ICF, PROTOCOL, AND STATISTICAL ANALYSIS PLAN

Study Title: Effects of Delayed Cord Clamp and/or Indomethacin on Preterm Infant Brain Injury

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Combined Consent and Authorization to
Participate in a Research Study

Delayed Umbilical Cord Clamping and Indomethacin in Preterm Infants

When we say "you/your" we are referring to you as an expecting mother and your baby.

WHY ARE YOU BEING INVITED TO TAKE PART IN THIS RESEARCH?

You are being invited to take part in a research study about possible brain injury that often happens in babies that are born prematurely. You are being invited to participate because it is likely that your baby is going to be born early or prematurely.

Infants born early, especially born at less than 29 weeks of gestational age, are at a high risk for various medical problems, including bleeding in the brain. It is possible, but unproven, that a short delay (of 45 seconds) in clamping the umbilical cord at the time a baby is born, may reduce the risks of this brain bleeding. It is also possible that a medicine called 'indomethacin' could be effective in reducing brain bleeding, either alone or in combination with a delayed umbilical cord clamp. This research study will investigate the potential benefits of delayed cord clamp, indomethacin, or their combined use to reduce how often or how severe brain bleeding occurs in babies born prematurely.

If you volunteer to take part in this study, you will be one of more than 400 people to do so at the Kentucky Children's Hospital, the University of Kentucky. If you volunteer to take part in this study your baby may be enrolled in our research study, but only if it is born before 29 weeks gestational age.

WHO IS DOING THE STUDY?

The person in charge of this study is Peter J. Giannone, MD, Division of Neonatology, Department of Pediatrics, at Kentucky Children's Hospital. Other members of the Neonatology Research Team will assist at different times during the study.

WHAT IS THE PURPOSE OF THIS STUDY?

Babies born prematurely are at risk of bleeding into the fluid spaces of the brain, called the ventricles and the tissue around the ventricles, known as the parenchymal white matter, in the first few days of life. If major bleeding in the brain occurs a baby may end up with long term problems like low IQ and cerebral palsy. Neonatologists, or doctors that take care of sick babies, have been trying to prevent the bleeding in the brain problem for many years, but so far there is no known way to prevent the bleeding. **This is a research study in which we are trying to find the best and safest way to prevent bleeding in premature babies' brains to prevent brain damage.**

One possible way to reduce brain bleeding is to wait before cutting a premature baby's umbilical cord after birth. Studies have shown that waiting 45 seconds to cut the umbilical cord may be helpful to prevent brain bleeds. No studies have shown that waiting to cut the umbilical cord has any negative effects on the baby. The obstetrician (doctor) delivering your baby will either: 1) cut the cord immediately following delivery and hand the baby over to the pediatricians to stabilize --- immediate cord clamping; or 2) wait 45 seconds before cutting the cord, then hand the baby over to the pediatricians --- delayed cord clamping.

Another possible way to reduce brain bleeding is by giving the baby a medicine called indomethacin. This medicine has been approved by the Food and Drug Administration and used for many years in premature babies for other reasons, but not to prevent brain bleeds. Studies have shown that this medicine can reduce the risk of premature babies bleeding in their brains but does have side effects and risks. Most of these side effects are temporary, such as renal failure, but can be severe, such as a rupture of the intestine where a hole forms in the bowel. By doing this study, we hope to learn if either delayed cord clamping by itself, indomethacin by itself or both together is the best and safest way to prevent bleeding in premature babies' brains.

ARE THERE REASONS WHY YOU SHOULD NOT TAKE PART IN THIS STUDY?

If you or your baby has one of the following situations, you should not take part in this study.

- Any congenital anomalies (or birth defects) of newborn;

- You are not planning to have your baby resuscitated but have comfort care and hold your baby;
- Problems with the placenta that may cause bleeding
- If the obstetrician identifies complications in the mom (such as breathing problems, other);
- If the obstetrician identifies complications in the baby (such as decrease in heart rate, other)

WHERE IS THE STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST?

The research procedures will be conducted at the University of Kentucky Medical Center, Kentucky Children's Hospital. Your baby will be admitted to the Neonatal Intensive Care Unit (NICU) right after delivery. The research procedures (blood draws, head ultrasounds, head MRI) will be conducted while your baby is in the NICU. After your baby goes home, you will need to bring your baby to the NICU Graduate Clinic at 3 different times (at about 3 months, 6-9 months, and 18-24 months of corrected gestational age) for neurodevelopmental assessments. Corrected gestational age means the age your baby would have been if he or she wasn't early. This is a standard procedure for all premature babies at UK. Each of those visits will take about 2 hours. The total amount of time you will be asked to volunteer for this study is approximately 12hrs over the next two years.

WHAT WILL YOU BE ASKED TO DO?

If you volunteer to participate in this research, the study will start when your baby is actually born. The Labor Hall staff will record the number of seconds between the baby's birth and the time till the umbilical cord is cut. After that, the baby will receive normal resuscitation and be taken to the NICU.

After being admitted to the NICU, your baby will be randomized to receive indomethacin or a placebo. Randomize means that your baby's group will be picked by chance, like a coin toss. The indomethacin and placebo will look alike, and the medical staff taking care of your baby won't know if there is medicine or not. You would have a 50% chance of being in any group. Your baby will receive the same NICU care, no matter which treatment he or she receives.

In the delivery room, 1 to 5 mL (1/5 - 1 teaspoon) of blood will be kept from the umbilical cord after the baby is born. This will be collected by the obstetrical nurse following the delivery of the infant, then immediately given to the study team (Dr. Giannone or other member of the study team). The blood will be used for research tests. Once the baby is in the NICU, the medical team will place IV's that will help give the baby nutrition and help with monitoring of various vital signs (heart rate, blood pressure). Special IV's will also be placed which blood can be taken from. These IV's are standard or normal care in the NICU for premature babies because blood tests are done several times a day in the beginning, then weekly. In this study, we will obtain half a milliliter (mL) or 1/10 of a teaspoon of blood from your baby at different times for research tests. The blood will be drawn from a special IV OR during a routine heel-stick. As premature babies get better, the special IV's are removed and blood is drawn from the heel. The study blood tests will occur at 7 times: 1) on admission, 2) 24 hours old, 3) 48 hours old, 4) 72 hours old, 5) 1 week old, 6) 1 month old, and 7) 2 months old. The total blood used for research is 3.7 mL, or less than 1 teaspoon.

Head ultrasounds (HUS) are done in the NICU on every baby less than 29 weeks gestational age. Ultrasound is a type of x-ray using sound waves – a wand, or probe is used on the skin to see inside the body. This test has been used for many years, safely, even used during pregnancy to see the baby. The HUS is used to see if a baby has any bleeding in the brain, which occurs in about half of the babies less than 29 weeks. In a head ultrasound, the wand is put on the soft spot (top of a baby's head), and can detect even a small amount of bleeding. Usually babies have two or more HUS, (depending on how sick they are) which are normally done on day 7 and day 30. In this study, your baby will have 4 ultrasounds, on days 3, 7, 14 and 30. By doing the ultrasound earlier (day 3), brain bleeds may be detected earlier. Your baby will also have a head MRI which will be performed at 38 weeks corrected gestational age.

After a premature baby is taken to the NICU, he or she is watched very closely, and is attached to monitors. One of the monitors is a pulse oximeter (attached by a soft sticky fabric is placed around the hand or foot of the baby, with a red light that shines). This is used to measure oxygen in the body. This monitor is used for most of every baby's stay in the NICU. For this study, it will be used until your baby goes home, and he or she may have 2 monitors. Babies in this study will have a "sleep study" performed at 2 different times (one and two months old), if he or she is stable. This will measure the oxygen levels and how well the baby is breathing at rest.

All premature infants less than 29 weeks are routinely followed in the NICU Graduate Clinic after discharge. Your baby will receive the standard testing that is done for all babies in the Graduate Clinic.

Timeline	Procedure	Basic Care	Research
at delivery	umbilical cord blood collection (1-5mL)		X
1, 24, 48, & 72 hours, 7, 30 & 60days	blood draw (0.5mL) at each time point (total less than 1 tsp all together	X	X
72 hours or 3 days	head ultrasound		X
7 days, 14 days, & 30 days	head ultrasound	X	X
38 weeks of corrected GA	head MRI		X
30 & 60 days	Sleep Study		X

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

Most of your information is in the medical record (both you and your baby's). If more information is needed, you may be asked questions about your pregnancy, and it is possible that you could feel upset. If you don't want to answer a question, you don't have to, and the study coordinator will be available to discuss this with you further.

There have not been any studies to show that a delay of cutting the cord has hurt a baby in any way. One possible problem might be the baby could get cold. Even without the delay, some babies get cold after being born because they're so small. The medical team at the delivery will use warm blankets, a plastic bag, and a warm bed to prevent your baby from getting cold. Another potential risk is that the bilirubin levels (the chemical that makes babies turn yellow) is increased following a delay in cutting the umbilical cord due to increased number of red blood cells (about a 30% increased chance). Almost all premature babies get high bilirubin levels because of immature liver function. Babies are treated with special lights – usually in the first week of life.

Indomethacin is a drug approved by the FDA (Food and Drug Administration) and given to babies born prematurely to close PDA (a blood vessel in the heart) as well as other reasons. This medication has been shown to reduce brain bleeding in babies born very prematurely, but it has some side effects. The side effects including 1) the amount of urine (pee) becoming low, 2) damage to the intestine, 3) bleeding problems, 4) low blood sugar, and 5) low salt and potassium levels. These side effects happen only while the baby is receiving the indomethacin, and go away when the drug is done. If the problems are serious, the medicine will be stopped. There is no way to give absolute assurance either that your baby will or will not experience any of these or other side effects. Your doctors will watch all babies in the Neonatal Intensive Care Unit closely for all problems.

Placing IV lines in a vein may cause soreness, bruising, pain, possible fainting, or bleeding at the puncture site. This is a routine requirement for babies in the NICU, for the purpose of collecting blood and making measurements that are used to make medical care decisions. Infection is a rare possibility. In this study, we will only obtain blood from an existing IV site or during a heel stick for routine labs.

Being in the group that does not get DCC or indomethacin may have higher risk of bleeding in the brain than the other groups. This, however, is currently the standard care of treating preterm babies and the reason this research is being done.

There is always a chance that any medical treatment can harm you, and the investigational treatment in this study is no different. In addition to the risks listed above, you may experience a previously unknown risk or side effect. If you are worried about anything while in this study, please contact the Principal Investigators or the study coordinator.

WILL YOU OR YOUR BABY BENEFIT FROM TAKING PART IN THIS STUDY?

It is possible, but not guaranteed, that you or your baby will get benefit from taking part in this study. We hope to learn something that could help premature babies. At the present time, we do not know the best and safest way to prevent bleeding in the baby's brain. We are trying to figure out if the timing of the umbilical cord clamping is related to changes in blood cell levels in the babies, and if those changes help prevent bleeding in the brain that babies born too early are at risk for developing, if the medicine indomethacin is better at preventing brain bleeding or both together are the best and safest way to prevent brain bleeds in babies born early. The data collected from this study will be compared to see what might work best for reducing brain bleeding. Your willingness to take part may, in the future, help doctors better understand and/or treat others who have your condition.

DO YOU HAVE TO TAKE PART IN THE STUDY?

If you decide to take part in the study, it should be because you really want to volunteer. You will not lose any benefits or rights you would normally have if you choose not to volunteer. You can stop

at any time during the study and still keep the benefits and rights you had before volunteering. If you decide not to take part in this study, your decision will have no effect on the quality of medical care you receive.

IF YOU DON'T WANT TO TAKE PART IN THE STUDY, ARE THERE OTHER CHOICES?

If you do not want to be in the study, there are no other choices except not to take part.

WHAT WILL IT COST YOU TO PARTICIPATE?

You and/or your insurance company, Medicare or Medicaid will be responsible for the costs of all care and treatment you receive during this study that you would normally receive for your condition. These are costs that are considered medically reasonable and necessary and will be part of the care you receive if you do not take part in this study. The tests and procedures that are done just for research purposes are paid by the study.

WHO WILL SEE THE INFORMATION THAT YOU GIVE?

Only the Principal Investigators and the Neonatology Research Team will have access to the information provided and we will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. You should know, however, that there are some circumstances in which we may have to show your information to other people. For example, the law may require us to show your information to a court or to tell authorities if you report information about a child being abused or if you pose a danger to yourself or someone else.

In the event of any publication regarding this study, the identity of you or your baby will never be revealed. Your information will be combined with information from other people taking part in the study. When we write about the study to share it with other researchers, we will write about the combined information we have gathered from the many babies enrolled in the study. You will not be personally identified in these written materials. We may publish the results of this study; however, we will keep your name and other identifying information private.

Reason(s) why the use or disclosure is being made: We will need to contact you in the future, need to locate medical records and lab values, and may need to contact your physician if there are dangerously abnormal results found in this study. You may decide not to authorize the use and disclosure of your PHI. However, if it is needed for this study, you will not be able to be in this study. If you agree to be in this study and later decide to withdraw your participation, you may withdraw your authorization to use your PHI. This request must be made in writing to Dr. Peter J. Giannone. If you withdraw your authorization, no new PHI may be collected and the PHI already collected may not be used unless it has already been used or is needed to complete the study analysis and reports.

Dr. Giannone and the study team will keep a database of all subjects who participate in a research study. This database may be used to contact people about future studies. Only Dr. Giannone will have access to this database. The database will not be disclosed or sold to others outside UK.

Officials at the Food and Drug Administration, the National Institutes of Health and the University of Kentucky may look at or copy pertinent portions of records that identify you.

You will need to provide your social security number. This is in order for you to be compensated for your time. If you do not provide this number, you will not be compensated. If you earn $100 or more by participating in any research, it is potentially reportable for tax purposes.

CAN TAKING PART IN THE STUDY END EARLY?

If you decide to take part in the study you still have the right to decide at any time that you no longer want to continue. You and your baby will not be treated differently if you decide to stop taking part in the study. However, if you decide to stop being in this study you must call the Principal Investigators or the study coordinator to see if there are any medical issues about stopping.

The individuals conducting the study may need to withdraw you from the study if the principal investigators believe participating in this study is not the best choice of care. This may also occur if you are not able to follow the directions they give you, if they find that your being in the study is more risk than benefit to you, or if the agency funding the study decides to stop the study early for a variety of scientific reasons.

ARE YOU PARTICIPATING OR CAN YOU PARTICIPATE IN ANOTHER RESEARCH STUDY AT THE SAME TIME AS PARTICIPATING IN THIS ONE?

You may take part in this study if you are currently involved in another research study. It is important to let the investigator/your doctor know if you are in another research study. You should also discuss with the investigators before you agree to participate in another research study while you are enrolled in this study.

It is important that health care providers know about all medicines that you are taking. This includes the medicine being tested in this research study. Because of this, we plan to tell your primary care doctor (if you have one) that you are in this research study. This is done so care can be taken in prescribing other medicines and looking at any unexplained symptoms that may occur. You cannot take part in this study if you do not want us to tell your primary care doctor.

WHAT HAPPENS IF YOU OR YOUR BABY GETS HURT OR SICK DURING THE STUDY?

We believe that there is very little chance that injuries will happen as a result of being in this study. If you believe your baby is hurt or gets sick because of something that is due to the study, you should call Dr. Peter Giannone at 1-859-323-2662 immediately. Dr. Peter Giannone will determine what type of treatment, if any, that is best for your baby at that time.

It is important for you to understand that the University of Kentucky does not have funds set aside to pay for the cost of any care or treatment that might be necessary because you get hurt or sick while taking part in this study. Also, the University of Kentucky will not pay for any wages you may lose if you are harmed by this study. In most cases, this care will be billed to your health insurance company or whoever usually pays for your health care at the usual charges, but some insurance companies will not pay for care related to a study. If the care is provided at Kentucky Children's Hospital, we make no commitment to pay for the medical care provided to you. If no one else pays for your care, you may have to pay for the cost of this care. This does not mean that you give up any of your legal rights to seek compensation for your injuries by signing this form. A co-payment/deductible from you may be required by your insurer or Medicare/Medicaid even if your insurer or Medicare/Medicaid has agreed to pay the costs). The amount of this co-payment/deductible may be substantial.

WILL YOU RECEIVE ANY REWARDS FOR TAKING PART IN THIS STUDY?

You will not be paid to be in this study at admission. However, we will provide $25 food and travel stipends at each post-discharge NICU Graduate Clinic visit (at 3-4, 6-9, and 18-24 months corrected age; these are the standard follow-up times in our hospital). In addition, your will receive gift certificates ($25) if you contact the study team with address or phone number change.

WHAT IF YOU HAVE QUESTIONS, SUGGESTIONS, CONCERNS, OR COMPLAINTS?

Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have questions, suggestions, concerns, or complaints about the study, you can contact the investigators, Dr. Peter Giannone at 1-859-323-2662 or Ms. Vicki Whitehead at 1-859-323-5530. If you have any questions about your rights as a volunteer in this research, contact the staff in the Office of Research Integrity at the University of Kentucky between the business hours of 8am and 5pm EST, Mon-Fri at 1-859-257-9428 or toll free at 1-866-400-9428. We will give you a signed copy of this consent form to take with you.

WHAT IF NEW INFORMATION IS LEARNED DURING THE STUDY THAT MIGHT AFFECT YOUR DECISION TO PARTICIPATE?

If the researcher learns of new information in regards to this study, and it might change your willingness to stay in this study, the information will be provided to you. You may be asked to sign a new informed consent form if the information is provided to you after you have joined the study.

POTENTIAL FUTURE USE

The Neonatology Research Team would like to keep the leftover blood collected during the main study participation but is not used for other tests for that study. No additional blood or tissue will be taken. If you agree, the blood samples may be used in future research, which could help other babies and their families in the future.

Researchers may also need health information about the people who provide specimens. We are also asking for your consent to place information from your medical record and/or research record in a database to be used for research. Your name and address will not be placed in the database.

Please read each sentence below and think about your choices. After reading each sentence, mark “yes” or “no.” If you have questions, please talk to the investigator or staff. Remember, no matter

what you decide to do about the storage, or banking, and future use of your blood samples, you may still take part in the main study. If you answer yes to either choice below you also give your authorization for your accompanying health information to be used and disclosed along with the blood. The sample(s) (blood) that you or your baby are giving will no longer belong to you and might be used in studies that lead to new products for research, diagnosis or treatment.

1. Do you give permission for your blood samples to be kept by Dr. Peter Giannone's study team in a central location/specimen bank at Kentucky Children's Hospital (MS472) until they are used up but no longer than 10 years for use in future research to learn more about how to prevent, detect, or treat brain bleeding in babies?

   ☐ Yes ☐ No _____Initials

2. Do you give permission for your blood samples to be used for future research about other health problems, for example, causes of preterm delivery?

   ☐ Yes ☐ No _____Initials

Genetics research and banking:

In addition to the main study, you are being asked to volunteer in a genetic sub-study to study your genes, or DNA (deoxyribonucleic acid). Genes are made up of DNA. Your DNA is like a huge database of chemical bases that carry the "blue prints" or instructions to tell each and every cell in your body what they should do. Genes can influence the likelihood that you will get certain diseases. Your participation in this sub-study is optional. You can still be in the main study even if you do not wish to participate in this sub-study.

Please read each sentence below and think about your choice. After reading each sentence, mark "yes" or "no." If you have questions, please talk to the investigator or staff. Remember, no matter what you decide to do about the storage, or banking, and future use of your DNA samples, you may still take part in the main study.

You give your permission for your DNA to be stored in a central location/DNA bank at Kentucky Children's Hospital for future use by the study investigators. We plan to store (or bank) the DNA samples until they are used up but they will not be kept longer than 7 years. You give authorization for your accompanying health information to be used and disclosed as marked below:

1. Do you give permission for your DNA samples to be kept by Dr. Peter Giannone's study team for use in future research to learn more about how to prevent, detect, or treat brain bleeding in babies?

   ☐ Yes ☐ No _____Initials

2. Do you give permission for your DNA samples to be used for future research about other health problems, for example, causes of preterm delivery?

   ☐ Yes ☐ No _____Initials

Contacting Research Subjects for Future Studies

Do you give your permission to be contacted in the future by the Neonatology Research Team regarding your willingness to participate in future research studies about how to prevent, detect, or treat brain bleeding and related health issues?

☐ Yes ☐ No _____Initials

WHAT ELSE DO YOU NEED TO KNOW?

There is a possibility that the data/specimens collected from you and your baby may be shared with other investigators in the future. If that is the case the data/specimen will not contain information that can identify you unless you give your consent/authorization or the UK Institutional Review Board (IRB) approves the research. The IRB is a committee that reviews ethical issues, according to federal, state and local regulations on research with human subjects, to make sure the study complies with these before approval of a research study is issued. NIH is providing financial support and/or material for this study.

AUTHORIZATION TO USE OR DISCLOSE YOUR IDENTIFIABLE HEALTH INFORMATION

Information collected for this study may include information that can identify you or your baby. This is called "protected health information" or PHI. By agreeing to be in this study, you are giving permission to Dr. Peter J. Giannone and the study team to collect use and disclose your PHI for this research study unless otherwise allowed by applicable laws. Information collected is the property of the Principal Investigators and NIH (sponsor). The reason why this PHI is collected, and what information will be used is listed below. The PHI will only be shared with the groups listed, but if you have a bad outcome or adverse event from being in this study, the Principal Investigators and staff or other health care providers may need to look at your entire medical records.

The privacy law, HIPAA (Health Insurance Portability and Accountability Act), requires researchers to protect your health information. The following sections of the form describe how researchers may use your health information.

Your health information that may be accessed, used and/or released includes:

- Names (individual, relatives); Address (including city, state, zip code and county); Telephone/Fax Numbers; Birth Date; Admission Date; Discharge Date; Date of Death; E-mail Addresses/URLs; Medical Record Numbers; social security number and a unique study identifying code.
- Your age, how many pregnancies you've had, your race, treatments, medicines and tests during and after this pregnancy
- Your baby's information which will include dates: birth data, results from tests, x-rays, ultrasounds, labs, treatments, complications and diagnoses

The Researchers may use and share your health information with:

- The University of Kentucky's Institutional Review Board/Office of Research Integrity.
- Law enforcement agencies when required by law.
- University of Kentucky representatives.
- UK Hospital
- Sponsor (NIH)
- Information Drug Services (IDS)
- Food and Drug Administration (FDA)
- Primary physician will be contacted if researcher in the course of the project learns of a medical condition that needs immediate attention.

The researchers agree to only share your health information with the people listed in this document. Should your health information be released to anyone that is not regulated by the privacy law, your health information may be shared with others without your permission; however, the use of your health information would still be regulated by applicable federal and state laws.

You will not be allowed to participate in the research study if you do not sign this form. If you decide not to sign the form, it will not affect your:

- **Current or future healthcare at the University of Kentucky**
- **Current or future payments to the University of Kentucky**
- **Ability to enroll in any health plans (if applicable)**
- **Eligibility for benefits (if applicable)**

After signing the form, you can change your mind and NOT let the researcher(s) release or use your health information (revoke the Authorization). If you revoke the authorization:

- You will send a written letter to: Dr. Peter Giannone at 800 Rose Street, MN470, Lexington, KY40536 to inform the study team of your decision.
- Researchers may use and release your health information **already** collected for this research study.
- Your protected health information may still be used and released should you have a bad reaction (adverse event).
- You may not be allowed to participate in the study.

You understand that you will not be allowed to review the information collected for this research study until after the study is completed. When the study is over, you will have the right to access the information.

The use and sharing of your information has no time limit.
If you have not already received a copy of the Privacy Notice, you may request one. If you have any questions about your privacy rights, you should contact the University of Kentucky's Privacy Officer between the business hours of 8am and 5pm EST, Mon-Fri at: (859) 323-1184.

You are the subject or are authorized to act on behalf of the subject. You have read this information, and you will receive a copy of this form after it is signed.

1-MOTHER'S CONSENT (REQUIRED):

_____ _____
Signature of research subject's legal representative (Mother) Date

_____ _____
Printed name of research subject's legal representative (Mother) Representative's relationship to research subject

MOTHER'S CURRENT AGE: _____
Note: Assent from legal guardian required if mother is 14-17 yrs of age

2-FATHER'S CONSENT (REQUIRED IF THIS PERSON IS REASONABLY AVAILABLE):

_____ _____
Signature of research subject's Father Date

IF THE FATHER'S SIGNATURE IS NOT INCLUDED AND HE IS 'NOT REASONABLY AVAILABLE' DEFINE THE REASON(S) BELOW:

☐ Unknown
☐ Incompetent
☐ Deceased
☐ Only the mother has legal responsibility for the care and custody of the child
☐ Other reason (note provided here)_____
_____
_____
_____

3-MOTHER'S LEGAL GUARDIAN (REQUIRED IF ENROLLED MOTHER IS 14-17 YRS OF AGE)

_____ _____
Signature of mother's legal guardian Date

_____ _____
Printed name of mother's legal guardian Representative's relationship to mother

4-AUTHORIZED STUDY REPRESENTATIVE (REQUIRED)

_____ _____
Name of [authorized] person obtaining Informed consent/HIPAA authorization Date

_____
Signature of Investigator

Study Protocol:

1. **Objectives:**

Primary GOAL: Compare efficacy and safety of prophylactic indomethacin, DCC, and their combination, in affecting the incidence and severity of IVH in infants less than 29wks GA at birth.

- **Aim 1A):** Test for differences in primary outcome measure: 'fraction of survivors with no severe IVH or PVL' among the 4 groups, using head ultrasound (1-30d), and head MRI at 38wks adjusted GA.
- **Aim 1B):** Test the hypothesis that DCC improves effectiveness and/or decreases toxicities of indomethacin.
- **Aim 1C):** Test for treatment dependent differences in neurocognitive follow up evaluations and investigate relationships between short term brain imaging results and later neurocognitive performance across treatment groups.

Secondary GOAL: Investigate mechanistic effects of prophylactic indomethacin, DCC, and their combination in infants less than 29wks GA.

- **Aim 2A):** Test the hypotheses that blood volume status and/or hemodynamic stability are different between groups (at 0,1,2,3,7, 30, and 60 days of life).
- **Aim 2B):** Test the hypothesis that biomarkers of inflammation and vascular endothelial stress are different between groups (at 0,1,2,3,7, 30, and 60 days of life).
- **Aim 2C):** Test the hypothesis that numbers, or apoptotic frequency, of circulating progenitor cells with endothelial or neuronal phenotype capacity are different between groups (at 0,1,2,3,7, 30 and 60 days of life).
- **Aim 3):** Determine relationships between clinical outcomes (Aim1) and blood measurements (Aim 2) among treatment groups.

2. **Study Design:**

The study design of this research project is a randomized, blinded investigation of 4 treatment groups:

1) Control (no intervention);

2) DCC alone;

3) Prophylactic indomethacin alone;

4) Combination of DCC/indomethacin

Infants randomized to block randomization with blocks of size 8 each to randomly assign the subjects into one of the 4 groups. The randomization table will be prepared in advance by the statistician and clinicians will be blinded to this choice prior to the delivery. Investigational Drug Services will provide indomethacin dosing solutions and placebo dosing solutions that are matched in appearance and volume for each study participant. Please note that randomization will occur in all cases, but the attending Neonatologist at delivery and bedside will have authority to define care; if this physician has an objection to the randomization of indomethacin treatment for a specific patient that patient will be excluded from the study. Similar provisions are afforded to the delivering Obstetrician regarding delay of umbilical cord clamp randomization and treatment.

3. **Study Population:**

It is anticipated that this study will begin July, 2014 and will enroll subjects through June 31 of 2019. Follow-up in NICU Graduate Clinic will continue thru June 31, 2020. We are going to enroll 400 infants during that time, male and female all inborn at the University of Kentucky. The population is primarily Caucasian (80%), African American (15%), Asian (3%), and a Hispanic population of about 2%. All races and ethnicities will be included if subjects parent(s) are able to understand English (spoken or written). Pregnant women are considered to also be research subjects in this trial. When the pregnant mother is a

legal adult (18yrs or older) their consent will include their participation as well as their infant. Pregnant women whom are less than 18yrs and greater than 14yrs of age and qualify for enrollment criteria will also be invited to participate in this study. In these cases assent will be obtained from the pregnant mother (who is considered a legal minor), and consent will be obtained from the pregnant mother's legal guardian.

Inclusion criteria

- Neonates with a GA less than 29 6/7weeks (based on last menstrual period and/or early pregnancy ultrasound)
- Consent obtained prior to delivery

Exclusion criteria

- neonates with congenital anomalies such as congenital heart disease
- a prenatal plan to withhold neonatal care
- maternal risks identified by obstetrician, including severe hemorrhage (placenta abruption or placental previa), hypertensive crisis
- if the attending obstetrician or neonatologist decides that infant requires immediate resuscitation for either maternal or newborn well-being
- any prenatal indocin use within 72 hours before birth

4. Subject Recruitment Methods and Privacy: **Recruitment:**

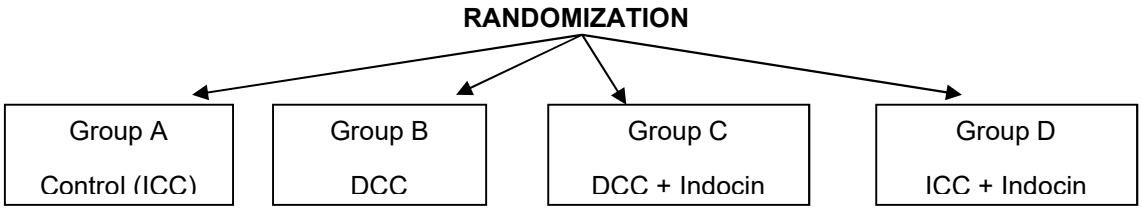
A total of 400 premature infants born <29 6/7weeks GA will be recruited from the obstetrics admission area and randomized to one of the 4 treatment groups; n=100 each will complete the study (control; DCC alone; Indomethacin alone; DCC + Indomethacin). This facility admits over 800 sick newborn infants/yr and sees a majority of the high-risk obstetrical patients in the Lexington area and beyond. Informed consent will be obtained prior to patient enrollment, by Dr. Giannone (PI) or another member of the research team experienced at consenting mothers for research studies. There will also be an on-call schedule at night to screen potentially eligible women, enroll them, and attend the births. Women enrolled will be assigned to either immediate or delayed cord clamping groups via a computer generated random number system to assure permuted block randomization. When notified of a subject's impending birth, the research coordinator will open the randomization card, review the protocol with the attending/fellow/or resident obstetrician, and time the cord clamping.

5. Informed Consent Process:

We will consent mothers who are in preterm labor. After consent forms are reviewed, the families will be asked to participate in the study. Consent will be obtained from the parent/legal guardian by a research nurse or the PI/designee trained in the informed consent process. All conversations related to patient participation/research procedures will be performed in private locations.

6. Research Procedures:

Following enrollment and consent at time of delivery the infant will be placed in one of four groups (per the following algorithm)



For the immediate cord clamping groups the obstetrician will clamp the umbilical cord immediately (<10sec) after birth, per standard procedure. For the delayed cord clamping groups the obstetrician will clamp the cord at 45 sec after delivery and hold the infant in a sterile bag (thermoregulatory bag) 10-15in below the mother's introitus at vaginal delivery or at/below the level of incision if a cesarean section is performed. A stopwatch will identify the time when the infants' buttocks are delivered from the vagina or the

uterus (or head if breech); time elapsed will be counted in 10sec intervals for the OB by the investigator. Infants in the DCC group will be placed into a sterile bag to minimize heat loss at the sterile field by the delivery OB. Per standard NICU clinical protocol, infants in the ICC groups will be placed in the thermoregulatory bag once they are placed on the neonatal warming bed.

Infants will be brought to NICU once the infant is determined stable by the neonatal resuscitation team. The enrolled infant will then be cared for in the NICU using standard practices and started on either Indocin 0.1 mg/kg/day for 3 days (Groups C and D) or placebo (Groups A and B), depending on group assignment. Randomization of indomethacin is "blinded" to medical care team (physicians, nurses, clinic personnel). Maternal and infant clinical data and infant blood samples will be collected at specified times for measurement of mechanism-based endpoints. In addition to routine assessment of renal function via standard clinical meaures of BUN and creatinine, renal function status will also be monitored by serum Cystatin C levels (0.1mL) at 7 and 30 DOL (these will be considered research measures, since they are not routinely measured in NICU patients). Measurements will be done with ELISA (50uL serum required) at Bauer's research lab. The oxygen saturation (SpO2) of all infants is continuously monitored upon admission to the NICU by a Masimo pulse-oximeter. Monitoring starts right after birth until discharge from the hospital. The SpO2 and heart rate waveforms stored in the Masimo pulse-oximeter will be downloaded for analysis. Some NICU beds have a different pulse oximeter (Nelcore) which does not allow for data downloads. If a patient is admitted to bedspace not equipped with a Masimo pulse oximeter, one will be placed but screen and alarms turned off. This is a minimal risk procedure and of no additional cost to the patient for data consistency purposes. A neonatal sleep study/pneumogram (respiratory inductance plethysmography, high-resolution pulse-oximetry and electrocardiography) will be performed on all subjects who are not intubated or on non-invasive ventilation at 30 and 60 days of life. In case infants remain intubated or on non-invasive respiratory support at 60 (55 – 65) DOL, a pneumogram will be performed as soon as that level of respiratory support is weaned or discontinued. The pneumogram is non-invasive and is of minimal risk to patients. All data collected will be measured/recorded by persons who are essentially 'blinded' to the delivery/treatment at birth.

If for any medical reason the bedside/attending Obstetrician or Neonatologist has concerns and wishes to become 'unblinded' to the infant's enrollment, the study participant will be removed from the study. We expect that the only practitioner who is unblinded in the study is the specific Neonatologist at the particular infants' delivery, who most likely is not performing the daily care for the newborn after admission based on the structure of our delivery room teams and NICU service teams. All study infants will routinely be followed-up at UK NICU Graduate Follow-up Clinic after discharge since these clinicians have the primary responsibilities for this standard clinical service in our institution. Research oversight for neurodevelopmental follow-up will be the responsibility of Dr. Sithisarn. The TIMP and Bayley Scales of Infant Development-III (BSID-III) will be administered at 3-4, 6-9, and 18-24 months corrected age; these are the standard follow-up times for collection of such data in our clinic, and the tests proposed has been optimized and defined for these age intervals. All exams will be performed by the same therapist trained in high-risk newborn neurodevelopmental exams to avoid performance biases, and will be blinded to the treatment group. Data will be reviewed quarterly by the research team. Money will be given to families ($25) for expenses (gas, parking, food) for each of the three follow-up evaluations. Frequent phone contact with project personnel has been another technique successfully used in past research by Dr. Giannone and colleagues. Project personnel will contact participants every 2 weeks until the first measurement (3-4 months), and then monthly until all measurements are complete for the follow up groups (n=400 completed). Participants will be asked to give multiple phone numbers (work, family, and friends) to make contact more feasible; participants who move or change phone numbers will receive gift certificates ($25) if they contact research personnel with their new address or phone number. Some attrition (~5%) is anticipated and is built into the sample size calculation.

In summary, a total of 3.7 ml of blood (7 time points during the 0-60 days of life, 0.5mL each, and 2 time points with 0.1ml each), 4 head ultrasound measurements (day of life (DOL) three, first week of life, DOL fourteen and at 30 DOL or later), a head MRI at 38 weeks corrected GA will be obtained from each subject as well as 3 measurements of neurodevelopment (TIMP and Bayley III). Enrolled patients will receive a study number to protect confidentiality of demographic information. Data to be collected is shown on the attached data collection instrument. Only the Co-PIs and study coordinators will know the identities

of the patients and be responsible for the storage of collected data. All data will be de-identified.

Studies shown below are marked as part of standard care or research. The 14 day ultrasound is variable, depending on findings on the 7 day ultrasound. Our standard unit policy is to repeat at 14 days if our routine 7 day ultrasound shows a significant hemorrhage.

Timeline	**Procedure**	**Standard Care**	**Research**
at delivery	umbilical cord blood collection (1-5mL)		X
At admission, 24, 48, & 72hr, Days 7, 30, & 60	blood draw (0.5mL) at each time point	X	
72 hours	head ultrasound		X
7 & 30 days	head ultrasound	X	
7 & 30 days	Serum Cystatin C (0.1mL)		X
14 days	head ultrasound	X	X
Days 30 & 60 if not on vent, or as soon as possible when extubated	neonatal sleep study/pneumogram		X
38 weeks of corrected GA*	head MRI*		X
3-4 months of corrected GA	Test of Infant Motor Performance (TIMP)	X	
6-9 months of corrected GA	Bayley III	X	
18-24 months of corrected GA	Bayley III; Brief infant Toddler Social Emotional Assessment (BITSEA), MCHAT(pass/fail)	X	

*, Please note that at the initial start of this 5 year study our plan for head MRIs in all enrolled patients is considered a research effort. Note however that the use of head MRI in extremely premature infants that are known to or suspected to have brain lesions is currently common-place and at other institutions is standard care. Therefore it is possible (and perhaps likely) that the routine use of this imaging modality will in coming years become 'standard care' for all preterm infants that are at very high risk of cranial bleeds (e.g. the exact patient group in this study) at our institution. If this becomes the case we will provide amendments to the IRB to account for this issue.

Statistical analysis plan

All aspects of data analysis/statistics will be directed by Dr. Aric Schadler, the Director of Pediatric Data Analytics for the Kentucky Children's Hospital-Office of Pediatric Research, and Dr. Arnold Stromberg, the Allen-Anderson Endowed Professor of Department of Statistics, University of Kentucky.

Aim 1: Compare efficacy and safety of prophylactic indomethacin, DCC, and their combination, in affecting the incidence and severity of IVH in infants less than 28wks gestational age.

• **Aim 1A)** Test for differences in **primary outcome measure:** 'fraction of survivors with no severe IVH or PVL' among the 4 treatment groups, using head ultrasound at1-30d and head MRI at 38wks adjusted gestational age.

• **Aim 1B)** Test the hypothesis that DCC improves effectiveness and/or decreases toxicities of indomethacin.

• **Aim 1C)** Test for treatment dependent differences in neurocognitive follow up evaluations and investigate relationships between short term brain imaging results and later neurocognitive performance across treatment groups.

Aim 1 Statistical analyses: ***[all analyses performed as 'intention to treat' where appropriate]:*** To investigate the primary hypothesis in Aim 1A (treatment-related outcome differences with respect to the primary endpoint fraction of cases surviving without severe IVH, grade 3 or 4) we will use head ultrasound data from day of life 0-30, and use two distinct approaches. First, based on the qualitative data used to compute the sample size, we will employ a bivariate analysis to identify the significant covariates, followed by a multiple logistic regression analysis to test for differences between the four groups in the presence of significant covariates. Secondly, we will develop severe IVH or PVL data and employ survival analysis statistics (log rank Mantel-Cox test). If the assumptions are validated, we will use the Cox proportional hazard model for this survival data. In addition we will compare head MRI results (measured at the single time point of 38wks adjusted gestational age using a multiple regression model that will incorporate significant covariates. Aim 1B is concerned with comparison of Groups 3 and 4 only (e.g. Indomethacin vs. Indo-DCC) and the procedures developed for the 4 group analysis are also applicable here. For Aim 1C we correlate later performance with the short term brain imaging results in the framework of a multiple regression model where the 4 groups will be used as predictors and will be compared. In all settings we will also perform separate analyses of IVH alone, and PVL alone.

Aim 1 also involves comparisons of nominal/ordinal and continuous variables associated with neurodevelopment and toxicity where single or repeated measures on the response are available. Approaches similar to the one described above will be taken where we first build a statistically valid model that controls for all influential covariates and test the relevant hypotheses in the context of the model. Aim 1B corresponds to the testing of the interaction effect between the ICC/DCC effect and the use of prophylactic indomethacin. Other hypotheses correspond to the main effects. We will also compare the variables that reflect renal function/status (as assessments of potential indomethacin toxicities, other specific comorbidities and hospital stay related variables across the 4 groups.

Aim 2: Investigate mechanistic effects of prophylactic indomethacin, DCC, and their combination in infants less than 28wks gestational age.

• **Aim 2A)** Test the hypotheses that blood volume status and/or hemodynamic stability are different between groups (at 0,1,3,7,and 30 days of life).

• **Aim 2B)** Test the hypothesis that biomarkers of inflammation and vascular endothelial stress are different between groups (at 0,1,3,7,and 30 days of life).
• **Aim 2C)** Test the hypothesis that numbers, or apoptotic frequency, of circulating progenitor cells with endothelial or neuronal phenotype capacity are different between groups (at 0,1,3,7,and 30 days of life).

Aim 2 Statistical analyses: ***Comparisons of biochemical and hemodynamic variables across treatments:*** For Aim 2 we compare the blood sample variables (Hct, HB, Ferritin, CRP, IL6, TNF, s-ICAM, progenitor cell numbers, and apoptotic cell percentages) and summary measures of cardiovascular variables (blood pressure, pressor support and others) using a multivariate linear regression model (for continuous responses) and a multivariate logistic regression model (for nominal variables such as the need for inotropic/vasopressor support) regression analyses where influential demographic variables associated with mothers and infants will be used as covariates. The differences in the pattern of changes over time of the repeatedly measured cardiovascular status variables such as the blood pressure levels will be examined using repeated measures Analysis of Variance techniques and mixed effect ANOVA models. Influential covariates will be used for building a suitable predictive model using variable selection methods to identify the optimal variable set. Using this model, the hypotheses regarding the differences between the 4 groups and their interaction effect with time will be examined.

Aim 3: Determine relationships between clinical outcome measures (Aim1) and blood measurements (Aim 2) among treatment groups.

Aim 3 Statistical analyses: We will use our complete data set to investigate predictors of brain injury (IVH, or PVL), predictors of poor neurocognitive outcomes and the influence of treatment group on these predictive variables. Ultimately we will use the data collected in this project to identify which of our measured variables are the most 'useful' from a clinical perspective in forecasting outcomes in these high-risk patients. Significant variables for prediction will be identified using bivariate methods. Correlations will be utilized for two continuous variables. ANOVA, independent samples t-tests and independent samples nonparametric methods will be employed as appropriate for a nominal explanatory variable and a continuous variable. Pearson's chi-square and Fisher's exact statistic will be used as appropriate when both variables are nominal. For each outcome variable, appropriate multivariate modeling will be constructed using the identified significant variables from the bivariate analysis along with the grouping variables to ascertain the best reduced models for prediction. An alpha level of 0.05 is used for inclusion into the models. Any non-normally distributed continuous data will be corrected through appropriate transformations. Statistical software SAS 9.4 (SAS Institute, Cary, NC) and IBM SPSS Statistics (IBM Corp., Armonk, N.Y., USA) will be used for analyses. We have powered our study using chi-square tests for equality of proportions, but adopted analysis strategy that controls for influential covariates will generally improve the power. ***In all settings we will also perform separate analyses of IVH alone, and PVL alone.***