivery of the baby. 4) Infants with cardiac conditions including cardiomyopathies, serious arrhythmias and congenital heart defects. 5) Infants transferred from outside hospital after 72 hours of life. 6) Renal failure or oliguria (defined as urine flow rate <0.5 ml/kg/hour in the 8 hours prior to randomization). Anuria is acceptable if less than 24 hours of life. 7) Admission platelet count <50,000 mm^3. 8) Clinical bleeding such as continuous, active oozing from puncture site. 9) Participation in other clinical trials while subjects participate in this study and for 7 days after the last dose of study drug. 10) Infants with cyanotic heart disease diagnosed by fetal echocardiography during pregnancy or infants who show persistently low oxygen saturations as per attending neonatologist despite adequate ventilator assistance.

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3.0 ABBREVIATIONS

AA		Arachidonic Acid
AE		Adverse Event
ANOVA		Analysis of Variance
BPD		Bronchopulmonary Dysplasia
BQNN		Brooklyn Queens Neonatal Network
BUN		Blood Urea Nitrogen
C		Drug Concentration
CBC		Complete Blood Count
CC		Coordinating Center
CLD		Chronic Lung Disease
COX		Cyclooxygenase
CRF		Case Report Form
CRYOROP		Cryotherapy for Retinopathy of Prematurity
Dll4		Delta-like ligand 4
DNA		Deoxyribonucleic acid
DOL		Day of Life
DSMB		Data and Safety Monitoring Board
DSMP		Data Safety Monitoring Plan
ECM		Extracellular matrix
ECs		Endothelial Cells
ELBW		Extremely Low Birth Weight
ELGANs		Extremely Low Gestational Age Neonates
FDA		Food and Drug Administration

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FiO_2		Fraction of Inspired Oxygen
g/dL		Grams per Deciliter
GA		Gestational Age
GCP		Good Clinical Practice
HIPAA		Health Insurance Portability and Accountability Act
IEC		Independent Ethics Committee
IGF-I		Insulin-like growth factor-I
IND		Investigational New Drug
IRB		Institutional Review Board
IVH		Intraventricular Hemorrhage
Kg		Kilogram
LFT		Liver Function Tests
mL		Milliliter
MMPs		Matrix Metalloproteinases
NEC		Necrotizing Enterocolitis
NICHD		National Institute of Child Health and Disease
NICU		Neonatal Intensive Care Unit
NIH		National Institute of Health
NSAIDs		Non-Steroidal Anti-Inflammatory Drugs
NYPD-PRC		New York Pediatric Developmental Pharmacology Research Consortium
OIR		Oxygen-Induced Retinopathy
PCA		Postconceptional Age
PD		Pharmacodynamic
PDA		Patent Ductus Arteriosus

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PG		Prostaglandins
$PGF_{2\alpha}$		Prostaglandin $F_{2\alpha}$
PGI_2		Prostaglandin I_2, Prostacyclin
PI		Principal Investigator
PK		Pharmacokinetic
RNA		Ribonucleic acid
ROP		Retinopathy of Prematurity
ROS		Reactive Oxygen Species
SAE		Serious Adverse Event
Shh		Sonic HedgeHog
STOP-ROP		Supplemental Therapeutic Oxygen for Prethreshold Retinopathy of Prematurity
SUNY		State University of New York
TIMP-1		Tissue Inhibitor of Metalloproteinases
TxA_2		Thromboxane A2
VEGF		Vascular Endothelial Growth Factor
VEGFR-1		Vascular Endothelial Growth Factor-1

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4.0 FLOW CHART OF STUDY PROCEDURES

Informed consent/Privacy Acknowledgement	**Baseline**	**Treatment**	**Post-Treatment**
Adverse Events		X	X
Body Weight	X	X	X
Clinical laboratory tests (renal, liver, hemogram)	X	X	
Collect extra scavenged blood from blood gases	X	X	X
Collection of placenta, cord blood, cord tissue	X		
Demographics (BW, GA, Apgar score, maternal history, diagnoses, O_2 and respiratory support, blood gases.	X		
Medical Baseline Conditions	X		
Neurodevelopment Assessment			
Ophthalmology exam			X
Pain Scoring	X	X	
Pertinent Medical History	X		
Physical examination	X		
Plasma and urine sampling	X	X	X
Record all concomitant medications	X	X	X
Record all morbidities			X
Record all non-medication treatments (surgical, other procedures)	X	X	X
Record results of all exams (head/abdominal US, CT scans, MRIs, echo, etc.)	X	X	X
Recording Surgical Interventions	X	X	X
ROP Assessment			

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Study Drug Dosing			
Vital Signs	X	X	

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5.0 INTRODUCTION

5.1 Retinopathy of prematurity (ROP)

ROP is the most common cause of blindness in children. It is a developmental retinal vascular disorder characterized by abnormal growth of retinal blood vessels in the incompletely vascularized retina of ELGANs, <28 weeks (w); <1250 grams (g). In 2010, Brooklyn, NY had 170 of 209 (81.3%) neonates born < 1,000 grams developed ROP (all stages), with 12/209 (5.7%) progressing to Stage 3+ treated with Laser or Avastin, leading to significant impairment of vision and a lifetime of darkness. The incidence and severity of ROP increase with decreasing birth weight and gestational age. The multicenter Cryotherapy for Retinopathy of Prematurity (CRYOROP) study indicated that the incidence of ROP increased from approximately 40% in infants with birth weights 1101 to 1200 g to more than 90% in those with birth weights 501 to 600 g (1,2). ROP increased from approximately 35% in infants with gestational age of 31 weeks to more than 95% in those with gestational age of 24 weeks. Jacobson et al. (3) reported that 97.4% of 114 neonates born <25 weeks gestation had ROP, 74.6% developed proliferative disease (stage ± 3) and 63.2% were treated with retinal ablation. Impaired visual acuity (<0.33) was found in 32.6% of boys and 9.2% of girls, who were blind living in darkness or had severe visual handicaps (4). In New York, 27.3% (1839 of 6745) of infants with birth weight <1200 g developed ROP (4). The incidence of any ROP was 68% among infants of <1251 g (5) with a higher rate in Brooklyn, NY. Although the overall incidence of ROP has decreased, it still remains high in ELGANs at some centers (5), with threshold ROP occurring in 5% of ELGANs in the US, with as much as 30% of them becoming blind (6,7). Although milder ROP (Stage 1 & 2) regress, visual problems requiring early use of corrective lenses are common. The proven risk factors for the development of ROP are immature retinal vasculature, hyperoxia, and circulatory and respiratory instability (8). Other factors include low birthweight, early gestation, PDA, IVH, BPD/CLD, NEC, and sepsis (9). Because ROP continues to be a major problem, and due to its multi-factorial nature, a better understanding of the underlying mechanisms is vital for its prevention. Current methods for treatment and prevention are highly invasive leading to increased pain and distress in these micropremies. The search for new, effective, and targeted pharmacotherapies warrants a high priority. Our proposed studies will investigate whether targeted, early, topically applied NSAIDs or systemic Ibuprofen potentiates Caffeine in preventing the cascade of events leading to severe ROP and a lifetime of blindness and darkness

5.2 Role of Oxygen Saturation Control

Prematurity and hypoxia/hyperoxemia are two underlying factors in the development of ROP. Previous studies have suggested that the incidence of ROP is lower in preterm infants with exposure to reduced levels of oxygenation than in those exposed to higher levels of oxygenation (10-15). Giving supplemental oxygen to maintain oxygen saturation 96% to 99% in the STOPROP study did not decrease progression of ROP but did increase exacerbation of chronic lung disease, use of diuretics and oxygen therapy (16). Currently, it is unclear what range of oxygen saturation is appropriate to minimize ROP without increasing adverse outcomes. A randomized controlled

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trial comparing target ranges of oxygen saturation of 85 to 89% versus 91 to 95% in ELGANs showed more deaths before discharge in the lower-oxygen-saturation group (19.9% vs. 16.2%; p=0.04), whereas severe ROP among survivors occurred less often in this group (8.6% vs. 17.9%; p<0.001) (17). The increase in mortality is a major concern, since a lower target range of oxygen saturation is increasingly being advocated to prevent ROP. Clearly, a balance between the maximum reduction in severe ROP and no increase in mortality remains a challenge for neonatal care givers. There are wide ranges of oxygen saturation targets in various NICUs (18) and the critical oxygen saturation ranges increasing the risk of ROP is still unknown. However, the practice of targeting O_2 saturations at 88% to 92% coupled with education of neonatal staff and better oxygen monitoring has been associated with further reductions in severe ROP (19). This approach is currently in practice in our NICU's and will be maintained in this trial.

5.3 Pathophysiology

The retina is oxygenated from retinal and choroidal circulations. The choroidal circulation is formed from arteries that pierce the sclera and form smaller branches that supply the choriocapillaries (20,21). In humans, formation of retinal vasculature occurs in utero, where the arterial oxygen tension is <30 mmHg. The inner retinal vasculature, which nourishes the inner portion of the retina, starts developing by the 16th week of gestation and is mature at term (22). The retina is highly susceptible to oxidative damage for several reasons: 1) it is high in polyunsaturated fatty acids, which are targets for oxygen free radicals; 2) it processes light, and photoexcitation can initiate free radical formation and peroxidation reactions and; 3) it has a high metabolic rate and requires a high blood flow for oxygen consumption (23-25). The retinal cells of premature infants are further subjected to altered perfusion, metabolic acidosis, and respiratory failure. During these adverse events, the retina derives its oxygen from the choroidal circulation and constricts in response to oxygen tension. The choroidal circulation autoregulates in response to changes in oxygen tension; therefore excess oxygen passes from the choroid to the retina, causing it to constrict to the point of obliteration. The development of ROP is thought to occur in two phases: Phase 1) vaso-obliteration: when the premature newborn infant is exposed to high levels of oxygen, normal vasculogenesis is disturbed, causing the immature retina to constrict and leading to obliteration of retinal capillaries, ischemia, retardation of vessel development, and apoptosis of the retinal cells; and Phase 2) vaso-proliferation: this phase is initiated when the infant is placed in room air. It is during the second phase of ROP that retinal hemorrhages, retinal folds, and retinal detachment occur (26). **This form of ROP may not be applicable to ELGANs (<1000 g) who experience frequent arterial oxygen desaturations during mechanical ventilation and oxygen therapy.** Seventy to ninety percent of all ELGANs experience recurrent, clinically significant apnea during the first weeks of life. These apneic episodes last several seconds and result in arterial oxygen desaturations. Apneas of less than 10 seconds duration can result in a reduction in oxygen saturation of 40% (27). The relationship between fluctuating arterial oxygen saturation and the risk for severe ROP has been demonstrated in humans (15,27,28) and animal models (2,29-36). These studies demonstrate that **the two phases of ROP occur simultaneously in ELGANs and suggest a "new" model for the development of the disease.**

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The mechanistic events of ROP may be activated early through constant cycling of hyperoxia/hypoxia, and may be irreversible. **Therefore, it is vital to identify infants at most risk for severe ROP and identify safe, effective pharmacotherapies and dosing regimens to prevent severe ROP.**

5.4 Role of COX in ROP

Exposure to high levels of oxygen induces the formation of highly reactive oxygen species, or ROS (37). This free radical formation appears to play an important role in the pathophysiology of ROP (38-40). Prostanoids play a key role in choroidal blood flow autoregulation (41). Generation of ROS is also known to induce VEGF, a potent angiogenesis factor involved in ROP (42) and prostanoids (43,44). The COX enzyme, which exists in three isoforms, regulates the production of prostanoids (45,46). The constitutively expressed COX-1 is responsible for maintaining basal PG levels, while the inducible COX-2 is involved primarily in inflammatory processes (47,48). COX-3 is a splice variant of COX-1 that retains the intron-1 gene sequence at the mRNA level which encodes a 30 amino acid sequence inserted into the N-terminal hydrophobic signal peptide of the enzyme protein (49). Recent data have shown that the inducible COX-2 correlates with VEGF expression (50) and with vascular function (51), and may contribute to angiogenesis (52,53). COX-2 can be expressed on ischemic stimuli in the retina (54). COX-2 exerts angiogenic effects in tumors (55,56) and corneal neovascularization (57). COX-2 inhibition has been shown to decrease retinal neovascularization in ischemic models (58). We have previously shown that COX-2 inhibition during hyperoxia preserves VEGF receptor (VEGFR)-1 receptor mRNA expression in newborn rabbit retinas (59). Increasing evidence suggests that free radicals such as superoxide anion are increased and contribute to tissue injury (60). Certain prostanoid compounds, the F-isoprostanes, are generated by oxygen free radical lipid peroxidation of arachidonic acid (AA), independent of the COX enzyme (61). One of these isomers, 8-isoPGF$_{2\alpha}$, is a potent mitogen and constrictor of vascular smooth muscle, and is highly correlated with oxidative stress (62). Recently, studies have demonstrated that 8-iso-PGF$_{2\alpha}$ is functionally linked to and activates the thromboxane A_2 (TxA_2) receptor (63,64). TxA_2, a potent product of AA metabolism in human platelets, is dependent on the COX enzyme and has been shown to be associated with oxidative injury in ROP (65,66). Prostacyclin (PGI2) appears to play a significant role in reversing the vasoconstrictor effects of TxA_2, an effect that may be impaired in ROP (67). Studies by Stuart et al. (67,68) have demonstrated marked reductions in kitten retinal 6-keto prostaglandin F$_{1\alpha}$, (6-ketoPGF$_{1\alpha}$, the stable metabolite of PGI_2) during hyperoxia and recovery in room air. Prostaglandin $F2_a$ ($PGF2_a$) has been shown to cause potent vasoconstriction, and participates in endothelial cell death and vasoobliteration of porcine retinal vessels (68). Nonselective COX inhibitors inhibit subretinal neovascularization (69) and improve oxygen-induced retinopathy (OIR) in mice (70) and rats (71), and suppress VEGF and IGF-I in the retina (72). The importance of the COX/PG influence in ROP provides one of the key mechanisms for the benefits of NSAIDs for the treatment and/or prevention of ROP (73). We have shown that ibuprofen decreases OIR in newborn rats but Indomethacin worsens it (Figure I). While both indomethacin and ibuprofen exert no effect on retinal vasoconstrictor TXB_2, Ibuprofen increases

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the vasodilator prostacylin which may partly ameliorate NSAID-induced retinal vasoconstriction during oxygen therapy. Higher doses of Indomethacin in preterm newborns increase risk of ROP (74). Thus, Ibuprofen was chosen over Indomethacin for the prevention of ROP. An overwhelming majority of human and animal studies have focused on rescue treatment of ROP when damage to the retina has already occurred. The current proposal will evaluate the therapeutic benefits of prophylactic use of topical or systemic NSAIDs potentiated with Caffeine, thus providing a novel, safe, and effective pharmacotherapy for ROP prevention.

5.5 Role of VEGF in ROP

As the retina develops, astrocytes and neural precursors spread out and migrate away from the existing blood vessels. As they move further away from the blood vessels, the astrocytes, which are hypoxic sensors, encounter hypoxic conditions. Hypoxia stimulates astrocytes to produce VEGF, forming a VEGF concentration gradient that stimulates angiogenesis and growth of new vessels toward the VEGF-producing astrocytes. The growing blood vessels follow the astrocytes and continue to migrate outward. A threshold concentration of VEGF is needed to inhibit apoptosis of endothelial cells (ECs) and stabilize the new vessels. The soluble $VEGF_{121}$ may be involved in maintenance and stability of the new vessels. In ROP, hyperoxic conditions cause the astrocytes to decrease VEGF production, leading to regression of vessels and impaired retinal angiogenesis. VEGF has been shown to play a key role in the development of ROP (75-84) Levels of VEGF in the vitreous humor correlate with the degree of neovascularization. Aiello et al. (82) demonstrated disease-related increase in VEGF protein in the vitreous and aqueous of human active neovascular retinopathies. Pierce et al. (83) showed that upregulation of the VEGF message in an oxygen induced retinopathy (OIR) mouse model can be reversed by placing the animals back in oxygen. VEGF mRNA is upregulated in tissue ischemia subsequent to oxygen-induced retinopathy. VEGF protein results in hyperpermeability of the endothelial cells and mediates plasma leakage. Increased VEGF may contribute to the edema observed at the transition zone in retinas with OIR and ROP (76). VEGF acts as a strong (perhaps the primary) angiogenic stimulus for preretinal neovascularization in the oxygen-injured retina (78). Various proteases (plasmin and matrix metalloproteinases or MMPs) cleave membrane-bound VEGF to produce diffusible isoforms. This appears to be a primary and key step involved in the cascade of events leading to ROP. Proteolysis of VEGF increases its gradient and triggers an angiogenic switch (84,85). MMPs degrade extracellular matrix (ECM), making it possible for retinal vessels to migrate and proliferate. The type IV collagenases (MMP-2 and –9) and tissue inhibitor of metalloproteinase (TIMP)-1 appear to play a key role in retinal neovascularization (86). The role of MMPs and its co-expression with COX-2 in inflammation is well established (87,88). These observations enable us to hypothesize that preserving the VEGF gradient during hyperoxia may be more beneficial than preventing vaso-proliferation. **Given the association between COX and VEGF, we will demonstrate the protective effects of early, locally applied NSAIDs potentiated with Caffeine to protect the immature retina from ROP. We will examine the efficacy of NSAIDs/caffeine pharmacotherapy for preserving VEGF and balancing MMP/TIMP.**

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5.6 Notch/Dll4 Signaling and Haploinsufficiency in ROP

Heterozygotes sometimes display an intermediate phenotype which can result from loss-of-function mutation in a gene where the amount of protein is not sufficient for normal cellular function. Such genes are called “haploinsufficient” which indicates that one copy of the gene is insufficient for proper function (89). Loss of function of the Notch receptors or their ligands has diverse and often severe clinical, physiological and biological consequences in humans (90). During normal retinal vascular development, suppression of Dll4/Notch signaling markedly enhances angiogenic sprouting and promotes the formation of a denser primary capillary network (91). Therefore, Dll4 acts as a negative regulator and potent endogenous inhibitor of vascular sprouting. Notch activation downregulated VEGFR-2 in EC cell lines, while Dll4 haploinsufficiency caused upregulation of VEGFR-2 in the retina of neonatal mice (92). In contrast, Jagged1 (another Notch ligand) is proangiogenic and down-regulates Dll4/Notch signaling (93,94). Dll4 is highly expressed in the embryonic eye (95) and developing retinal vasculature. Gene inactivation in mice shows that Notch signaling is critical for vascular development. Treatment with COX inhibitors down-regulated Jagged1, an effect that was restored by prostaglandin E2 or exogenous COX-2, demonstrating that activation of Notch1 signal pathway occurs in part through COX-2 (96). The regulation of Notch/Dll4 signaling on VEGF and angiogenesis allow us to speculate that ELGANs who develop severe ROP are haploinsufficient for Dll4, causing increased Notch/Jagged1 signaling and enhanced retinal vascular sprouting and is a molecular target for drug intervention.

5.7 Critical barriers: Pharmacologic Interventions

Caffeine citrate is a methylxanthine used as first-line pharmacotherapy for reducing the frequency of apneic episodes. Caffeine is one of the most frequently prescribed drugs in ELGANs (approximately 93%) during their hospitalization. Caffeine significantly reduced the risk of severe ROP in ELGANs (97). The mechanism of caffeine effects on severe ROP remain to be determined, but may involve effects on regulators of angiogenesis such as sonic hedgehog (Shh) (98,99), VEGF, and MMPs (100). We have shown that caffeine increased retinal VEGF during the vaso-obliterative phase of OIR (Figure 2A), which may allow normal vascularization in the phase of oxygen induced obliterative effect: In contrast, caffeine at therapeutic doses suppressed VEGF during vasoproliferative phase (Figure 2B), which may lead to decrease neovascularization. Although low serum IGF has been associated in babies with ROP (101) caffeine probably does not exert its protective effect via this mechanism since serum IGF is suppressed by therapeutic caffeine during vasoproliferative phase. Caffeine potentiates the anti-inflammatory effects of COX inhibitors in activated microglia which may also occur in the retina (102). Caffeine has been used in combination with NSAIDs for many decades to enhance their analgesic effects, suggesting that caffeine may be an effective adjuvant to ibuprofen (103,104). Caffeine also has some antioxidant properties and prevents oxidative stress in the lens (105). NSAIDs have been shown to be beneficial for ROP. A retrospective chart review found that Indomethacin use for PDA was protective for ROP (106). These findings were corroborated by a Cochrane metanalysis (107). It is

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suggested, but not proven, that the effect of indomethacin on ROP may be related to the infant's postnatal age (108). This may indicate that the exact timing of treatment may be crucial for efficacy of the drug. Topical application of Ketorolac also prevents severe ROP (109); however, this effect is controversial, since another study showed no benefit (110). Other pharmacologic interventions include inositol (111,112), beta blockers (113), atocopherol (114,115), and recently, intravitreal VEGF inhibitors (116-127). The use of intravitreal Avastin is highly invasive, and causes pain, distress, and discomfort. In addition, there are numerous reports of retinal detachment, vitreous and retinal hemorrhage, and choroidal rupture with intravitreal bevacizumab (117-125). Intravitreal Avastin appeared to spread via the bloodstream into the fellow eye when injected into an 8-year old patient (124). These adverse events suggest that the immature retina may be vulnerable to VEGF blockade and may cause breakdown of the blood-ocular barrier (127). More importantly, if Avastin, even at low doses, effectively inhibits neovascularization and interferes with the immature blood-ocular barrier it is possible that it may cross into the neonate's brain and suppress the normal angiogenesis of the developing brain. Given the role of astrocytes in production of VEGF and maintaining the integrity of microvasular ECs, the use of anti- VEGF therapies, such as Avastin, will undoubtedly have adverse effects on other related cells such as astrocytes, pericytes and microglia with possible long-term neurological repercussions. Clearly, a fuller understanding of the biomolecular mechanisms regulating ROP, as well as the use of alternate pharmacologic interventions that is safer, less invasive, and as effective, is needed.

5.8 Improvement of Scientific Knowledge and Clinical Impact

Seventy to ninety percent of all ELGANs experience recurrent, clinically significant apnea during the first weeks of life. These apneic episodes last several seconds and result in arterial oxygen desaturations. Apneas of less than 10 seconds duration can result in a reduction in oxygen saturation of 40%. The relationship between hyperoxemia and hyperoxemia and significant increase in severe ROP has been established (128). What is NOT known is the number of apneic episodes or arterial oxygen desaturations per day that results in severe ROP. We believe that a "critical" number is essential for the cascade of events that lead to severe ROP. This project will address this issue and provide new information for identification of these infants at risk. The impact of these studies will be substantial. We will correlate the number of arterial oxygen desaturations with serum VEGF levels. This has not been previously done. Finally, we will examine the genetic profile of these infants to determine if the propensity for severe ROP is due to Dll4 haploinsufficiency, and whether pharmacologic interventions can reverse it. If our proposed aims are achieved and hypotheses proven, our studies will provide guidelines for identification of infants at risk for severe ROP long before it develops in order to implement early treatment. Our studies will contribute to prevention of a long life of blindness, darkness and visual impairment. Prevention of blindness at the beginning of life will surely impact the quality of life for these children.

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6.0 STUDY OBJECTIVES

This study will evaluate the safety, tolerability and PK-PD of, and to compare and contrast, IV Ibuprofen with Caffeine and Ketorolac eye drops with Caffeine in ELGAN infants <28 weeks GA for 14 days duration to treat and preferably prevent ROP associated with prematurity and ELGAN. The specific aims of this trial are:

6.1 Aim 1: To establish the synergistic effect of local ophthalmic NSIADs and systemic caffeine as optimal therapies for the attenuation and/or prevention of severe ROP.

Hypothesis: Ocular Ketorolac or systemic Ibuprofen potentiated with systemic Caffeine will prevent or diminish the severity of ROP. We will: a) Evaluate the safety, tolerability, and efficacy of early postnatal local ophthalmic NSIADs for prevention of severe ROP in ELGANs. b) Determine the pharmacokinetics, pharmacodymanics and pharmacogenomics of NSAIDs potentiated with caffeine for prevention of ROP.

6.2 Aim 2: To identify a "critical" number of arterial oxygen desaturations as a key risk factor for severe ROP.

Hypothesis: A "critical" number of daily arterial oxygen desaturations during the first two weeks of life is a key risk factor for severe ROP. We will: a) Further define the role of VEGF, IGF, MMPs, and ROS in ROP and correlate the levels with the number of arterial oxygen desaturations. b) Establish and identify whether increased serum VEGF in infants with severe ROP is the diffusible isoform $VEGF_{121}$. This isoform is formed from VEGF proteolysis by plasmin and MMPs. MMPs also cleave Notch/Dll4, which acts as a regulator of VEGF signaling.

6.3 Aim 3: To determine whether infants at risk for severe ROP are haploinsufficient for the delta-like ligand 4 (Dll4).

Hypothesis: ELGANs at risk for severe ROP will have different pattern of gene expression specifically related to the Notch signaling pathway, as has been previously shown in animal models. We will: a) Examine cord blood, cord tissue, and placental tissue to compare the gene profile of VEGF and Notch signaling pathways among infants who develop severe ROP and those who do not; and b) Determine whether NSAIDs and/or Caffeine will confer protective benefits on Notch/Dll4 signaling and prevent the development of severe ROP.

7.0 INVESTIGATIONAL PLAN

7.1 Overall Study Design/Centers

This is a phase 2b, randomized, open label, multi-center, safety, tolerability and efficacy study comparing 3 interventions for possible prevention of ROP. The trial will be conducted in at least 5 investigational sites including the Neonatal networks (SUNY Downstate and the Brooklyn-Queens Neonatal Network sites, SUNY Stony Brook). An independent DSMB will assess safety during the

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study. This study will monitor for safety while on study drug and for 7 days after last dose of drug. An exploratory study to determine the role of pharmacodynamic, drug concentrations (as surrogate of PK profile) and pharmacogenomics will also be conducted in this patient population.

One hundred and twenty preterm infants (<28 weeks gestation; <1250 grams) between 0 and 72 hours of life will be randomized to receive either:

1) Caffeine citrate IV (20 mg/kg loading dose followed by 5 mg/kg/day maintenance dose) plus placebo saline IV (1 ml/kg followed by 0.25 ml/kg) for 5 days plus sterile normal saline (one drop two times a day) for 14 days (n=40);
2) Caffeine citrate as described in group 1 plus Ibuprofen (10 mg/kg loading dose followed by low dose ibuprofen 2.5 mg/kg/day) for 5 days plus sterile normal saline (one drop two times a day) for 14 days (n=40); and
3) Caffeine citrate plus saline IV placebo as described in group 1, and Ketorolac (Acuvail) eye drops (one drop two times a day) for 14 days (n=40).

All patients will receive Caffeine citrate for Apnea of Prematurity as per standard of care. Therefore, Caffeine citrate is not a study drug. The doses of Caffeine citrate are as per standard of care for Apnea of Prematurity. The final blood and urine samples will be obtained at 21 days post treatment. This will also be the final day of the study. It should be noted that for Ketorolac the 21-day post treatment can range from 35-38 days of life depending on the day of life at the initiation of the study drug. On the other hand, the 21-day post treatment for Ibuprofen can range from 26-29 depending on the day of life at the initiation of the study drug.

7.2 Table 1. Study Design

Group and Intervention	Caffeine	Ibuprofen IV	Ketorolac eye drops
Group 1 (n=40)	+	Saline (placebo)	sterile normal saline (placebo)
Group 2 (n=40)	+	+	sterile normal saline (placebo)
Group 3 (n=40)	+	Saline (placebo)	+

Treatment with Ketorolac will last 14 days and treatment with Ibuprofen will last 5 days. Caffeine will be administered as per standard of care. Birth weight will be used for dosing of Neoprofen and IV Saline up to day of life 8. The primary outcome will be measured at 36 weeks post-conceptional age. Each infant will be followed until death or discharge. Discharge will be defined as release of the infant to go home. For infants transferred rather than being discharged home, study staff will call to determine the primary outcome. Transfer includes release of the infant to outside institutions or other units within the same hospital. Plasma PK will be evaluated using a

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limited sampling scheme. Cord blood, cord tissue and placental tissue will be used to assess haploinsufficiency for Notch/Dll4 signaling. Patients can still be enrolled in the study, if cord and placental samples cannot be obtained at the time of delivery.

7.3 Rationale for Study Design.

Phase 2, open-label, randomized, multi-center studies in infants and premature infants are necessary to determine treatment and preventative strategies for ROP. This study was designed to: a) target infants at the highest risk of ROP in a large number of centers with variable rates of ROP (all stages and severe ROP or stage 3+); and b) assess whether caffeine plus systemic or ophthalmic NSAID will decrease ROP among infants most at risk for ROP. The study is designed to determine whether the novel treatment regimens are safe and potentially effective for ROP prevention and to obtain requisite data for the development of a Phase III efficacy/safety randomized blinded trial. Since caffeine is used extensively in NICUs as standard of care for ELGANs, no placebo group is included.

7.4 Rationale for Drug and Dose Selection.

Neonates at high risk for ROP as outlined by the American Academy of Pediatrics, Section on Ophthalmology; American Association for Pediatric Ophthalmology and Strabismus; and American Academy of Ophthalmology (129) will be enrolled. We have published the first pharmacokinetic data on IV Ibuprofen (130) and caffeine in newborns (131,132). Our published data on intravenous ibuprofen suggest doses of 10 mg/kg loading dose and 5 mg/kg/day maintenance dose are safe for closure of PDA during the first week of life. All published data indicate safety of a three-day therapy. However, the safety of a longer course (2 weeks) after birth is not known. We will provide these data which will be extremely useful in the design of larger trial. Lower doses (2.5 mg/kg) for a 5-day treatment will be used to maximize safety. Ketorolac eye drops (0.25 mg tid) have been previously evaluated in pilot trials in a limited number of neonates (109). There are no data on the synergistic effects of NSAIDs and caffeine for severe ROP. No ibuprofen ophthalmic preparation is commercially available. Caffeine potentiates the anti-inflammatory effects of COX inhibitors in activated microglia which may also occur in the retina (102). Caffeine has been used in combination with NSAIDs for many decades to enhance their analgesic effects, suggesting that caffeine may be an effective adjuvant to Ibuprofen (103,104). Caffeine also has some antioxidant properties and prevents oxidative stress in the lens (105). NSAIDs have been shown to be beneficial for ROP. A retrospective chart review found that Indomethacin use for PDA was protective for ROP (106). These findings were corroborated by a Cochrane met-analysis (107). It is suggested, but not proven, that the effect of Indomethacin on ROP may be related to the infant's postnatal age (108). This may indicate that the exact timing of treatment may be crucial for efficacy of the drug. Topical application of Ketorolac also prevents severe ROP (109), however, this effect is controversial, since another study showed no benefit (110). Other pharmacologic interventions include inositol (111,112), beta blockers (113), atocopherol (114,115), and recently, intravitreal VEGF inhibitors (116-127). The use of intravitreal

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Avastin is highly invasive, and causes pain, distress, and discomfort. In addition, there are numerous reports of retinal detachment, vitreous and retinal hemorrhage, and choroidal rupture with intravitreal bevacizumab (117-125). Intravitreal Avastin appeared to spread via the bloodstream into the fellow eye when injected into an 8-year old patient (124). These adverse events suggest that the immature retina may be vulnerable to VEGF blockade and may cause breakdown of the blood-ocular barrier (127). More importantly, if Avastin, even at low doses, effectively inhibits neovascularization and interferes with the immature blood-ocular barrier it is possible that it may cross into the neonate's brain and suppress the normal angiogenesis of the developing brain.

7.5 Baseline Study Procedures

Signed and dated informed consent, record demographics (birth weight, gestational age, maternal history) medical history (diagnoses, and medical baseline conditions: oxygen and respiratory support, blood gases), physical examination will be performed within 24 hours prior to administration of study drug, vital signs will be recorded within 24 hours prior to administration of study drug. Clinical laboratory tests (renal function, liver function, hemogram) will be performed within 72 hours prior to the first dose of IV study drug. If these labs are obtained within 72 hours prior to enrollment in accordance with local standard of care, the results will be used for the baseline values for the study. Laboratory values closest to enrollment will be used if there are multiple tests. If obtaining laboratory tests is not in the best of interest of the infant, it will be deferred. Infants will be assessed for pain using the Neonatal Infant Pain Scale (NIPS) by two independent individuals. All subsequent tests will be standard of care.

7.6 NIPS and N-PASS Assessment.

NIPS (Newborn Infant Pain Score) and N-PASS (Neonatal Pain, Agitation and Sedation Scale) are used as behavioral and physiological assessment tools for newborn pain. NIPS includes five behavioral components and one physiological component (respiration) and will be assessed by two independent individuals before treatment and once every day for 5 days during Ibuprofen or placebo treatment. NIPS is used for acute pain and N-PASS is designed for prolonged pain and include behavioral, facial and physiologic assessments. Each component of the NIPS and N-PASS will be scored as the highest level achieved by the infant every day. NIPS is widely used for neonatal pain assessment but N-PASS have the added capability of including crying even in intubated infants on mechanical ventilation **(Appendix V).**

7.7 Inclusion Criteria Selection of Study Population

Neonates at high risk for ROP as outlined by the American Academy of Pediatrics, Section on Ophthalmology; American Association for Pediatric Ophthalmology and Strabismus; and American Academy of Ophthalmology (129) will be enrolled. Inclusion criteria are:

1) all infants with a birth weight of less than 1250 grams;
2) all infants with a gestational age of 28 weeks or less;

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3) all infants who required oxygen therapy and/or ventilator support within the first 2 days of life; and
4) All patients receiving Caffeine citrate for Apnea of Prematurity as per standard of care.

7.8 Exclusion Criteria for Study Population

Exclusion criteria are:

1) Infants with major congenital anomalies, congenital sepsis, chromosome abnormalities (incl. duct-dependent cardiac anomalies).
2) Fused eyelids
3) Maternal antenatal NSAID exposure < 72 hours before delivery of the baby.
4) Infants with cardiac conditions including cardiomyopathies, serious arrhythmias and congenital heart defects.
5) Infants transferred from outside hospital, after 72 hours of life.
6) Renal failure or oligouria (defined as urine flow rate <0.5 ml/kg/hour in the 8 hours prior to randomization). Anuria is acceptable if less than 24 hours of life.
7) Admission platelet count <50,000 $mm^{3.}$
8) Clinical bleeding such as continuous, active oozing from puncture site.
9) Infants with cyanotic heart disease diagnosed by fetal echocardiography during pregnancy or infants who shows persistent low oxygen saturations as per attending neonatologist despite adequate ventilator assistance.

Participation in other clinical trials while subjects participate in this study and for 7 days after the last dose of study drug.

7.9 Patent Ductus Arteriosus (PDA) Treatment

About 40 to 80% of ELGANs <1250 grams will develop PDA and most will be treated with either Indomethacin or Ibuprofen as per standard of care. This will contaminate the treatment group. If the standard of care protocol is not to treat during the first week of life, then leave the baby on Ibuprofen because it is not a treatment drug for PDA in this study. If the standard of care protocol is to administer Indomethacin, then stop Ibuprofen for 24 hours (the t½ of Ibuprofen is 22 hours), administer Indomethacin as standard of care. If the standard of care is to administer Ibuprofen, then increase the dose to the standard of care dose and follow the standard of care protocol. Since Ibuprofen treatment is for 5 days only, there will likely be no need to resume the study drug.

7.9.1 Withdrawal from Study

Infants may be withdrawn from treatment or from the study at any time. Reasons for infant withdrawal from the study include, but are not limited to:

1. Infant's parent or legal guardian chooses to withdraw the infant for any reason

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2. AEs, conditions, or intercurrent illnesses that preclude compliance with the protocol, particularly if continuation would pose a risk to the infant's safety
3. Clinical seizure occurring between informed consent and 1st administration of study drug
4. The investigator determines that it is in the infant's best medical interest to be withdrawn.
5. Patient is transferred to another hospital during treatment. However, the patient must be followed up for AEs and SAEs until death or discharge.

Detailed reasons for infant withdrawal because of lack of efficacy or because of pre-determined safety concerns are given in the appropriate sections of this protocol. Withdrawn infants will be followed for safety end points to the extent possible.

7.9.2 Parental/Guardian Permission

Prior to the start of any study-related procedure, a signed and dated informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained and documented in the infant's medical record **(Appendix I)**. Once it has been determined that the infant meets all inclusion criteria and no exclusion criteria, the infant will be assigned a subject identification number that will be used on the subject's CRFs and will be considered enrolled.

8.0 STUDY DRUG ADMINISTRATION

8.1 Method of Assignment to Study Groups (Randomization)

Randomization to treatment arms will be stratified by each center. To obtain a patient number and the randomized treatment randomization will be conducted by the pharmacist using sealed envelopes in blocks of 6. This is an open-label study. Twins enrolled in the study will be randomized together to the same treatment.

8.2 Study Procedures During Study Drug Treatment

The following procedures or evaluations will be performed during the treatment phase and the data recorded as indicated: a) study drug dosing information; b) adverse events felt related to study drug (definite and probably related) and all serious adverse events; c) all concomitant medications, antimicrobials administered through the last dose of study drug therapy; d) examinations and interventions for PDA during study period; e) results of any examinations (head/abdominal ultrasound, CT scan, MRI, echocardiogram, ophthalmology exam, etc.) performed to assess the diagnosis or status of any adverse event must be documented and recorded in the CRF; f) any non-medication treatment (surgeries, procedures) associated with the patient's underlying disease will be recorded in the CRF.

8.3 Drug Supplies

The Sponsor will provide the study drugs (Ketorolac and Neoprofen) and the placebos (Saline eye drops and Saline I.V.) to the local pharmacies. Caffeine will not be provided as it is not a study drug and is used per standard of care. The local pharmacy at each center will distribute the

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study drugs/placebos to the NICU and the drugs/placebos will be administered by the attending Neonatologist.

8.4 Dosing Weight.

Birth weight will be used for dosing of the study drug until 8 days of life (DOL) for Neoprofen and IV Saline. Adjustments for Ketorolac and saline eye drops are not necessary.

8.5 Treatment Compliance

Treatment compliance will be evaluated by review of information documented on study drug administration and drug accountability forms. Day 0 of study is the day before dosing of the study drug. Pre-treatment blood and urine samples will be collected at Day 0. A final blood and urine sample will be collected at 21 days post treatment or Day 21. This is the final day of the study.

8.6 Method for Administration of Study Drug

For administration of Ketorolac, patients will be dosed with one drop of study medication per eye by the site PI or sub-PIs beginning Day 1 of enrollment into the study. The date and time will be recorded by the study personnel. Approximately 15 minutes after each dose, the study personnel will perform the NIPS and/or N-PASS pain score. The second dose will be administered 12 hours later. For each of the doses, the right eye will receive the study medication first, immediately followed by the left eye. Approximately 15 minutes after each dose, the NIPS and/or N-PASS pain score will be performed. Approximately 30 minutes later vital signs will be recorded. One sterile unopened vial will be used for each dosing. The remaining opened vial will be returned to the site Pharmacist and discarded appropriately following documentation. The placebo (sterile normal saline will be administered similarly. Neoprofen will be administered according to the local pharmacy guidelines.

8.7 Storage and Disposition of Supplies

The clinical supplies will be stored at controlled room temperature according to the manufacturer's recommendations. Ketorolac (Acuvail) and Ibuprofen (Neoprofen) will be stored at 15°- 30°C and protected from light in the manufacturers' containers. Investigational products are for investigational use only, and are to be used only within the context of this study. Study drug will be maintained under adequate security. Drug accountability logs will be kept by the PI and the Research Pharmacist.

8.8 Drug Accountability

The investigator or his/her designee will verify that study drug supplies are received intact and in the correct amounts. The investigator or his/her designee will document this verification by signing and dating the appropriate shipment request/receipt document. An accurate inventory of study drug will be kept by the site. An overall accountability of the study drug will be performed and verified by the clinical research associate (CRA) throughout the study and at the site close-out visit. All used and unused supplies must be inventoried, accounted for, and according to the institution's standard operating procedures (SOPs) following review by the CRA. The investigator agrees not to supply study medication to any persons not enrolled in the study.

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8.9 Discontinuation of Study Drug

If the study drug is discontinued because of safety, the patient must be followed until resolution of the safety event. The study drug will be discontinued if any one of the following occurs:

1. The patient develops severe AE (renal failure, necrotizing enterocolitis Stage 2A, Bell's criteria)
2. The patient develops an unacceptable hepatic adverse event as defined by: AST or ALT >3X upper limit of normal (ULN) OR conjugated hyperbilirubinemia (direct bilirubin >5.0 mg/dL) OR other evidence of liver failure;
3. The parents or legal guardian declines further study participation;
4. The investigator decides it is in the patient's best interest to discontinue treatment with study medication.

Study drug may be held if a patient develops a serious adverse event related to study drug.

9.0 SCHEDULE AND DESCRIPTION OF STUDY PROCEDURES

9.1 Baseline Study Procedures (Before Start of Study Drug/Placebo)

1. Obtain signed and dated informed consent/Authorization.
2. Obtain cord blood, cord tissue and placental tissue. Patients can still be enrolled in the study if cord and placental samples cannot be obtained at the time of delivery.
3. Record demographics (BW, GA, diagnoses), medical history
4. Medical baseline conditions (O_2 and respiratory support, blood gases). FiO_2 range recorded at the time of delivery and continued for 2 weeks or until baby off ventilator. Number of desaturations and lowest saturations will be recorded. Any and all corrective actions for pulseox alarm (RT, nurse, attending) will be documented (i.e. saturation%, length of time of desat, corrective action, stable FiO_2 or FiO_2 required for infant to maintain normoxia, desat due to handling, procedure, apnea, etc., arterial blood gas parameters closest to time of desat).
5. Record maternal history and medications (age, parity, use of tocolysis, antenatal steroids and number of courses, presence of fever, antibiotic treatment, preeclampsia, placental abruption, chorioamnionitis, gestational diabetes, and any other pregnancy complication)
6. Physical examination will be performed within 24 hours prior to administration of study drug.
7. Birth weight will be used for dosing of Neoprofen and IV Saline through DOL 8.
8. Vital signs will be recorded within 24 hours prior to administration of the study drug
9. Clinical laboratory tests within 72 hours prior to first dose of IV study drug (or standard of care if done at least 72 hours prior to study drug). Lab tests closest to enrolment will be used. The following clinical laboratories will be performed within 72 prior to the first dose of study drugs per standard of care. If these labs have been processed within 72 hours prior to enrollment, the results will be used as baseline values for the study. Use of laboratory values closest to enrolment if there have been multiple tests. If obtaining laboratory is not in the best interest of the infant, they may be deferred.

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a) Renal function tests

b) Liver function tests

c) Hematology

d) Serum chemistry

10. Record all surgeries prior to the first dose of the study
11. Record all procedures. Patients randomized to IV ibuprofen will have cardiac echo to rule out PDA-dependent cardiac abnormalities.
12. Pain score (NIPS)

9.2 Study Procedures During Study Treatment (Study Days 1-14)

The following procedures or evaluations will be performed during the treatment phase and the data recorded as indicated:

1. Record study drug dosing information (Neoprofen/placebo will be administered for 5 days and Ketorolac/placebo will be administered for 14 days. Final blood and urine sample will be 21 days post treatment).
2. Assess and record AEs felt related to study drug (definite and probably related) and all SAEs
3. Observe eyes patients randomized to Ketorolac for any adverse events (i.e. corneal haziness or cloudiness) on a daily basis at 30 minutes post treatment
4. Record all concomitant medications administered through the last dose of the study drug
5. Record all surgical procedures during the study drug therapy
6. Birth weight will be used for dosing of Neoprofen or IV Saline until DOL 8
7. Clinical laboratories will be performed as standard of care and are to be reported on a weekly basis in the case report forms (CRFs)
8. A limited PK sampling scheme will be employed such that no more than 300 µL (total 5 samples) of blood is obtained from each patient for PK analyses.
9. Surviving infants will have a final blood sampling on 21 days post treatment.
10. Results of any examinations (head/abdominal ultrasound, CT scan, MRI, echocardiogram, ophthalmology, etc.) must be documented and recorded in the CRFs
11. Any non-medication treatment (surgery, procedures) associated with the infant's underlying disease will be recorded in the CRFs
12. Cord blood, cord tissue and placental tissue will be used to assess haploinsufficiency for Notch/Dll4 signaling.
13. Pain score will be done daily for 5 days during Ibuprofen or IV Saline treatment.

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14. Record FiO_2 range for 14 days or until baby off ventilator, number of desaturations and lowest saturations, any and all corrective actions for pulseox alarm (RT, nurse, attending)

9.3 Post-Treatment/End of Study Procedures (21 days post treatment)

1. 72 hours after the last dose of the study drug: Assess and record status of AEs related to the study drug (definitely related and probably related)
2. 7 days after the last dose of the study drug: collect one blood and urine sample for PK analyses
3. 14 days after the last dose of the study drug: collect one blood and urine sample for other analyses
4. 21 days after the last dose of the study drug: collect one blood and urine sample for other analyses
5. Weekly thereafter until death or discharge: Assess and record all SAEs
6. 36 weeks post-conceptional age: Record ophthalmology examination results
7. Blood and urine samples: to be shipped to appropriate labs for processing (PK, proteomics, metabolomics, biochemical analyses)
8. Record all surgical procedures during the study drug therapy through last study day (21 days post treatment)
9. Record all concomitant medications administered through last study day (21 days post treatment)
10. Record all non-medication treatment (surgery, procedures) associated with the infant's underlying disease through last study day (21 days post treatment)
11. Record results of any examinations (head/abdominal ultrasound, CT scan, MRI, echocardiogram, ophthalmology, etc.) through last study day (21 days post treatment)
12. Death or discharge: Morbidities of special interest will be recorded including:
    a) Necrotizing enterocolitis
    b) Spontaneous intestinal perforation
    c) Intraventricular hemorrhage
    d) Periventricular leukomalacia
    e) Retinopathy of prematurity
    f) Patent ductus arteriosus
    g) Chronic lung disease/bronchopulmonary dysplasia
    h) Sepsis
    i) Corneal toxic lesions
    j) First day of full feeds

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k) Length of hospitalization

l) Drug toxicity

9.4 Other Data Collection

Data will be collected prospectively in real time, and analyzed according to different birth weight categories: 1) <750 grams; 2) 751-1000 grams; and 3) 1001-1250 grams. Growth will be assessed in terms of body weight, length, and head circumference at birth and weekly thereafter (as per standard of care) until the baby is off oxygen therapy. Infant data will include race, gender, gestational age, Apgar score, cord gases, pre- and postnatal steroids, transfusion, indomethacin treatment, surfactant treatment, indication for delivery, mode of delivery, % saturation, fraction of inspired oxygen (FiO_2), duration of oxygen use, use of diuretics, and adverse events such as chronic lung disease, intraventricular hemorrhage, sepsis, necrotizing enterocolitis, patent ductus arteriosus, infection, mortality, pulmonary status, ventilator parameters, and number of hypoxic episodes. For each infant who received an eye examination, the maximum stage of ROP, the presence or absence of "plus" disease (dilated and tortuous vessels in at least 2 quadrants), and the need for treatment, the rate of progression to threshold in at least one eye and final status of eyes at 3 months (i.e. retinal detachments or folds and macula ectopia) will be recorded. Maternal data will include use of tocolytics, antenatal corticosteroids and number of courses, presence of fever before and after delivery, antibiotic treatment, presence of preeclampsia, placental abruption, chorioamnionitits (temperature >38^{o}C during labor), and any other complication.

9.5 Oxygen Saturation Monitoring

The range of FiO_2 and the lowest daily FiO_2 will be recorded at the time of delivery and documentation will continue for two weeks or until the baby is off the ventilator, whichever comes first. The oxygen saturation goal limits will be standard for all centers (90%-95%) as measured with a pulse oximeter and electronically stored data will be downloaded weekly. Below these ranges will be considered de-saturation (hypoxia), and above these ranges will be considered hyperoxia. The number of de-saturations, as well as the duration of FiO_2 increase will be recorded. When an infant shows a low alarm for oxygen saturation <90%), the FiO_2 and monitor settings will be recorded. The infant and monitor will be evaluated to determine whether the alarm is due to:

a) motion artifact;

b) lines disconnected; and

c) heart rate and respiratory effort.

When corrective action is taken, the respiratory technician, the nurse, or the attending physician will document parameters such as:

a) how low is the saturation;

b) length of time of de-saturation;

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c) corrective actions such as changes in monitor settings, alarm;

d) weaning FiO_2 if oxygen saturation is high (>95%), how much and how long;

e) stable FiO_2 (FiO_2 required for infant to maintain normoxia);

f) desaturation due to handling, procedure, spontaneous, apnea.

Arterial blood gases closest to the time of desaturation will also be recorded.

9.6 Specimen Collection

All specimens (blood, urine, cord blood, cord tissue, placental tissue) will be collected as outlined in the Standard Operating Procedures (Appendix L) in the Manual of Operations.

9.6.1 Blood. To determine whether ocular ketorolac crosses the blood-ocular barrier and enters the systemic circulation and to determine a potential relationship between efficacy/toxicity and plasma ibuprofen, plasma drug levels of Ketorolac and Ibuprofen will be obtained within 24 hours prior to drug or placebo (0 pre), and at 1, 7, 14, and 21 days post treatment, within a 15 minute period before or after the initial dosing time. For example, if the dose is administered at 9:00 am, then there is 15-minute window of 8:45 am to 9:15 am where the next dose can be administered, and blood samples can be collected. The maximum number of samples per infant for this study will be 5 at 0.3 mL/sample. The total blood volume will be 1.5 mL. Samples will be collected in EDTA tubes, centrifuged and the result plasma stored at -70- to 80^{o}C until shipment to SUNY Buffalo, Dr. Jun Qu's U54 Proteomics Core Laboratory for Ketorolac and Ibuprofen levels (see Appendix L of the Manual of Operations).

9.6.2 Urine. For renal function, urine will be collected within 24 hours prior to drug or placebo (0, pre), and 1, 7, 14 and 21 days, within a 15 minute period before or after the initial dosing time. For example, if the dose is administered at 9:00 am, then there is 15-minute window of 8:45 am to 9:15 am where the next dose can be administered, and urine samples can be collected. Urine samples will be collected with the use of a sterile cotton ball. The cotton ball will be placed into the appropriate tubes, labeled appropriately and frozen at -70 to -80^{o}C until shipment. Samples will be shipped to SUNY Downstate Medical Center, Brooklyn, NY, U54 Translational/Retinal Core Laboratories for ELISA and other assays (142, 143), (see Appendix L of the Manual of Operations).

9.6.3 Mixed Cord Blood. For collection of mixed cord blood samples, the discarded umbilical cord will be double clamped and the blood samples will be aspirated with the use of a 19 gauge needle attached to a 10 mL syringe. Informed consent to perform these sample collections will be obtained. Mixed cord blood will be collected in non-preservative tubes, centrifuged and the resulting serum will be stored at -70 to -80^{o}C until shipment to SUNY Downstate Medical Center, Brooklyn, NY, U54 Translational/Retinal Core Laboratories for ELISA assays, and PCR assays for Notch/Dll4 haploinsufficiency (see Appendix L of the Manual of Operations).

9.6.4 Cord and placental tissue. Cord and placental tissue will be collected into sterile DNAse/RNAse free tubes containing RNA/DNA stabilization solution (RNA later, Qiagen). Placental biopsies will be taken from 3 sites in the placenta: 1) the maternal-fetal interface; 2) the fetal side; and 3) the intervillous space. Two samples from each site will be taken, and one complete section of the placenta will be taken for a total of 7 placental samples. Each sample will

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be first rinsed in ice-cold sterile normal saline prior to placement in the respective tubes. Samples #1 will be approximately 5 mm in size and 200 mg in weight. This will be placed in specialized tubes containing ceramic beads and sterile normal saline. Samples #2 will be approximately 5 mm in size and 200 mg in weight. This will be placed in specialized tubes containing ceramic beads and *RNA Later*. The complete section will be placed in specialized containers with 10% phosphate buffered formalin.

For umbilical cord samples, a total of 6 samples will be taken from 2 sites: 1) closest to the baby; and 2 closest to the placenta. Samples will be first rinsed in sterile normal saline prior to placement in the respective tubes. From each site, sample #1 will be approximately 5 mm in size or 200 mg in weight will be taken from the inner cord (vessels), rinsed, and placed in specialized tubes containing ceramic beads and sterile normal saline. Sample #2 will be approximately 5 mm in size or 200 mg in weight will be taken from the inner cord (vessels), rinsed, and placed in specialized tubes containing ceramic beads and *RNA Later*. Sample #3 will be a complete section of the cord approximately 1 cm in size and 1 gram in weight. This will be placed in specialized containers with 10% phosphate buffered formalin.

For each patient there will be 7 placental samples and 6 cord samples. The tubes will then be appropriately labeled. Samples in the tubes containing ceramic beads will be stored in a -70^{o}C to -80^{o}C freezer until overnight shipment on dry ice. Samples in the 10% formalin containers will remain at room temperature until shipment. (See Appendix L of the Manual of Operations for more detailed instructions on shipping). Cord and placental biopsies will be harvested with sterile disposable scalpels provided by the study site.

9.7 Specimen Shipping

The specimen shipping form will be provided and will list all patients, and their identifying information, whose specimens are being sent to SUNY Downstate Medical Center or to SUNY Buffalo. Shipping supplies (i.e. appropriate boxes and labels) will also be supplied. However, dry ice must be purchased at the site and billed to your U54 account. The shipping forms must be completed by both the staff at the study site center and the staff at SUNY Downstate Medical Center receiving laboratory. It is used to document and identify all specimens that are shipped out by the center. A copy of this form will accompany the shipment from the study site to SUNY Downstate Medical Center or to SUNY Buffalo. This form is not entered into the computerized database. The following information should be completed on each shipment log.

1. Center Number
2. Number of cord blood, patient blood, urine, cord tissue, or placental tissue samples enclosed
3. Initials of staff at the shipping center completing the form
4. Specimen numbers of specimens contained in the shipment

Please note that there are regulations for shipping samples on dry ice that must be followed. Therefore, only individuals who are certified for shipping hazardous materials must undertake this task. If there are no individuals at the site, the person identified to ship the samples must take the required shipping certification course as mandated by the FAA. The samples should be shipped on a Monday (or Tuesday after a Monday holiday) and at the end of the study. For the week of

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Christmas and New Year's, the study coordinator will contact Khadija Sikriti, MD at (929) 295-2834 (mobile); (718) 270-4657 (office); Khadija.sikriti@downstate.edu (email) who will discuss with the nurse coordinator and the technologist the ideal time for sample shipment. Batch the samples in an outer bag, and then pack in dry ice to be shipped to the SUNY Downstate Medical Center Clinical Research Office or SUNY Buffalo Proteomics Core Laboratory. The Specimen Shipment Log should be filled out and included in the shipment. For further shipping instructions please refer to (see Appendix L of the Manual of Operations).

9.8 PD Measurements

Measurements of VEGF, sVEGFR-1, HIF1α, Notch-1, Notch-4 Dll4, IGF-I, and MMPs will be conducted using enzyme immunoassays. All EIA assays will be carried out with the use of commercially-available immunoassay kits in the Translational/Retina Core Laboratories at SUNY Downstate Medical Center, Brooklyn, NY.

10.0 DEFINITIONS AND DETAILED DESCRIPTIONS OF ASSESSMENTS AND ENDPOINTS

10.1 Efficacy Assessment

The primary endpoint for the study is ROP (all grades) and severe ROP (Stage 3+ disease) or need for laser or Avastin. Eye examinations will be done at standard of care through discharge and once, at 50 weeks PCA, solely for the study. Parents will bring subjects back to clinic for eye examination at 50 weeks PCA ±7 days. All infants will undergo routine eye examination by a pediatric ophthalmologist according to the International Classification for ROP (135). The rate of mild (stage 1), moderate (stage 2) and severe ROP (stages >3) will be calculated as the number of infants diagnosed with ROP over the number of infants receiving retinal examinations. ROP will be classified according to the three components outlined by the International Classification for ROP: 1) The zone in which ROP occurs; 2) the stage of ROP; and 3) the presence or absence of plus disease. Zone 1 is the most posterior (an area within twice the distance from the optic nerve head to the fovea; Zone 2 is ROP outside of zone 1; and Zone 3 is ROP only present on the temporal side of the eye. Stage 1 is a line of demarcation from the vascularized region of the retina and avascular zone. In stage 2 the line becomes a ridge that protrudes into the vitreous. In stage 3, the extraretinal vascular proliferation occurs when the ridge and neovascular tufts are seen in the posterior to the ridge. Disease occurs when the vessels posterior to the ridge become dilated and tortuous. Stage 4 has scarring and fibrosis when neovascularization extends into the vitreous, causing traction of the retina and retinal detachment. Stage 5 is total retinal detachment.

10.2 Safety

The number and percentage of infants having treatment-emergent AEs will be tabulated, with a breakdown by group. Descriptive statistics will be provided for clinical chemistry and hematology data, including change from baseline. All subjects who received at least one dose of study product will be included in the safety analyses. AEs will be summarized and tabulated by severity, and relationship to therapy. Deaths and premature termination will be tabulated and summarized. Changes in laboratory parameters will be tabulated and summarized. Laboratory data, such as hematology and serum chemistry data will be tabulated by dosage and age group. Summary statistics for changes from baseline will be presented. AEs will be summarized in tabular form by dosage and age group. Continuous laboratory measurements will be described at each visit

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using univariable descriptive statistics (mean, median, etc.); observed values and changes from baseline will be summarized. Lab tests reflective of liver toxicity (e.g., ALT, AST) will be further summarized in terms of the most extreme values and largest changes from baseline (in the appropriate direction) observed from start of study drug through the end-of-therapy lab. Vital signs and physical exam results will be listed.

10.3 Safety Assessments

Secondary outcome such as renal function will be determined by: a) Urine output (ml/kg/hr) daily; b) serum electrolyes, creatinine and blood urea nitrogen as per standard of care; and c) daily fluid requirement. Gastrointestinal function will be determined by daily assessment for: a) necrotizing enterocolitis using Bell's criteria; b) GI bleeding; c) trophic feeding; d) time to full feeds (100 ml/kg/day); and e) time to discontinue total parenteral nutrition. Liver function studies done weekly as standard of care will include a) serum bilirubin total/direct; b) AST, ALT, alkaline phosphatase. Hematologic parameters: done as per standard of care include platelets, hemoglobin, hematocrit, white blood count. Other secondary outcomes will include Intraventricular hemorrhage (Papile's criteria), Perinventricular leucomalacia by sonogram as per standard of care, and ocular examination for: corneal lesions. Corneal toxic lesions have been reported with NSAIDs such as nepafenac (136) and case reports of reversible toxic keratolysis in adults with prolonged ketorolac treatment (137). However, recent randomized trials on ketorolac and diclofenalac eye drops showed anti-inflammatory efficacy with high tolerability and no adverse effects (138,139). Daily examination by the neonatal staff of the cornea for haziness or other lesions confirmed by an ophthalmologist at least once a week or whenever an AE occurs will be done. Corneal lesions or haziness will require prompt discontinuation of the ophthalmic study eye drops. Pain scores will be determined using NIPS. Safety assessments will include physical examination, clinical laboratory values as available per standard local care.

10.4 Safety First Plan

The protocol will rely on three mechanisms for safety:

1. The DSMB whose role is outlined below and in the DSMB charter;
2. AE and SAE reporting mechanisms in accordance with FDA guidance outlined below

10.5 Data Safety and Monitoring Plan (DSMP)

A DSMP will be established in compliance with NIH/NICHD policies for the protection of human subjects in clinical studies. The DSMP outlines procedures for reporting SAEs and AEs of Special Interest to the New York Pediatric Developmental Pharmacology Research Consortium (NYPD-PRC) and dissemination of this information from the Coordinating Center (CC) to the DSMB.

10.6 Data and Safety Monitoring Board (DSMB)

The DSMB will be empowered to monitor safety during the conduct of the study. No efficacy data will be provided to the board, but the board will review safety data. The DSMB will be comprised of independent reviewers who are not involved in the conduct of the study and will be blinded to study drug information. Any two members of the DSMB can ask that the study be halted at any

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time for safety. The DSMB will meet after 10%, 25%, 50% and 75% of the subjects have been enrolled in the study. Safety monitoring will be done throughout the study on an ongoing basis. The DSMB or the investigator may terminate the study at any time based on safety concerns. The DSMB consists of Virginia Delaney, MD, MPH (Neonatologist-Epidemiologist), Ron Thomas, PhD (Biostatistician), Nadine Lahage, MD (Neonatologist and DSMB chair).

11.0 ADVERSE EVENTS

11.1 Adverse Events Definition

An AE is defined as any untoward medical occurrence such as a sign(s), symptom (s), and/or laboratory finding(s) concurrent with the use of a drug in humans. AEs include worsening of any baseline symptoms. The event may/may not necessarily have a causal relationship with the administration of the drug. AEs may be reported by the subject, or detected by the investigator, or other competent observer. The investigator will also evaluate any change in laboratory values. If the investigator determines a laboratory abnormality to be clinically significant, it is considered a laboratory AE; however, if the laboratory value abnormality is consistent with a current diagnosis, it may be documented accordingly. Adverse events related to study drug (definite or probably related) will be recorded from the time of first dose of study drug until 72 hours following the last dose of study. All serious adverse events will be recorded from the time of first dose of study drug until 30 days after the last dose of study drug. Adverse events felt related to study drug and all serious adverse events ongoing at the time of the last dose of study drug will be followed up for as long as necessary to adequately evaluate the patient's safety or until the event stabilizes. If the event resolves during the study or follow-up period, a resolution date should be documented on the case report form. All serious adverse events will be reported promptly (within 24 hours of knowledge) to CC by phone or fax. If the initial notification is by phone, the investigator will follow that notification with a written serious adverse events report form by fax within 24 hours.

11.2 AE Reporting.

AEs will be recorded from the time of informed consent until 72 hours following the last dose of study drug for non SAEs and until 30 days after the last dose of study drug for SAEs. Any AE that occurs between the time informed consent is obtained and the initial dose of study, that is considered related to a protocol specified procedure, must be reported. An initial serious adverse event form must be as complete as possible, including details of the current serious adverse event, and provide an Investigator assessment of the causal relationship between the event and the investigational product. Information not available at the time of the initial report will be documented on a follow-up serious adverse events form and reported to the local institutional review board (IRB) as institutional requirement. The CC will be responsible for receiving and reviewing event forms, data basing events into Clintrace, coding events using the MedDRA dictionary, writing clinical narratives, contacting sites for missing/additional information and generating regulatory forms (MedWatch and CIOMS) for those events that are assessed as study drug related by the investigator and determined by the CC monitor to be unexpected.

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11.3 Procedures for Assessing, Recording and Reporting AEs

Throughout the duration of the study, the investigator will closely monitor each subject for clinical evidence of drug intolerance and monitor all clinically obtained laboratory values for laboratory evidence of AEs. AEs not explained by the infant's underlying illness which occur during the course of the study will be reported in detail on the appropriate CRFs and followed until resolution or until it becomes stable. All SAEs will be reported to CC within 24 hours. The description of the AE will include description of event, start date, stop date, intensity, if it was serious, and relationship to the study drug. The investigator must verify this information. The intensity or severity of AEs will be graded as follows:

- **Mild** - awareness of sign or symptom, but easily tolerated. Not expected to have a clinically significant effect on the subject's overall health and well-being. Not likely to require medical attention
- **Moderate** - discomfort enough to cause interference with usual activity or affects clinical status. May require medical intervention
- **Severe** - incapacitating or significantly affecting clinical status. Likely requires medical intervention and/or close follow-up

AEs that increase in intensity will be recorded with a stop date on the AE CRF of the milder AE equal to the date that the condition worsened. A new AE with a start date equal to the date of worsening will then be reported. AEs that decrease in severity need not be reported in this way. The start date will be the date entered above and the date of resolution should be reported as the stop date. The Investigator is responsible for assessing relationship to study medication using the following definitions:

- **Not related**: An AE that is due to a pre-existing illness or use of another drug, and is not related to the study drug.
- **Possibly related**: An AE that has little or no relationship to the study drug and there exists a more likely alternative cause.
- **Probably related**: An AE that is likely to be related to the administration of the study drug and an alternative cause less likely when compared to the study drug.
- **Definitely Related**: An AE that has a strong temporal relationship to the study drug. AE will recur with continued or repeated use of the study drug, and another cause is unlikely or less likely.

11.4 Follow-up of AEs

AEs will be followed until resolution or until stability is reached using good clinical practices.

11.5 SAEs

A serious adverse event will be any adverse event that results in any of the following outcomes: death, life-threatening adverse event, persistent or significant disability or incapacity, inpatient

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hospitalization or prolongation of existing hospitalization, congenital abnormality or birth defect, important medical event, life-threatening means that the patient or subject was, in the view of the investigator, at immediate risk of death from the adverse event as it occurred. It does not include an adverse event that, had it occurred in a more severe form, might have caused death. Persistent or significant disability/incapacity means that the event resulted in permanent or significant and substantial disruption of the subject's ability to carry out normal life functions. Important medical event is any medical event that may not result in one of the above outcomes, but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious adverse events.

11.6 Procedures for Assessing, Recording and Reporting SAEs

The Investigator will use the following definitions when assessing causality of an adverse event to study medication: a) "**NOT RELATED**": an adverse event that has no temporal relationship to trial drug or has a definite alternative etiology. Only serious adverse events of this category will be recorded on the CRFs; b) "**PROBABLY NOT RELATED**": an adverse event not commonly associated with the drug class; has little or no temporal relationship to trial medication; and a probable alternative etiology is apparent. Only serious adverse events of this category will be recorded on the CRFs; c) "**PROBABLY RELATED**": an adverse event not commonly associated with this drug class; has a temporal relationship to trial medication; and a possible alternative etiology exists or no alternative etiology is apparent, Both adverse events and serious adverse events of this category will be recorded on the CRFs; d) "**DEFINITELY RELATED**": an adverse event that has a temporal relationship to trial medication and/or reappeared on re-challenge; and no other etiology is apparent, Both adverse events and serious adverse events of this category will be recorded on the CRFs. All SAEs will be forwarded to the DSMB Chair within 1-2 business days. The NYPD-PRC Safety Surveillance will submit MedWatch/CIOMS forms to IRB and DCRI Regulatory Services for review and submission to regulatory authorities. NYPD-PRC Safety Surveillance will provide "Safety Alert" reports to NYPD-PRC Clinical Trials team to distribute to sites.

11.7 Dose Interruptions for SAEs

If the patient experiences a serious adverse event felt to be related to study drug, the study product must be held at the discretion of the investigator. For a serious adverse event felt to be related to study drug, study drug can be held until the adverse event resolves. Resume drug at the original dose. If the same SAE recurs, study drug should be held and the Medical Monitor (phone: tbd) will be contacted. For a SAE not felt to be due to study drug, no interruption is necessary.

11.8 Follow-up of SAEs

The investigator must complete and submit a follow-up SAE form when important follow-up information (diagnosis, outcome, results of specific investigations, etc.) becomes available after submission of the initial form. Follow-up forms should be submitted according to the same process used for reporting the initial event as described above (i.e., within 24 hours of knowledge). All SAEs and AEs of Special Interest will be followed until resolution, stabilization or

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30 days after the last subject is enrolled, whichever occurs, first. The investigator will be responsible for reporting SAEs to the local IRBs in accordance with local guidelines.

11.9 IRB Summary Safety Reports

As required by the NIH "Guidance of Reporting Adverse Events to Institutional Review Boards for NIH-Supported Multicenter Clinical Trials," the DSMB's summary safety reports will provide feedback at regular and defined intervals to the IRBs. After each meeting of the DSMB, the executive secretary will send a brief summary safety report to each investigator. The report will document that a review of data and outcomes across all centers took place on a given date and will summarize the Board's review of the cumulative adverse experiences reported from all participating sites without specific disclosure by treatment arm. It will also inform investigators of the study the Board's conclusion with respect to progress or need for modification of the protocol. The clinical site investigators are required to transmit the report to their local IRB as soon as they are received.

11.9.1 Table 2: AE Reporting

		Record on CRF	Report to FDA	Report to NYPD-PRC Safety Surveillance
AE	Related (definite or probable	Yes	No	No
AE	Not related	No	No	No
SAE	Related (definite or probable	Yes	Yes (expedited)	Yes
SAE	Not related	Yes	Yes	Yes

12.0 STATISTICAL METHODOLOGY

12.1 Planned Sample Size

The primary objective of this study is to assess the safety and potential efficacy of caffeine plus NSAIDs to prevent ROP. We will enroll 40 evaluable neonates for each group (total n=120). Evaluable subjects are those who fulfill inclusion and exclusion criteria and survived for 36 weeks PCA eye examination. The estimated rate of ROP (all stages) for neonates <1250 grams in the BQNN Network is 58% and severe (Stage 3 plus) is 5.7%. We hypothesize that early synergistic intervention is safe and will decrease the incidence of ROP in the combination therapy treated patients by 50%. Assuming an ROP incidence of 57% (all stages), a total of 120 EVALUABLE neonates or 40 infants in each group would be needed to have a statistical power of 80% to detect a 50% relative reduction in the risk of ROP at an alpha =0.05 beta =0.20. **This is a small, adequately powered phase II, safety, efficacy, pharmacodynamic trial which will generate requisite data needed for the design and implementation of a conclusive randomized phase 3 trials.** Randomization will be done using sealed envelopes at each site in blocks of 6.

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The participating NICUs of the Brooklyn Neonatal Network, SUNY Stony Brook NICU admit at least 350 ELGANs < 1250 grams per year, all of which will be potential subjects in this study.

12.2 Populations for Analysis

The full analysis set is defined as all randomized patients who receive at least one dose of the study drugs (intent to treat modality). These infants will comprise the population for the safety analysis, and if any PK samples are obtained, their blood, urine and/or cord blood will be evaluated in the PK analysis.

12.3 Statistical Methodology

All data will be collected and analyzed by the Bioinformatics Core Laboratory at the State University of New York (SUNY) Eye Institute. Additional data analysis and ad-hoc consultations will be provided by Dr. Ron Thomas (Wayne State PPRU biostatistician 2004 -2010) who has worked closely with PI (Aranda) in previous clinical trials. Descriptive, parametric and nonparametric statistics will be done for the birth weight categories. Contingency tables will be analyzed using Chi-square. The Fisher's exact test will be used if an individual cell has <5 observations. Comparison of continuous variables between patients with no ROP and patients with ROP will be done by the Student's t-test for normal variables and the Wilcoxon rank sum test for non-normal variables. For comparisons of continuous variables between >2 groups (i.e. birth weights), analysis of variance (ANOVA) will be used for normal variables and Kruskal-Wallis test will be used for non-normal variables with the appropriate adjustments for multiple comparisons. The analyses of the primary outcome and of all other dichotomous outcomes will be adjusted with the use of a logistic-regression model that will include terms for treatment and center. If no treatment effect, the quotient of the estimated coefficient and its standard error will be used as a z-test statistic for the null hypothesis. For all relevant binary outcomes, logistic regression will be used to include adjustments for gestational age, and other relevant variables. Odds ratios and confidence intervals will be calculated for all outcomes of the groups. All statistical tests will be 2-sided. Data will be presented as mean ±SD where applicable. All p-values will be two-sided and a value of <0.05 will be considered significant.

12.4 Demographics and Baseline Characteristics

Infant and maternal demographic and baseline characteristics such as age, gender, race, primary underlying disease, etc. will be summarized and compared among treatment groups. Descriptive statistics such as number of observations, mean, median, 95% confidence interval, standard deviation, standard error, minimum, and maximum will be presented by dosage group for continuous variables (such as age, weight, etc.). Other descriptive statistics such as counts, proportions, and/or percentages will be presented by dosage group to summarize discrete variables (such as race, sex, ROP stage, stage +3, mortality rates, etc.). The number of infants completed, and discontinued early from study, and the reasons for discontinuation, will be summarized by dosage. Demographic and baseline characteristics will be summarized by group and dosage. Variables include race, age, sex, and selected clinical variables recorded prior to initiation of study drug. Study drug administration will be summarized in terms of number of days of dosing, and reasons for final discontinuation of study drug.

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12.5 Efficacy

The primary endpoint for the study is ROP (all grades) using International ROP Classification and severe ROP (Stage 3+ disease) or need for laser or Avastin. It is estimated that approximately 20% of the analysis population will be twins. Therefore, a generalized estimating equation method will be used with a repeated statement to account for correlated data when analyzing the treatment effect on the primary endpoint. Patients with missing outcomes will be treated as failures. The parameters for handing missing data will be detailed in the Statistical Analysis Plan. Eye examinations will be done at standard of care through discharge and once, at 50 weeks PCA, solely for the study. Parents will bring subjects back in to clinic for eye examination at 50 weeks PCA ± 7 days. Ideally, an objective technique such as retinal digital photographs (RetCam) read by 2 independent investigators would decrease variability; however, such a technique employed in multiple sites will substantially increase cost and may not be feasible for this study. Since this clinical protocol will only be implemented at year 2, participating centers will be encouraged to procure RetCam for their sites during year 1. Otherwise, all infants will undergo routine eye examination by a pediatric ophthalmologist according to the International Classification for ROP (135). The rate of mild (stage 1), moderate (stage 2) and severe ROP (stages $\geq$3) will be calculated as the number of infants diagnosed with ROP over the number of infants receiving retinal examinations. ROP will be classified according to the three components outlined by the International Classification for ROP: 1) The zone in which ROP occurs; 2) the stage of ROP; and 3) the presence or absence of plus disease. Zone 1 is the most posterior (an area within twice the distance from the optic nerve head to the fovea; Zone 2 is ROP outside of zone 1; and Zone 3 is ROP only present on the temporal side of the eye. Stage 1 is a line of demarcation from the vascularized region of the retina and avascular zone. In stage 2 the line becomes a ridge that protrudes into the vitreous. In stage 3, the extraretinal vascular proliferation occurs when the ridge and neovascular tufts are seen in the posterior to the ridge. Disease occurs when the vessels posterior to the ridge become dilated and tortuous. Stage 4 has scarring and fibrosis when neovascularization extends into the vitreous, causing traction of the retina and retinal detachment. Stage 5 is total retinal detachment. Participating ophthalmologists will meet annually to optimize uniformity in ROP assessment.

The secondary endpoints are as follows:

1. Necrotizing enterocolitis
2. Spontaneous intestinal perforation
3. Intraventricular hemorrhage
4. Periventricular leukomalacia
5. Patent ductus arteriosus
6. Chronic lung disease/bronchopulmonary dysplasia
7. Sepsis
8. Corneal toxic lesions
9. First day of full feeds
10. Length of hospitalization
11. Drug toxicity

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Treatment group comparisons will be made on the secondary efficacy endpoints as described above.

13.0 TERMINATION OF THE STUDY

The clinical trial progression will be monitored by CoVeris Pharmaceutical and Clinical Consultants (Alex J. Coimbre, BA, MPS, CCRA 919-341-0484). The Steering Committee has the right to terminate the study at its discretion with written notice to the institution and Principal Investigator. The investigator or institution has the right to terminate this study at its discretion with written notice to the Steering Committee or its designee. The study may be terminated by the NYPD-PRC PI if there is evidence of an investigator failing to maintain adequate clinical standards or evidence of an investigator or staff failing to comply with the protocol. Possible reasons for termination of the study include, but are not limited to:

a) Unsatisfactory enrollment with respect to quantity or quality

b) Inaccurate enrollment with respect to quantity or quality

c) Inaccurate or incomplete data collection

d) Falsification of records.

e) Failure to adhere to the protocol

13.1 Protocol Deviations

When a deviation from the protocol is deemed necessary for an individual infant, the investigator or other responsible physician must contact the NYPD-PRC PI or clinical monitor immediately, unless a delay would endanger the subject, so that a timely decision can be made as to whether or not the infant should be enrolled or continue in the study. The deviation from the protocol will be authorized only for that particular infant. A description of the departure from the protocol and the reason(s) for it must be recorded on the appropriate CRF or the provided protocol deviation log sheet. Additionally, sites will adhere to local IRB reporting rules for protocol deviations.

14.0 ADMINISTRATIVE AND REGULATORY CONSIDERATIONS

14.1 Prior to Initiation of the Study

The following will be provided to the Steering Committee or their representative, prior to the shipment of the study medication and initiation of the study:

1. A completed US Food and Drug Administration (FDA) form 1572 or other form required by the local regulatory agency as appropriate
2. Curriculum vitae for the investigator and sub-investigators signed and dated within 2 years of current date.
3. The “Investigator Agreement” page of the protocol and any applicable amendment(s) signed and dated by the investigator.
4. An IRB/independent ethics committee (IEC) membership list and IRB assurance number.

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5. Written verification of IRB/IEC approval of the protocol, amendments, if applicable, and informed consent/authorization form (or separate authorization document) in compliance with US federal regulation 21 CFR Part 50 and 21 CFR Part 56 (Human subjects and IRBs) and for clinical sites in the US, 45 CFR Parts 160 and 164 (HIPAA Privacy Rule; HIPAA 1996), effective date 4/14/03.
6. Approved copy of informed consent/authorization (or separate document).
7. Written documentation of financial disclosure in compliance with US Federal regulation 21 CFR Part 54.
8. A duly executed Research Agreement, if applicable.
9. Documentation of laboratory certification and normal reference ranges.

Investigators will not be allowed to administer study drug to patients in the study prior to an initiation visit between appropriate investigational staff and the Steering Committee representative(s). This meeting will involve a detailed review of the protocol and case report form (CRF).

14.2 Institutional Review Board/Independent Ethics Committee Approval

Prior to its implementation, this protocol, including any subsequent amendments, must be approved by an IRB/IEC constituted according to FDA regulations. Documentation of the approved IRB/IEC's initial and continued study approval or the withdrawal of such approval will be immediately forwarded to the Steering Committee or its designee.

14.3 Informed Consent and Assent

The principles of informed consent in the current edition of the Declaration of Helsinki (See Appendix K of Manual of Operations) should be implemented before protocol-specified procedures are carried out. Informed consent will be obtained and documented in accordance with U.S. 21 CFR Part 50.25, §§ 116, 117 and 408 of 45 CFR Part 46 and all other applicable regulatory requirements. Prior to any study procedures being performed, the investigator or his/her designee will inform the subject's legally authorized representative (e.g., parent, guardian) of all aspects pertaining to study participation. Information should be given in both oral and written form whenever possible and deemed appropriate by the IRB. The subject's legally authorized representative (parent or guardian) must be given ample opportunity to inquire about details of the study. The description of the study procedures will include the purpose of the research and procedures, risks and benefits of the research, alternative procedures, confidentiality, legal rights, parental or guardian permission, the contact person and phone number if there are any questions, and the voluntary nature of participation. It will be emphasized that participation is voluntary and participants may withdraw from the study at any time without any effect on standard care. The investigator or his/her designee, and the subject's legally authorized representative must both sign and date the informed permission form. An original signed informed permission form will be retained in the site study records. The subject's legally authorized representative will receive a copy of the signed and dated informed permission form

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and a copy of the signed assent (if applicable). The parental/guardian permission form generated by the investigator must be approved (along with the protocol) by the IRB and be acceptable to the Steering Committee. Permission forms must be in a language fully comprehensible to the subject's legally authorized representative. Permission shall be documented by the use of a written consent form approved by the IRB and signed and dated by the subject's legally authorized representative. The written parental/legal guardian permission document will embody the elements of informed consent as described in the Declaration of Helsinki, the Code of Federal Regulations, and the ICH Guidelines and will comply with local regulations. This form may be read to the subject's legally authorized representative, but, in any event, the investigator shall give the representative adequate opportunity to read it before it is signed and dated. Permission must be documented by the dated signature of the subject's legally authorized representative. The signature confirms the permission is based on information that has been understood. Each signed permission form must be kept on file by the investigators for possible inspection by Regulatory Authorities, and NICHD or its designees.

14.4 Protection of Personal Health Information

Prior to any study-related procedures, the investigator or designee is obligated to obtain from each patient, or the patient's legally authorized representative (i.e. parent/legal guardian), a signed and dated written authorization consistent with FDA regulations, and the HIPAA Privacy Rule, and applicable State and local laws. In the US, HIPAA Privacy Rule Authorization language must be included in the Informed Consent/Authorization form (where the Informed Consent and Authorization are combined in one document) and it must be IRB/IEC approved. The patient health information (PHI) of the patients will be protected according to the HIPAA regulations. Measures to protect against and minimize risk of breach of confidentiality will be: Master list of subject's name, medical record number and medical information will be kept under strict confidentiality on a password protected computer which will only be accessed by authorized personnel. After each sample is collected it will be placed in a labeled container with the patient's medical record number to prevent breech of confidentiality. All PHI will be kept in a locked cabinet with only the PIs and research personnel having access. All electronic PHI will be encrypted and kept in password protected computers with only the PIs and research personnel having access.

14.5 Amendments and/or Changes to Informed Consent/Authorization

Amendments to this protocol must be agreed upon in writing between the investigator and the Steering Committee. Written verification of IRB/IEC approval will be obtained before any amendment is implemented which affects patient safety or the evaluation of safety and/or efficacy. Modifications of the protocol that are administration in nature do not require IRB/IEC approval but will be submitted to the IRB/IEC for information. If there are changes to the Informed Consent/authorization, written verification of the IRB/IEC approval must be forwarded to the Steering Committee. An approved copy of the new Informed Consent/Authorization must also be forwarded.

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14.6 Ethical conduct of the trial

This study will be conducted according to the protocol, the applicable FDA and HHS Code of Federal Regulations, Good Clinical Practice, the Declaration of Helsinki, and the ICH Harmonized Tripartite Guideline for Good Clinical Practice. It will also adhere to the ethical principles outlined in The Belmont Report.

14.7 Source Documents

Source documents are defined as original documents, data and records. They may include hospital records, clinical and/or office charts, laboratory data/information, pharmacy dispensing records, radiology procedures, CPAP and ventilator recordings. The investigator(s)/institution(s) will permit study-related monitoring, audits, IRB/IEC review, regulatory inspection(s), and will provide direct access to source data documents.

14.8 Case Report Forms (CRFs)

Data for individual subjects will be recorded on case report forms (CRFs) provided by the NYPD-PRC. All entries must be complete. A CRF must be completed for each subject enrolled, including those removed from the study. If a subject is removed from the study, the reason for removal must be noted on the CRF by the investigator. The principal investigator must review and approve each CRF. CRFs must be current to reflect subject status at each phase during the course of the study. Subjects are not to be identified on the CRFs by name; appropriate coded identification and subject initials must be used. The investigator must keep a separate log of subject names and addresses. If requested as part of an FDA inspection, this log may be shown to the FDA investigator, but no copy should be provided so that confidentiality is protected. Because of the potential for errors and inaccuracies in entering data onto CRFs, laboratory and other test results must be kept on file with the subject's study dossier. Case report forms and copies of test results must be available at all times for inspection by the CRA for the site and the FDA.

14.9 Steering Committee

The Members of the Steering Committee will include the faculty and research expertise of several members of the PIs' site (or Co PIs where applicable) from subcontract sites. The NICHD Project Officer's input will also be requested in special circumstances. The NICHD Project Officer will be a non-voting member of the Steering Committee. The Steering Committee will hold regular teleconferences. All Steering Committee members (or in special circumstances, their designee) will be required to participate in these meetings/teleconferences. The Steering Committee will seek and accept advice from the NICHD, CTCC (Clinical Trials Coordinating Center) and the DSMB, and will receive implementation recommendations from regular study coordinators' teleconference, to which it may delegate authority for minor implementation decisions. It will adopt a publication policy acceptable to all sites and will supervise the publication of results. Should a problem at any given site arise, the PI (and Co-PIs) at that site will be contacted by one or more members of the Steering Committee to discuss the problem and to develop a plan for its resolution. A timeline and action-plan will be developed. This plan will be reported back to the Steering Committee. The timeline and outcome will then be monitored by the Steering Committee.

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15.0 RESPONSIBILITIES OF THE CLINICAL INVESTIGATOR

The investigator is obligated to conduct this study in accordance with US Federal regulation 21 CFR 312.60-69 as specified on the signed form FDA 1572, applicable state laws, and the International Conference on Harmonization: Good Clinical Practice: Consolidation Guideline. The investigator is responsible for informing the IRB/IEC of any safety issues related to the study and the study drug including reports of SAEs, if required, and all investigational new drug (IND) safety reports. Prior to initiation of the study, the following will be done:

a) Approved IND for use of Ketorolac (Acuvail) in preterm newborns. IV Ibuprofen was approved for newborn in 2006;

b) completed U.S. Food and Drug Administration (FDA) 1572 form or other form required by local regulatory agency, as appropriate;

c) curricula vitae for the investigator and sub-investigators signed and dated within 2 years of current date;

d) An IRB/IEC membership list or IRB assurance number;

e) written verification of IRB/IEC approval of protocol, amendments, if applicable, and informed consent/authorization form (or separate authorization document) in compliance with U.S. federal regulation 21 CFR Part 50 and 21 CFR Part 56 (Human Subjects and IRBs) and, for Clinical sites in the U.S., 45 CFR Parts 160 and 164 (HIPAA Privacy Rule; HIPAA 1996), effective date 4/14/03;

f) approved copy of informed consent/authorization (or separate document);

g) written documentation of financial disclosure in compliance with U.S. federal regulation 21 CFR Part 54;

15.1 Data Quality Control and Assurance

Prior to the initiation of the study, an investigator's meeting will be held with the BPCA-CC and MPODS network personnel, the investigators and their study coordinators for the study. This meeting will include a detailed discussion of the protocol, performance of study procedures, CRF completion, simulation of study procedures and specimen collection methods, as applicable. In addition to the investigators' meeting, the study personnel at each site will be trained on the study procedures at a study initiation visit. The CRAs will monitor each site throughout the study. At each visit, 100% source document review will be made against entries on the CRF and a quality assurance check will be performed to ensure that the investigator is complying with the protocol and all applicable regulations. After completion of the entry process, computer logic checks will be run to check for such items as inconsistent study dates and outlying laboratory values. Any necessary correction will be made to the database and documented via addenda or audit trail. A manual review of selected line listings will also be performed at the end of the study.

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15.2 Site Monitoring Visits

The clinical trial progression will be monitored by CoVeris Pharmaceutical and Clinical Consultants (Alex J. Coimbre, BA, MPS, CCRA 919-341-0484). The Steering Committee or its designee will monitor the study progress, as frequently as is necessary to assure compliance of Good Clinical Practices and protocol procedures and to monitor completion of CRFs. Arrangements for monitoring visits will be agreed to in advance of planned visits, except in cases of emergency. FDA or other health authority representatives reserve the right to visit sites at any time. Monitoring visits to the sites will be made before, during, and at close of the study to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of data collected on the CRFs. Participating sites and investigators will guarantee access to source documents and CRFs to the CRAs. The principal investigator and relevant site personnel will be available during the monitoring visits and will set aside sufficient time for the process.

15.3 Quality Assurance and Regulatory Agency Audits

The study sites may also be subject to quality assurance audits by the NICHD or its designees and appropriate regulatory agencies.

15.4 Ensuring Confidentiality

A study number will be assigned for each subject. Data forms will be identified by subject number and initials. The database will not contain any personal identifiers other than subject number and initials.

15.5 Record Retention

It is the investigator's responsibility to retain all records and documents (including electronic data capture materials) pertaining to the conduct of the study and the distribution of the investigational drug for two years after discontinuation of the IND. It is recommended, however, that records be retained for at least 5 years in the event follow-up is necessary to help determine any potential hazards to patients who took part in these studies. The Steering Committee will notify the investigator if the IND is discontinued. The investigator agrees to obtain Steering Committee agreement prior to disposal, moving, or transferring of any study-related records. Data generated by the methods described in the protocol will be recorded in the patient's medical records and/or study progress notes. Data may be transcribed legibly on CRFs supplied for each patient or directly inputted into an electronic system or any combination thereof. The Investigator and Steering Committee will mutually agree upon the storage format for the retention of electronic data. The investigator will agree to provide access to the office, clinic, and/or hospital records of all patients entered into the study. Access inspection of these records may be required by the Steering Committee personnel and/or their representative(s) at the time of each monitoring visit. In addition, all records may be subject to inspection by officials of the FDA and other health authorities. The investigator shall make accurate and adequate written progress reports to the IRB/IEC at appropriate intervals, not exceeding one year. The investigator shall make and accurate and adequate final report to the IRB/IEC within three months after completion or

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termination of the study. The Investigator shall report to the Steering Committee or their representative(s) any SAEs or deaths during the study, whether regarded as drug-related or not.

To enable evaluations and/or audits from Regulatory Authorities and NIH/NICHD or its designees, the investigators will keep records, including the identification of all medical charts and associated source documents and copies of all CRFs. The investigators will contact NIH/NICHD before disposing of any such materials.

16.0 USE OF INFORMATION AND PUBLICATION

16.1 Use of information

After the dataset for the study is finalized and main findings have gone into publication, the data from this project will be made available and shared through CD-ROM and/or a website. All project data will be stored without subject identifiers, so that the data that are shared cannot be linked back to any particular subject. The dataset will cover the outcome data on children collected over the course of the study.

16.2 Data sharing plan

The dataset for this study will cover course and outcome data on preterm infants collected over the period of study. The data sharing plan will follow guidelines as dictated by institutional rules and approval of local IRBs of participating research sites, local, state and federal laws and regulations including the Privacy Rule. The final data sharing plan that will be employed by the BEST protocols will be developed in conjunction with the individual site PI's, and the NYPD-PRC.

16.3 Publication Policy

Prior to a manuscript or abstract being submitted for possible publication or presentation, the Steering Committee and NICHD must review the contents of the submission. More specifically, manuscripts, abstracts, and poster submissions must be submitted to the Steering Committee, and NICHD at least 30 days prior to submission for publication or presentation. Financial support from the NIH-NICHD will be acknowledged in all publications.

17.0 INVESTIGATOR AGREEMENT

I have received and reviewed the package insert for IV Ibuprofen & Ketorolac

I have read the protocol and agree to conduct the study as outlined and in accordance with all applicable local, state, and federal regulation.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

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Signature of Principal Investigator Date

Name of Principal Investigator (printed or typed)

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