# Fenoldopam to Prevent Renal Dysfunction in Indomethacin Treated Preterm Infants

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**Specific Aims**

Failure of the ductus arteriosus to close after birth results in significant morbidity in premature infants. As many as 60% of newborns delivered prior to 28 weeks gestation require therapy to close their patent ductus arteriosus (PDA). Administration of non-steroidal antiinflammatory agents, such as indomethacin, is the preferred initial therapy. However, impairment of renal function commonly occurs as a result of indomethacin and may result in 1) fluid retention that may inhibit ductal closure; 2) limitation of nutritional fluid intake; 3) interruption of the three-dose course of treatment with resultant treatment failure; and 4) increased risk for long term renal injury. Alleviating the severity of renal impairment associated with indomethacin would significantly reduce neonatal morbidity. To date, no effective approach to attenuate the risk for renal impairment has been identified. Fenoldopam is a selective dopamine type 1 receptor agonist with the potential to dilate the renal vasculature, as it does not have the effect on vasoconstricting adrenergic receptors seen with dopamine. In critically ill adults and postoperative pediatric cardiac surgical patients, fenoldopam preserves renal function compared to placebo or dopamine. Studies in neonates are limited and suffer from retrospective design, small sample size and heterogeneous study populations. In late gestation fetal sheep, an animal model with a pattern of renal development stage similar to humans, we demonstrated fenoldopam significantly increased glomerular filtration, urine flow and sodium excretion. Demonstration of similar effects in the preterm infant would suggest fenoldopam as an important therapeutic intervention in this population.

Our primary hypothesis is that administration of fenoldopam, in doses previously demonstrated in preterm infants to not have significant systemic hemodynamic effects, will preserve renal function in preterm infants treated with indomethacin for pharmacologic closure of PDA. A secondary objective will be to determine fenoldopam pharmacokinetics in the premature population, which we hypothesize will differ from those published in older pediatric populations. Lastly, we hypothesize that urine and serum acute kidney injury (AKI) biomarkers will be superior to contemporary neonatal AKI definitions in their ability to identify renal injury. To address these hypotheses, we propose to undertake a randomized, blinded, placebo-controlled trial to pursue the following aims:

**Specific Aim 1: Evaluate the effect of fenoldopam on renal function in preterm infants administered indomethacin.** Specifically, we will study the effects of fenoldopam on indomethacin induced renal dysfunction using classic markers of acute kidney injury according to the neonatal specific AKI definition modified from the 2012 Kidney Disease / Improving Global Outcomes (KDIGO) criteria.

**Specific Aim 2: Determination of fenoldopam pharmacokinetic (PK) and pharmacodynamic (PD) profiles in preterm infants.** We propose to use a novel population based approach, in which the PK profile of fenoldopam can be determined with a limited number of serum samples from each infant. The fenoldopam PK profile will be used as the driving force to further build exposure-response models (i.e. PK/PD modeling), which will be used to examine the relationships between fenoldopam concentrations and drug effects, including systemic hemodynamics, urine output and markers of AKI. Efficacy of fenoldopam in promoting closure of the ductus arteriosus will be assessed.

**Specific Aim 3: Define whether newly identified biomarkers of acute kidney injury are more sensitive markers of renal dysfunction following indomethacin than traditional markers such as urine output and serum creatinine.** We will take advantage of commercially available sensitive and precise biomarker platforms (Meso Scale Discovery) for quantitative determination of urine and serum levels of 7 biomarker analytes. Additionally, we will define the molecular signature differences in the urine metabolome in infants before and after indomethacin therapy, and we will identify the effects of fenoldopam on the metabolic profile.

Collectively, these studies will 1) identify a medication that preserves renal function in the preterm infant, likely having an immediate impact on reducing short term morbidities associated with prematurity; 2) develop a PK/PD model of fenoldopam in this population to determine the most effective dosage regimen; and 3) define novel and early metabolomic markers of AKI and pathways of injury and repair. Avoidance of acute kidney injury in neonates will positively impact clinical care in this population and lessen the burden of chronic kidney disease later in life.

**B. Significance**

Patent ductus arteriosus (PDA) affects up to 60% of infants less than 28 weeks gestation.[5] While the indications for pharmacological closure are debated, avoiding the need for surgical closure is optimal.[6] The effectiveness of indomethacin, a non-steroidal anti-inflammatory agent (NSAID) and the most commonly used medication for PDA closure, is variably with 30% or more of PDAs failing to close with initial therapy. Furthermore, greater than 25% reopen after initial therapy, necessitating multiple courses of therapy.[7-9] There is concern regarding the use of indomethacin given its toxicity to the neonatal kidney.[10] In preterm infants 24 to 32 weeks gestation, a single course of indomethacin results in oliguria in up to 30% of patients.[9, 11] Even in infants without frank oliguria, there are often significant decreases in urine output and increases in serum creatinine. To avoid fluid overload, the volume of parenteral nutritional provided is often reduced before or during indomethacin therapy, resulting in a reduction in energy, protein and carbohydrate intake.

Treatment of PDA with indomethacin significantly reduces renal blood flow and glomerular filtration through inhibition of cyclo-oxygenase and decreased production of prostaglandin E, an important renal arterial vasodilator. To date, efforts to maintain renal blood flow following administration of indomethacin have failed. A meta-analysis reviewing the effects of dopamine in indomethacin-treated infants found no evidence to support to use of dopamine to prevent renal dysfunction.[12] The diuretic furosemide is known to increase renal prostaglandin production and has also been investigated in indomethacin-treated infants. Surprisingly, use of furosemide with indomethacin increased the incidence of acute kidney injury (AKI).[13]

Causes of neonatal AKI are multifactorial and include nephrotoxic agents such as indomethacin.[14] The incidence of AKI among neonatal intensive care unit patients varies widely, but is estimated to range between 8 and 24%; with rates even higher among premature infants.[15, 16] Neonatal AKI has been clearly identified as an independent risk factor for poor outcome, with associations existing between AKI and mortality and morbidity, including chronic kidney injury.[15, 17] Data from the Pediatric Health Information System database, representing 44 tertiary care children's hospitals, suggest that NICU patients exposed to NSAIDs had a 2.3-fold risk of AKI compared to non-exposed patients (J Misurac, personal communication). In view of the recent report that very low birth weight infants with AKI are at two-fold higher risk of death compared to those without AKI, efforts to prevent or ameliorate development of AKI in this population are urgently needed.[18]

There is increasing concern regarding the risk of chronic kidney disease later in life for infants born premature.[4, 19, 20] Disruption of normal nephrogenesis and a reduction in nephron number likely contribute to increased susceptibility to renal injury and increased risk of renal disease later in life.[19, 21, 22] Recent evidence suggests that although nephrogenesis continues after birth in preterm infants, kidney growth is altered in terms of nephron number and size. In autopsy samples from human preterm infants, a large number of glomeruli appear abnormal with scant capillarization and enlarged Bowman spaces.[23] In baboons and mice delivered prematurely, nephron number is significantly decreased compared to term-delivered, postconceptional age-matched controls.[24, 25] Additionally, preterm infants are at significant risk for acute kidney injury related to birth associated events such as maternal chorioamnionitis, episodes of hypoxemia, intravascular volume depletion, and exposure to nephrotoxins such as aminoglycosides and indomethacin.[19, 21] While classically taught that acute kidney injury is reversible, recent epidemiological studies link acute kidney injury in adults and children to the later development of chronic kidney disease.[26, 27] Although data are lacking in neonates, it is reasonable to assume that in the face of abnormally developed kidneys resulting from prematurity, there are deleterious effects of AKI on long-term kidney health. In 3-10 year old children born prematurely and diagnosed with and without neonatal AKI, those with AKI had significantly decreased renal volume.[4] Thus, identification of novel markers of AKI and measures to avoid kidney injury in the preterm infant population is critical.

Studies of pharmacologic agents in pediatric patients, let alone neonates, are lacking. We propose to study the renal sparing effects of fenoldopam and its pharmacokinetic/pharmacodynamics profile of fenoldopam. Our proposal, which is carefully designed and for which FDA Investigation New Drug (IND) approval has been obtained, will address a troubling information gap in the care of this unique and vulnerable population.

**C. Innovation**

The innovation of this proposal exists at multiple levels. **First**, we are using a currently marketed pharmaceutical with a well-defined profile in adults for a new and unique population. Fenoldopam mesylate is a selective dopamine receptor ($D_1$ receptor) agonist which induces vasodilation of renal, mesenteric and other arteries. Unlike dopamine, it has no affinity for vasoconstricting $\alpha$-adrenoreceptors and thus improves blood

flow to the renal cortex and medulla. Approved as a short-term antihypertensive agent, fenoldopam has also been widely promoted and studied in the adult population for prevention and treatment of AKI.[28-30] A meta-analysis of randomized placebo-controlled studies involving over 400 patients undergoing cardiac surgery found that fenoldopam reduced the risk of acute kidney injury by more than half (OR = 0.41; CI,0.23-0.74; p = 0.003).[31] A multicenter randomized controlled trial to evaluate the effects of fenoldopam on the need for renal replacement therapy in cardiac surgery patients who had already developed AKI was stopped early for futility, as no effect was seen.[28] *Such findings emphasize the importance of starting fenoldopam before the development of AKI or its inciting event occurs*. **Second**, the design of our studies allows for exact timing of the inciting risk for AKI—indomethacin administration—and for obtaining important study samples both before and after indomethacin. We will initiate fenoldopam therapy prior to the development of AKI or indomethacin administration, taking a preventive rather than therapeutic approach. **Third**, we will use a commercially available, sensitive and precise biomarker platform (Meso Scale Discovery) for quantitative determination of urine and serum levels of 7 biomarker analytes. We will determine whether these markers, singly or in combination, are superior in identifying AKI compared to conventional measures. **Finally**, we will define the molecular signature differences in the urine metabolome in infants before and after indomethacin therapy, and we will identify the effects of fenoldopam on the metabolic profile. These studies will identify novel and early metabolomic markers of AKI, which we anticipate will lead to identification of pathways of injury and repair.

## D. Approach

### D.1 Previous Work

Studies of fenoldopam for reducing the risk or severity of AKI in the pediatric population are limited. High-dose fenoldopam infusion (1 µg/kg/min) was found to significantly reduce the levels of AKI biomarkers in children undergoing cardiopulmonary bypass with a strong trend towards reduction in AKI (p = 0.08).[32] In contrast, a study of low-dose fenoldopam (0.1 µg/kg/min) in newborn patients undergoing bypass found no significant effect on AKI incidence.[33] A retrospective review of fenoldopam use in a single NICU in patients with established oliguria or anasarca found patients not on extracorporeal membrane oxygenation at the time of treatment (0.1µg/kg/min over 24 hours) had a significant increase in urine output.[2] Use specifically in the extremely preterm population has not been described. However, in fetal sheep, commonly used to model human kidney development, fenoldopam significantly increased urine flow rate, glomerular filtration rate and sodium excretion.[34] Taken together, the above findings substantiate the need for determining whether fenoldopam can prevent renal dysfunction in indomethacin-treated preterm infants.

### D.2 Study Design

We propose to conduct a randomized, blinded, placebo-controlled trial to determine whether administration of fenoldopam reduces renal dysfunction associated with indomethacin administration for closure of patent ductus arteriosus in preterm infants.

Patient Selection: Infants born between 23 0/7 and 27 6/7 weeks gestation (by obstetrical dating) and admitted to the University of Iowa Children's Hospital NICU are routinely screened for PDA by echocardiography during first few weeks of the postnatal period. If a PDA is present, the decision to attempt pharmacologic closure is at the discretion of the attending physician. If the decision is made to use indomethacin, the infant will be deemed eligible to be screened for enrollment in the study, and the clinical team will notify the designated research nurse. Echocardiographic screening for PDA will not be performed specifically for the purpose of this study.

Inclusion criteria include:

1) Gestational age at birth 23 0/7 to 27 6/7 weeks by best obstetrical dating
2) No previous exposure to indomethacin
3) Clinical determination made to use indomethacin to attempt closure of PDA
4) No known congenital abnormalities involving the kidneys, heart or lungs
5) No preexisting renal dysfunction, defined as serum creatinine >1.0 mg/dl, or oliguria, defined as urine output <1.0 ml/kg/hour over the previous 24 hours.

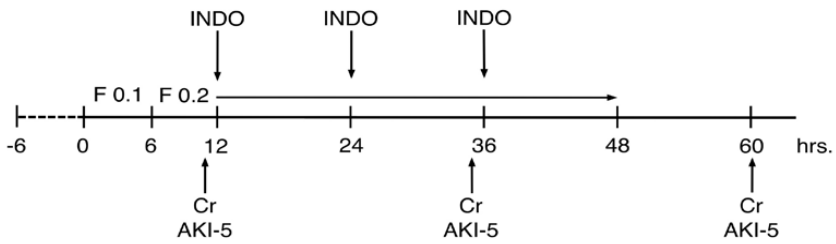
Exclusion criteria:

1) Enrollment in concurrent study in which interventions may contribute confounding variables
2) Infants with diagnosed or suspected renal or urinary tract abnormalities
3) Infants with umbilical cord or infant blood pH below 7.0 at any time before enrollment
4) Attending physician unwilling to have infant participate in study

Infants will be screened for eligibility for enrollment by a designated research nurse. Before being approached, the parents will have been informed by the care team about the presence of a PDA and the decision to attempt PDA closure using indomethacin. A log will be maintained of all screened infants indicating which are eligible and which were enrolled. Upon receipt of consent, treatment assignments will be made electronically using the Research Electronic Data Capture (REDCap) treatment randomization module using a randomized block design to assign treatment group and stratified for gestational age (23 - 25 6/7 and 26 - 27 6/7 weeks gestation). A block randomization strategy will be employed to achieve balance between study arms and reduce the opportunity for bias and confounding. Infants from multiple gestations will be randomized independently. Subjects will be analyzed by intention to treat, with the group to which they were randomized, regardless of subsequent events. Fenoldopam will not be used outside of the study.

Determination of status of ductus arteriosus:
After an echocardiogram is obtained at the discretion of the attending neonatologist as part of routine care, a decision regarding whether to treat or not is made. If the decision to treat with indomethacin is made, an echocardiogram will be obtained immediately prior to the first dose of indomethacin and again 24 hours after completion of the course of indomethacin therapy (routine in our NICU). The echocardiographer and cardiologist interpreting the echocardiogram will be blinded to the treatment group.

Time course for study **(Figure**):
Urine will be collected in 6-hour increments by extracting from special diapers (Cuddle-Buns Preemie Diapers, Trace Medical Equipment, Lisle, IL), measured, and frozen for later determination of urinary metabolomics as described below. Serum creatinine, which in our NICU is always measured within 12 hours prior to initiating indomethacin, will be recorded. Infants will receive either placebo (0.9% sodium chloride, 0.2 ml/kg/hr) or fenoldopam (30 µg/ml; 0.2 ml/kg/hr to provide 0.1 µg/kg/min). If, after 6 hrs there is not a clinically concerning decrease in blood pressure as determined by attending physician, the rate of fenoldopam infusion (or placebo) will be increased to 0.2 µg/kg/min (30 µg/ml, 0.4 ml/kg/hr). Increased urine output has been reported in neonates at median doses of 0.1-0.3 ug/kg/min while decreased blood pressure has been at doses above 0.2 ug/kg/min.[1, 2, 35] Thus to avoid systemic hemodynamic changes, we will limit dose to 0.2 ug/kg/min. This rate will be continued throughout the remainder of the study. Fenoldopam will be started 12 hours before the first dose of indomethacin and discontinued 12 hours after the third dose of indomethacin. Blood pressure will be monitored 15, 30, 45 and 60 minutes after starting fenoldopam or increasing the dose and hourly throughout the infusion. Infants will receive indomethacin therapy (3 doses, 0.2, 0.25, 0.25 mg/kg iv, every 12 h, respectively), in accordance with our current medical practice. Provision of parenteral and/or enteral nutrition, including fluid, electrolyte, lipid and energy intake will be at the discretion of the attending physician, though attempts will be made to hold these constant during the study period. Except for administration of fenoldopam or placebo, all clinical care decisions, including respiratory care, will be at the discretion of the attending physician. Infants will be weighed daily per our NICU routine.




- F 0.1, * Fenoldopam (0.1 ug/kg/min i.v.)
- F 0.2, * Fenoldopam (0.2 ug/kg/min i.v.)
- Cr, Serum creatinine
- AKI-5, Acute kidney injury biomarker panel 5 assay
- INDO, Indomethacin (0.2 mg/kg i.v.)
- Urine collected in 6-hour increments from -6 hr to 60 hr.
- Population-based PK/PD
  - 0-48 hr 1-2 samples/patient (random times)
  - >48 hr 2-3 samples/patient (random times)

**Figure. Timeline of study**

Determination of patient demographics: Routine clinical data will be abstracted from the infants' medical records by study personnel. We will use the standardized NICHD Neonatal Research Network (of which we are a part) Generic Database forms for data collection; this is routinely used for all <1500 g infants admitted to Network centers. These forms will be used as the source for maternal and infant demographic data, maternal/pregnancy history, including exposure to antenatal indomethacin, MgSO4, antenatal steroids, and clinical outcome data including potential confounding variables. We will ensure sex, gestational age, appropriateness of weight for gestational age, daily weight, daily fluid intake, daily energy intake, ventilator settings, supplemental oxygen requirements, urine output, presence of documented or suspected sepsis, age at time of treatment and treatment outcome are obtained during the course of the study as will the occurrence

of any attendant medical conditions, including intracranial bleeding, change in respiratory status, necrotizing enterocolitis, renal failure and development of retinopathy of prematurity.

**Specific Aim 1*: Evaluate the effect of fenoldopam on renal function in preterm infants administered indomethacin.***

Urine output will be recorded and expressed as ml/kg/h for the 12 hours prior to administration of fenoldopam, the 12-h during fenoldopam administration prior to starting indomethacin, and for each subsequent 12-h period until fenoldopam has been discontinued for 12 hours (**Figure**). Serum creatinine, measured by our hospital central laboratory, will be determined immediately prior to the administration of the initial and third dose of indomethacin as well as 24 hours after the final dose. The stage of AKI (0 – 3) will be determined according to the neonatal specific AKI definition as modified from the 2012 KDIGO definition (Table).[36]

**Table. Neonatal AKI definition**

| Stage | SCr criteria | Urine output (UOP criteria) |
|---|---|---|
| **0** | No change or rise < 0.3 mg/dl | UOP > 1ml/kg/h (over previous 24 h) |
| **1** | ↑ SCr of ≥0.3 mg/dl or ↑ SCr to 150-199% of baseline | UOP > 0.5 ml/kg/h and ≤ 1 ml/kg/h (over previous 24 h) |
| **2** | ↑ SCr to 200%-299% of baseline | UOP > 0.1 ml/kg/h and ≤ 0.5 ml/kg/h (over previous 24 h) |
| **3** | ↑ SCr to ≥ 300% of baseline or SCr ≥ 2.5 mg/dl or Receipt of dialysis | UOP ≤ 0.1 ml/kg/h ( over previous 24 h) |

Baseline SCr will be defined as the SCr value prior to indomethacin

**Specific Aim II: Determination of fenoldopam pharmacokinetic and pharmacodynamic profiles in preterm infants.**

To our knowledge, only a single study has examined pharmacokinetic (PK) and pharmacodynamic (PD) characteristics and the side-effect profile of fenoldopam in children.[35] In this study of 77 children from 3 weeks to 12 years of age, only doses above 0.8 µg/kg/min were associated with significantly reduced blood pressure. Understanding fenoldopam pharmacokinetics is critical for optimizing fenoldopam dosing regimens as well as establishing the relationship between the exposure of fenoldopam (i.e., pharmacokinetics) and the clinical outcome of renal function protection (i.e. pharmacodynamics). This study will provide important, novel information about this pharmacologic agent in the preterm population.

**Pharmacokinetic/Pharmacodynamic (PK/PD) Approach**

Because of their limited circulating blood volume and ethical restrictions placed on removal of blood from premature infants, it is not feasible to perform a traditional pharmacokinetic study. To evaluate the PK of fenoldopam, we propose to use a novel population-based approach, in which the PK profile of fenoldopam is determined using relatively few samples (1-3 samples) from each infant. In this approach, samples are collected randomly (i.e., not at predetermined time points) and subjects contribute different numbers of samples. This approach permits an estimation of PK parameters of fenoldopam and an explanation of variability using the nonlinear mixed-effects modeling approach.[37] Blood samples (100-200 µL) will be obtained from discarded blood from other clinical lab tests or collected during this routine clinical sampling.[38] We will target obtaining 1-2 samples per patient for time periods 0-48 hours and 2-3 samples per patient out to 60 hours, 12 hours after stopping fenoldopam. Fenoldopam concentrations will be measured using LC/MS/MS based on a sensitive method reported by Wang et al.[39]. A nonlinear mixed mathematic model (i.e. population-based approach) using specialized PK software NONMEM will be employed to fit the data to characterize the PK profile as previously performed by one of us (GA).[40] In this model, the first-order conditional estimation method with the interaction and a user-defined subroutine (ADVAN6) will be used to estimate the typical population PK parameters, random inter-individual variability, and residual variability between observed and individually predicted plasma fenoldopam concentrations. Final PK model selection will be based on the visual inspection of goodness-of-fit plots, the objective function value, and the precision of parameter estimation. The likelihood ratio test will be used for comparing nested models.

Pharmacodynamic effects of constant rate, fixed-dose fenoldopam infusion compared to placebo infusion on heart rate, mean blood pressure and urine output will be determined after 12, 24 36 hours of infusion and 12 hours after completion of infusion. Comparisons of hemodynamic and urine output data before, during, and after fenoldopam infusion will be made for each patient by linear regression analysis. Ductal patency will be assessed 24 hours after completion of the course of indomethacin to allow for determination of benefit of

fenoldopam on this outcome. Fenoldopam PK will be used as the driving force to further build exposure-response models (i.e. PK/PD modeling), which will be used to examine the relationships between fenoldopam concentrations and drug effects, including hemodynamics and urine output. The exposure-response analyses will be performed modeled using NONMEM software (NONMEM Project Group, UCSF, San Francisco, CA).

**Specific Aim III: Define whether newly identified biomarkers of renal dysfunction are more sensitive markers of renal dysfunction following indomethacin than traditional markers such as urine output and serum creatinine.**

We will take a two-faceted approach to the exploration of potential new biomarkers of renal dysfunction following indomethacin treatment in preterm infants. First, we will examine urinary levels of previously reported biomarkers in the preterm infant population. Askenazi et al recently reported increased urinary levels of NGAL and osteopontin in a population of VLBW infants (500-1500 grams) with AKI.[41] A prospective study of 140 pediatric patients (ages 1 month – 21 years) found urinary NGAL levels rose 48 hours *before* identification of AKI.[42] This proposed study provides a novel opportunity to evaluate the utility of potential biomarkers in this population since the timing of the inciting cause of AKI (indomethacin administration) is known and we will have pre- and post-administration urine samples, allowing longitudinal comparisons. Second, in parallel with determination of pre-described urine biomarkers, we will undertake an exploratory urinary metabolomic approach to identify previously suggested and novel markers of AKI in this population.

**Urine and Serum Biomarker Identification**
Seven biomarkers, including albumin, beta 2 macroglobulin, Cystatin C, EGF, NGAL, osteopontin and uromodulin, will be measured in urine and serum using the Meso Scale Discovery (MSD) Sector imager 2400 and MSD multiplex human kidney injury biomarker panel 5 assay. Urinary determinations will be made in samples obtained 6-12 hours after starting fenoldopam (prior to indomethacin), 6-12 hours after the second dose of indomethacin and 18-24 hours after the third dose of indomethacin while serum samples will be obtained immediately prior to the first and third doses of indomethacin, as well as 24 hours after the third dose of indomethacin (see figure). Analysis of these samples is as described below. Dr. Askenazi will serve as a consultant related to the identification of biomarkers using the MSD assay (see letter of support).

**Determination of urinary metabolomic markers of kidney injury**
Using high throughput technology, metabolomics examines thousands of low molecular weight metabolites that may change according to the pathologic state of the organism. Metabolites represent the downstream expression of genome, transcriptome and proteome, reflecting the phenotype at a defined time. The clinical utility involves biomarker discovery, defining as yet unrecognized biological therapeutic targets, linking metabolites to relevant standard indices and clinical outcomes. Urinary metabolomics will be performed on samples obtained 6-12 hours after starting fenoldopam (prior to indomethacin), 6-12 hours after the second dose of indomethacin and 18-24 hours after the third dose of indomethacin while serum samples will be obtained immediately prior to the first and third doses of indomethacin, as well as 24 hours after the third dose of indomethacin. Mass spectrometry (MS) is used to identify metabolites after separation by either gas chromatography (GC), or high-performance liquid chromatography (HPLC) as described by our group.[10] Evaluation of the urine metabolome will identify pathways of renal cell injury and novel diagnostic and therapeutic targets, filling a large unmet need in this area of medicine.

**Biomarker data analysis**
For the two primary measures of AKI, namely SCr and urine output, univariate analysis will be performed and the sensitivity, specificity, and positive and negative predictive value for each individual AKI biomarker will be calculated, as will receiver operator curves. Logistic regression models will also be created to determine the ability of multiple biomarkers to predict AKI. Urine biomarker studies in premature infants must be adjusted for gestational age, as levels are inversely related to gestational age independent of AKI status. The longitudinal design of our study, which includes determination of biomarker levels prior to and following the AKI-inducing event (indomethacin administration), allows us to examine changes from baseline in each patient, rather than relying on population normative values adjusted for gestational age.

**Metabolomic data analysis**
The MS spectra processing will be carried out using the XCMS software.[43] Peak detection, alignment and retention time correction are performed sequentially. Absolute value changes between the pre and post-

indomethacin administration means will be compared by fold-change analysis. Unsupervised principal component analysis (PCA) will performed using the prcomp software. Supervised partial least squares discriminates analysis (PLS-DA) will be performed to identify and characterize metabolic perturbations signatures induced by indomethacin. To assess the significance of class discrimination, a permutation test will be performed. Variable Importance in Projection (VIP) will be measured in PLS-DA, representing a weighted sum of squares of the PLS loadings.[44] Metabolic pathway analysis will be performed using Ingenuity Pathway Analysis (IPA) MetPA as previously described by us.[45]

**E. Power Calculation, Data Capture and Data Analysis Plan**

In 2014 and 2015, our NICU had on average 78 infants per year admitted between 23 0/7 and 27 6/7 weeks gestation. 70% of these infants receive at least one course of indomethacin. Based upon historical consent rates in our NICU and using conservative estimates, we will be able to enroll at least 20 patients per treatment group over an 18 month period. Review of infants in our NICU over the past 3 years as well as data from the published literature revealed an increase in serum creatinine of about 2 standard deviations following indomethacin treatment.[42] Based on a sample of 20 patients per group with $\alpha = 0.05$, the power to detect an increase in serum creatinine in the fenoldopam group compared to placebo by one standard deviation (0.3 mg/dl) is 86.9%. A REDcap database will be developed capturing demographic and prospective data (outlined above). The REDcap application interface allows direct importing to SAS® 9.4 software, which will be used for performing statistical analyses. Descriptive statistics for the study populations by treatment group will be generated, using means and standard deviations for the continuous measures (non-normally distributed variables will be normalized using a transformation) and percentages for categorical measures. For the three outcomes in Aim 1 (largest change in serum creatinine, largest decrease in 24h urine output, AKI stage), we will fit generalized linear models using the canonical link function for the distribution of each outcome. AKI stage will also be dichotomized by collapsing stages 1-3 together. The two predictor variables that will be used in every model are treatment and gestational age groups. Additional demographic predictors will be considered as control variables in the models. In order to determine the most appropriate collection of predictor variables for each outcome, we will fit all possible subsets of the control variables with each model fit having a corresponding corrected Akaike model information criteria (AICc).[46, 47] This measure is an extension of the Akaike information criterion (AIC) and is more appropriate when the sample size is small.[48, 49] The model with the smallest AICc will be deemed the most favorable in the collection and used for interpretation of its effects. The use of AICc is beneficial in that we can compare model fits of predictor sets as a whole, rather than assessing the significance of each independent variable in an attempt to specify the best model. This method ensures we select a statistically viable model by including important predictors that may impact the relationship between treatment or gestational age groups and the outcomes, while omitting spurious variables.

**F. Expected Outcomes, Potential Pitfalls and Alternative Approach**

We do not expect any major difficulties with our experimental approach. The investigators are well versed with the described methodologies. We recognize that with the projected sample size, and the published rates of AKI after indomethacin therapy that the absolute number of infants developing Stage 1 or higher AKI will likely be low. The lack of sensitivity and specificity of the current definitions of AKI have been well acknowledged. However, we anticipate that the incidence of AKI, as determined by significant increases in levels of urinary biomarkers will be much higher than that identified by SCr values and/or changes in urine output. The investigation of novel biomarkers will provide us with the opportunity to contribute to the discussion regarding new and refined definitions of neonatal AKI with important clinical implications. We further anticipate that fenoldopam with preserve urine output, obviating the need to reduce fluid intake, and thus nutritional intake. Additionally, we expect fenoldopam to increase the success rate of closure of PDA with indomethacin, as preservation of renal function will allow for completion of the 3-dose course of indomethacin, rather than interruption due to renal concerns. Development of hypotension may lead investigators/clinicians to suspect treatment with fenoldopam, though hemodynamic changes have not been reported at the doses used. The clinical team has the option to stop infusion of the investigative drug at any point.

**G. Recruitment Strategy**

Our team of research nurses is in constant communication with the NICU care team and will be informed when the decision is made to treat an infant diagnosed with a PDA with indomethacin. They will screen patients and present to the parents the details of the study and obtain informed consent. The extensive experience of the nurses results in effective communication about protocols and success in recruitment. Our team is highly successful in recruiting subjects for clinical trials in our NICU. Based on this extensive experience and absence

of invasive procedures in this study, we anticipate a consent rate of approximately 70% of eligible infants and an enrollment period of approximately 18 months. The dropout rate is likely to be minimal given the short duration of the study and lack of anticipated side effects from the intervention.

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