

The Renal Anhydramnios Fetal Therapy (RAFT) Trial

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STATEMENT OF COMPLIANCE

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Johns Hopkins Institutional Review Board (IRB) for review and approval. Approval of the protocol and all consent forms must be obtained before any participant is enrolled at a RAFT study site. Any amendment to the protocol will require review and approval by the Johns Hopkins IRB before the changes are implemented to the study. In addition, all changes to a consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	The Renal Anhydramnios Fetal Therapy (RAFT) Trial
Study Description:	<p>Early pregnancy renal anhydramnios (EPRA) occurs when a fetus does not make urine and, as a result, has no amniotic fluid. This condition can occur because the fetus has no kidneys or because the fetal kidneys do not function. EPRA is thought to be universally fatal at birth because amniotic fluid is required during pregnancy for the lungs to develop and function. The aim of this study is to determine whether replacement of amniotic fluid with isotonic fluid into the uterus can reverse the effects of EPRA such that affected fetuses have enough lung function to survive outside the womb. Instillation of fluid into the amniotic cavity occurs through a technique called amnioinfusion in which a small needle is inserted into the womb next to the fetus and isotonic fluid (normal saline or lactated ringers) including an antibiotic (oxacillin 1 gram in 500 mL, nafcillin 1 gram/500 mL or clindamycin 600mg/500 mL, depending on the hospital formulary) for prophylaxis is infused. This procedure needs to be repeated several times during the pregnancy to maintain fluid in the uterus. This study aims to determine if serial amnioinfusions are a feasible, safe and effective therapy for EPRA. This research has the potential to offer realistic hope to the approximately 1,500 families per year in the United States that receive this diagnosis.</p> <p>The goal of this research is to determine the feasibility, safety and success rate of serial amnioinfusions for EPRA caused by congenital bilateral renal agenesis (CoBRA) and by fetal renal failure (FRF) with kidney tissue present. EPRA occurs in at least 1 in 3000 pregnancies, and there are no reported cases of survival without prenatal intervention. Case reports have suggested that serial amnioinfusions have the potential to lead to postnatal survival, but there have been no systematic prospective trials demonstrating this. We <u>hypothesize</u> that serial amnioinfusions of isotonic fluid in patients with EPRA from CoBRA and FRF are feasible and safe and that this therapy will lead to enhanced survival and the opportunity for successful postnatal dialysis and renal transplantation.</p>

Objectives:

Primary Objective:

To determine the proportion of neonates surviving to ≥ 14 days and placement of dialysis access.

Secondary Objectives:

- 1) To determine the feasibility of serial amnioinfusions for EPRA.
- 2) To perform an exploratory study of the in-utero natural history of untreated EPRA.
- 3) To examine correlations between prenatal ultrasound, echocardiogram, MRI, and lung specific factors in amniotic fluid as predictive biomarkers of the success of RAFT for EPRA.
- 4) To determine the short term and long-term quality of life of patients and families that undergo RAFT.

Endpoints:

Primary Endpoint:

- 1) Survival to ≥ 14 days and placement of dialysis access.

Secondary Endpoints:

- 2) The quantity of amnioinfusions and interval after the initiation of amnioinfusions before rupture of membranes
- 3) Survival to discharge
- 4) Survival to kidney transplant
- 5) PEDs QL™ Pediatric Measurement Model for the Pediatric Quality of Life Inventory Scores™ (Lyon, France)

Study Population:

The study population will consist of four cohorts of maternal/fetal pairs where the single fetus has a confirmed diagnosis of isolated EPRA. One cohort will consist of maternal/fetal pairs with EPRA secondary to CoBRA, of which 35 will receive amnioinfusions (Cohort #1) and those not electing infusions will have expectant management (Cohort #2); and one cohort will consist of maternal/fetal pairs with EPRA secondary to FRF, of which 35 will receive amnioinfusions (Cohort #3) and those not electing infusions will have expectant management (Cohort #4). Mothers will be of good general health status and over the age of 18 (inclusive).

Phase:

1

Description of Sites/Facilities Enrolling Participants:

The planned participating sites enrolling participants will all be established fetal/neonatal RAFT centers with expertise in amnioinfusions for anhydramnios and expertise in neonatal dialysis and transplantation. There will be 7-15 sites, all within the United States.

Description of Study Intervention:

The study intervention will entail access to the uterine cavity with a 20-gauge or 22-gauge spinal needle and infusion of warmed isotonic fluid including oxacillin (1 gram in 500 mL), nafcillin (1 gram/500 mL) or clindamycin (600 mg/500 mL) depending on the hospital formulary for prophylaxis, or according to the institution's standard of care practice. Replacement of fluid into the amniotic sac will allow repressurization of the fetal tracheobronchial tree and facilitate lung growth.

Study Duration:

120 months

Participant Duration: Until fetal/pediatric death or 4 years after transplant

1.2 SCHEMA

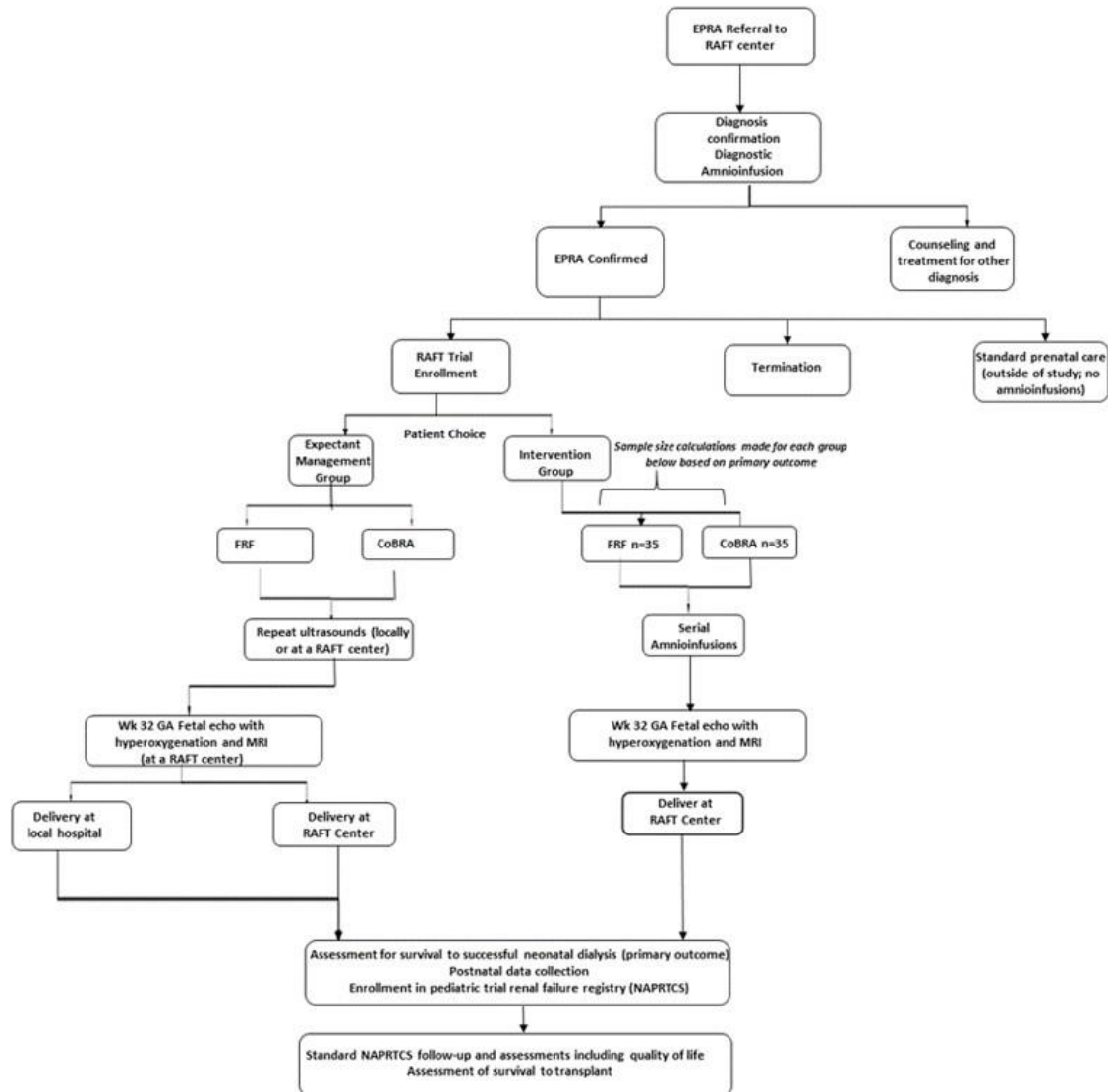


Figure 1. Experimental Design. Patients referred to a RAFT center for suspicion of EPRA will undergo counseling and diagnostic screening. If the diagnosis is confirmed, inclusion and exclusion criteria will be applied. If the parents do not wish to be part of the study, standard prenatal care will be offered. Once enrolled, participants will have the choice of the expectant management or serial amniotomies. Those in the intervention arm will be allowed to switch to expectant management if they do not wish to continue amniotomies. All participants in the intervention arm will deliver at a RAFT center. Participants in the expectant management arm will be encouraged to deliver at a RAFT center but may deliver at their local hospital. Additionally, all participants will be enrolled in North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) registry for long-term follow-up in the RAFT trial

1.3 SCHEDULE OF ACTIVITIES

Table 1. Intervention Group

	Screening Day -7 to -1	Enrollment/Baseline Up to 26 wk GA	27 wk GA	28 wk GA	29 wk GA	30 wk GA	31 wk GA	32 wk GA	33 wk GA	33 wk GA	34 wk GA	Weekly Assessments from 35 wk GA to birth	Delivery	Neonatal Period	Outpatient Up to 4 years of age	
Informed consent		X														
Demographics	X															
Medical history	X															
Patient choice of arm		X														
Administer study intervention		X as needed X ⁴														
Amniotic Fluid Collection		X	Pre- and Post each Amnioinfusion (if enough fluid is available)										X			
Concomitant medication review	X	X-----X														
Physical exam (including height and weight)	X	X			X			X			X			X		
Vital signs	X	X			X			X			X		X	X		
Height	X															
Weight	X	X		X		X		X		X		X		X		
Ultrasound	X	X	X	X	X	X	X	X	X	X	X	X		X ⁵		
Fetal MRI ¹	X							X								
Fetal echocardiogram ²	X	-														
Fetal echocardiogram with hyperoxygenation test ³								X								
Confirmation of diagnosis		X												X		
Genetic testing	X	-														
Adverse event review and evaluation		X-----X												X		
Meeting with required consultants	X	-												X		
Survival to >=14 days and placement of dialysis access														X		
Survival to 90 days														X		
NAPRTCS follow-up															X	
Transplantation															X	
Complete case report forms	-	X	X	X	X	X	X	X	X	X	X	X	X	X		

1. Fetal MRI scan during screening and at about 32 weeks GA. Second MRI should occur at minimum after the 4th amnioinfusion and at the latest 33 weeks GA.
2. Fetal echocardiogram at enrollment.
3. Second echocardiogram with hyperoxia should occur at minimum after the 4th amnioinfusion and at the latest 33 weeks GA.
4. Post-procedure office visit may be requested at discretion of medical provider if this is the institution's standard of care.
5. Head ultrasound of neonates is mandatory within first 7 days of life.

Table 2. Expectant Management Group

	Screening Day -7 to -1	Enrollment/Baseline Up to 26 wk GA	27 wk GA	28 wk GA	29 wk GA	30 wk GA	31 wk GA	32 wk GA	33 wk GA	33 wk GA	34 wk GA	Weekly Assessments from 35 wk GA to birth	Delivery	Neonatal Period	Outpatient Up to 4 years of age
Informed consent		X													
Demographics	X														
Medical history	X														
Patient choice of arm		X													
Administer study intervention															
Concomitant medication review	X	X-----X													
Physical exam (including height and weight)	X	X						X						X	
Vital signs	X	X						X					X	X	
Height	X														
Weight	X	X						X						X	
Ultrasound	X							X							
Fetal MRI ¹	X							X							
Fetal echocardiogram ²	X														
Fetal echocardiogram with hyperoxygenation test ³								X							
Confirmation of diagnosis		X												X	
Genetic testing	X														
Adverse event review and evaluation		X-----X												X	
Meeting with required consultants	X													X	
Survival to >= 14 days and placement of dialysis access														X	
Survival to 90 days														X	
NAPRTCS follow-up															
Transplantation															
Complete case report forms		X						X					X	X	

1. Fetal MRI scan during screening and at about 32 weeks GA.

2. Fetal echocardiogram at enrollment.

3. Fetal echocardiogram with hyperoxia test near 32 weeks gestation.

2 INTRODUCTION

2.1 STUDY RATIONALE

The goal of this clinical trial—which represents the first ever such trial performed in humans—is to test a novel strategy for the rescue of respiratory function in children who have been diagnosed prenatally with early pregnancy renal anhydramnios. During pregnancy, specifically by 16 weeks gestation, fetal urine production becomes the primary mechanism for sustaining amniotic fluid in the uterus. This fluid is critical for lung development. Complete lack of amniotic fluid, known as anhydramnios, has been shown to lead to lethal pulmonary hypoplasia in 100% of patients if it persists from prior to 22 weeks gestation.¹ Common causes of anhydramnios by 22 weeks gestation are congenital bilateral renal agenesis (CoBRA) or early fetal renal failure (FRF). EPRA complicates more than 1 in every 3000

pregnancies.² Several case reports have demonstrated that lung function can be rescued with serial fluid infusions into the uterus (amnioinfusion).^{3,4} A case series of nine patients, albeit with highly variable renal anomaly inclusion criteria, has also demonstrated promising rescue of pulmonary function.⁵ Though treatment with amnioinfusion may improve pulmonary morbidity, renal replacement therapy, initially by dialysis and eventually by renal transplant, is obligatory in these infants. Thus, fetuses with CoBRA have two fatal defects: lung hypoplasia and renal failure. In terms of neonatal renal failure, the outlook has significantly improved over the last decade. Large studies have demonstrated that peritoneal dialysis and renal transplant are highly successful strategies in neonates with little or no kidney function.⁶⁻⁸ Accordingly, there is a need to evaluate the efficacy of serial amnioinfusion to produce life-sustaining pulmonary function specifically in the subset of EPRA patients. This can be achieved only in a prospective trial where cases are enrolled with strict entry criteria. The aim of this study is to evaluate the efficacy of serial amnioinfusion to reliably prevent pulmonary hypoplasia in patients with isolated EPRA. It is unknown if serial amnioinfusions will give neonates enough pulmonary reserve to be candidates for renal replacement therapy with acceptable levels of morbidity. We hypothesize that serial amnioinfusions of isotonic fluid including oxacillin will reliably prevent lethal pulmonary hypoplasia in fetuses diagnosed with EPRA with minimal maternal morbidity. Furthermore, we hypothesize that greater than 25% of treated patients will be candidates for dialysis and eventual transplant. This research is of critical importance to determine the efficacy of renal anhydramnios fetal therapy (RAFT) and provide guidance for patients and physicians dealing with EPRA. We will test these hypotheses in the following specific aims: (1) to determine the safety and feasibility of serial amnioinfusions in EPRA; (2) to determine survival to successful dialysis rate in EPRA; (3) to determine survival to discharge and survival to renal transplantation; and (4) to determine the quality of life for surviving RAFT and non-RAFT patients/families.

Previous studies have shown that amnioinfusions can rescue lung function in patients with oligo- or anhydramnios.⁹ These studies have been in patients with preterm premature rupture of membranes. Retrospective case reports have suggested that serial amnioinfusions may have rescued lung function in a few cases of EPRA. We propose a phase 1 prospective multicenter cohort study to assess the feasibility, safety, and efficacy of serial amnioinfusions in pregnancies complicated by EPRA.^{3,5,10} This study is innovative because not only does it seek to validate the first viable therapy in this disease that is thought to be universally fatal, but it also seeks to find predictors of survival by measuring changes in biomarkers of treated and nontreated patients over time. These biomarkers may provide mechanistic insight into the therapy and in-utero disease natural history. No trial has specifically examined the effect of amnioinfusions on patients with no amniotic fluid secondary to renal disease. Moreover, no trial has examined the effect of amnioinfusions on a uniform cohort of isolated EPRA patients with prospective assessment of EPRA origin from CoBRA or FRF with kidney tissue present. Furthermore, this study will provide the most accurate picture to date of untreated EPRA. We further seek to go beyond simple survival and uncover what the quality of life is like for these patients and their caregivers. This study is innovative because it seeks not just to determine survival with a novel therapy but to specifically address *meaningful* survival.

2.2 BACKGROUND

EPRA complicates greater than 1 in 3000 pregnancies and has been considered to be universally fatal. The two main causes of EPRA are CoBRA or FRF with kidney tissue present. Fetuses with EPRA do not make urine and develop anhydramnios by 16-18 weeks gestational age (GA) once the placenta, membranes, and umbilical cord produce minimal amniotic fluid. Persistent absence of amniotic fluid from this gestational age, in turn, leads to lethal pulmonary hypoplasia.^{11,12} Pressurized fetal lung fluid is

essential for functional lung development. Absence of amniotic fluid allows lung fluid to escape the tracheobronchial tree during normal fetal glottis opening and closing. As a result, the fetal tracheobronchial tree becomes depressurized leading to underdevelopment. This phenomenon has been seen in large animal experiments of anhydramnios as well as in other human diseases that cause oligo- or anhydramnios such as preterm premature rupture of membranes and lower urinary tract obstruction.¹² Furthermore, increasing the pressure in the tracheobronchial tree has been shown to promote lung growth with tracheal occlusion.¹³ Therefore, the causative link between amniotic fluid levels and functional lung growth is extremely strong. This link provided the rationale for amnioinfusions to be attempted in both CoBRA³ and FRF.¹⁰ In the past, some centers have implanted a port into the maternal abdomen using open surgical techniques to deliver replacement amniotic fluid for severe oligohydramnios/anhydramnios.⁵ Although this technique has promise, it is not yet ready for systemic study because of its lack of approval by the Food and Drug Administration (FDA) and maternal invasiveness. No study has systematically and prospectively looked at the efficacy of serial amnioinfusion in patients with isolated EPRA, nor has a study been done that prospectively examines the natural history of untreated EPRA in utero. This paucity of generalizable knowledge in the setting of EPRA likely stems from the fact that even if EPRA fetuses did not have to contend with lung disease, they would have to face the morbidity and mortality of having no renal function. These neonates must undergo dialysis to survive and then, ultimately, require a kidney transplant. Furthermore, reconstructive urologic surgery is necessary for CoBRA patients to permit effective micturition.

Despite these renal and urologic challenges, large studies have shown that neonates who initiate dialysis within the first month of life have an overall 3-year survival of 80% with an above 90% 3-year survival after transplant.⁶⁻⁸ Furthermore, the advent and refinement of laparoscopic donor nephrectomy has made living kidney donation a reality with little morbidity for the donor.¹⁴ Thus, it is now possible for a parent to donate a kidney to a child after 1-2 years of temporizing dialysis. Contemporary data suggests that the renal mortality of EPRA can now likely be addressed by dialysis and transplant. A note of caution is, however, warranted because the intervened upon EPRA population is likely to have a higher degree of prematurity than was reported in these recent analyses of neonatal dialysis. It remains unknown if the pulmonary mortality of EPRA in both CoBRA and FRF patients can be *reliably* overcome with serial amnioinfusions and if serial amnioinfusions in this setting are feasible and safe. In addition, there are centers that have begun offering variations of this therapy to patients outside of the context of a study and/or without strict inclusion/exclusion criteria. Two prominent ethics reviews from 2017 and 2018 respectively concluded that any therapy for EPRA should be done in the context of an IRB-approved trial or study.^{15,16} For this reason, we have obtained single-center IRB approval and the approval for this multicenter study will elucidate generalizable knowledge about EPRA. A prospective trial with strict enrollment criteria will allow us to assess the safety and feasibility of serial amnioinfusions in EPRA. Additionally, it will allow us to determine the efficacy of serial amnioinfusions on survival to successful dialysis and to characterize the quality of life for EPRA survivors and their families.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

For each amnioinfusion (total risk depends on number of amnioinfusions administered):

1. Premature preterm rupture of membranes; 2-4% for each infusion
2. Preterm labor; 2-4% for each infusion
3. Preterm delivery; 2-4% for each infusion
4. Bleeding; the expected frequency of this outcome; <1%
5. Chorioamnionitis; the expected frequency; <1%

6. Placental abruption; <1%
7. Uterine rupture; <1%
8. Amniotic fluid embolism; <1%
9. Bleeding in the absence of placental abruption; <1%
10. Local wound infection; <1%
11. Minor discomfort from amniotic fluid in the abdomen; <5%

b) Fetal Risk

1. Death. The risk of spontaneous fetal death with isolated EPRA is reported to be as high as one out of every three fetuses. It is not known if RAFT will increase or decrease this risk.
2. Fetal injury; <1%
3. Chorioamniotic separation and amniotic band syndrome; <1%
4. Procedure failure; <1%
5. Morbidity after birth – Babies born with isolated EPRA after RAFT are likely to have considerable morbidity from the extensive treatment needed after birth. Examples are chronic lung disease and pulmonary infections from prolonged mechanical ventilator support, need for peritoneal dialysis and catheter-related complications, failure to thrive due to chronic illness, and complications of prematurity (intra-ventricular hemorrhage, retinopathy of prematurity, sepsis, necrotizing enterocolitis, and death). It is possible that fetal intervention would increase morbidity by allowing survival of a severely affected fetus that would otherwise have died, or by successfully treating pulmonary hypoplasia at the expense of complications of a preterm delivery.

c) Additional Risks

1. Some of the questions asked may make the pregnant woman/mother/father/partner uncomfortable or upset.
2. As with taking part in any research study, some privacy may be lost. All medical records and information will be handled in accordance with federal guidelines.

2.3.2 KNOWN POTENTIAL BENEFITS

The known potential benefits for the participants who choose intervention is the possible survival of their baby from an otherwise fatal disease. For parents who wish to continue a pregnancy complicated by isolated EPRA, this intervention may be the only potential way to prevent lethal respiratory failure.

Those parents that wish to participate in the study but not undergo RAFT will be providing important information by participating as a contemporaneous control group. This is of no benefit for these study participants but could potentially benefit participants who carry a fetus with EPRA in the future. Successful completion of this study with a positive result could promote this therapy as a viable option for the thousands of parents with a fetus with this disease every year. Furthermore, success of this study may be applicable to other fetal diseases in which there is a low amniotic fluid level and a high incidence of pulmonary hypoplasia. Successful completion of this study with a negative result will help prevent serial amnioinfusions from inappropriately becoming the standard of care for EPRA.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The intervention in this trial is the least risky and least invasive known method of providing therapy to fetuses with EPRA. As in nearly all fetal therapy trials, there is no direct benefit to the mothers who undergo the intervention. In this trial, where baseline fetal survival is thought to be nearly zero, participant exposure to risk is the only way to assess and attempt reliable fetal survival. Additionally, we have taken many steps to minimize risk:

1. All amnioinfusions will be performed by board-certified maternal fetal medicine physicians who are experts in fetal therapy.
2. All evaluations and screening procedures will be performed at an approved RAFT center, which has the infrastructure to provide private counseling rooms. Fetal therapy staff are trained in discussing complex fetal conditions with prospective parents. The RAFT centers also provide additional support to families, including licensed clinical social work services.
3. All research-related documentation will be kept in a locked research office in the RAFT center. Access will be restricted to the investigators and approved study research team members.
4. A secure password-protected, HIPAA-compliant REDCap database will be hosted on the Johns Hopkins University server for data entry from all participating RAFT sites.
5. High resolution ultrasound guidance, per routine clinical protocol, will be used to guide the infusion needle into the amniotic cavity.
6. Doppler ultrasound will be used to avoid maternal and fetal vessels during all procedures.
7. Sterility will be maintained at all times during all procedures.
8. Warmed fluid will be instilled to attain a maximal vertical pocket (MVP) of 4-5 cm.
9. NAPRTCS data is securely stored behind several servers at the Emmes Corporation, and NAPRTCS has its own rigorous data protection service.

3 OBJECTIVES AND ENDPOINTS

Table 3. Study objectives and endpoints

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine the proportion of neonates surviving to ≥ 14 days and placement of dialysis access. after serial amnioinfusions	Survival to ≥ 14 days and placement of dialysis access.	This endpoint was chosen because of its clinical significance in establishing efficacy of serial amnioinfusions to allow clinically meaningful respiratory survival that will then allow dialysis to occur.
Secondary		
To determine the feasibility of serial amnioinfusions for EPRA.	The quantity of amnioinfusions and interval after the initiation of	These are meaningful clinical outcomes that are likely to provide outcome variables in

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
To perform an exploratory study of the in utero natural history of untreated EPRA.	amnioinfusions before rupture of membranes.	future studies if serial amnioinfusions reliably lead to respiratory survival.
	Survival to discharge.	
To examine correlations between fetal ultrasound, echocardiogram, respiratory factors in amniotic fluid (AF), and MRI as predictive biomarkers for the success of RAFT for EPRA.	Survival to kidney transplant.	
To determine the short-term and long-term quality of life of participants and families that undergo RAFT.	PEDs QL™ Pediatric Measurement Model for the Pediatric Quality of Life Inventory Scores™ (Lyon, France).	The PEDs QL™ Pediatric Measurement Model is a robust standardized tool to determine pediatric quality of life. Long-term quality of life is critical to understanding whether this intervention is justified.

4 STUDY DESIGN

4.1 OVERALL DESIGN

Primary Objective:

- 1) To determine the proportion of neonates surviving to ≥ 14 days and placement of dialysis access after serial amnioinfusions.

Secondary Objectives:

- 2) To determine the feasibility of serial amnioinfusions for EPRA.
- 3) To perform an exploratory study of the in-utero natural history of untreated EPRA.
- 4) To examine correlations between prenatal ultrasound, echocardiogram, lung-specific factors in amniotic fluid, and MRI as predictive biomarkers for the success of RAFT for EPRA.
- 5) To determine short-term and long-term quality of life of participants and families that undergo RAFT.

This trial is a non-randomized multi-regional four group prospective cohort study of expectant management and novel intervention treatment, for both the EPRA and FRF diagnostic groups. To minimize bias, all patients who meet eligibility requirements will be offered study enrollment. This novel intervention will be offered multi-regionally to minimize the effects of operational bias. There are four study groups: (1) CoBRA and (2) FRF participants that undergo intervention, and (3) CoBRA and (4) FRF participants that undergo expectant management. Participants in all groups that survive will be followed until four years after transplant or death, whichever occurs first. This is a multi-center trial with 7-15 RAFT centers all within the United States. The study intervention is serial amnioinfusions.

There are no current sub-studies as part of this protocol; however, if our hypothesis is correct and serial amnioinfusions for EPRA are safe and lead to reliable survival to dialysis, there will be significant

opportunities to further optimize therapy. It will remain an open question whether larger but less frequent infusions are more successful than smaller more frequent infusions. This could be the basis for a prospective randomized trial.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Given the novel nature of the proposed intervention and the lack of reported survival without therapy, we do not believe it would be ethical to randomize these participants. Due to the fact there is currently no other viable approach for this condition, we believe all eligible participants should be offered the option of fetal intervention if they desire it after extensive counseling and understanding of all risks and potential complications. We do believe it is vital to understand the natural history of this disease in the modern era of neonatal resuscitation and for that reason we have included expectant management groups.

4.3 JUSTIFICATION FOR DOSE

Fetuses with EPRA caused by CoBRA or FRF are unable to produce amniotic fluid due to their lack of functional kidneys. Anhydramnios in the second trimester of pregnancy is almost unvaryingly lethal due to pulmonary hypoplasia without restoration of amniotic fluid volume. Restoration has been suggested to limit the risk of pulmonary hypoplasia. Participants who choose to receive intervention will return approximately every 2-12 days for amnioinfusion or frequently enough so that there is residual fluid to act as a target for the next amnioinfusion. It may be medically necessary to dose the participants at a shorter interval than every 2-12 days; this will be based on each participants' biological absorption rate and provider's assessment of the amount of amniotic fluid in the uterus. Amnioinfusate will consist of isotonic fluid including oxacillin 1 gram/500 mL or nafcillin 1 gram/500 mL depending on the hospital formulary for prophylaxis. If there is a penicillin allergy, clindamycin will be used 600 mg/500 mL. The procedure will be performed by ultrasound under local anesthesia for maternal comfort. The amnioinfusion will be performed with 20-gauge or 22-gauge needle, or according to the institution's standard of care practice, as guided by the clinical indication as the least invasive method to the mother. The maximal flow rates will be dependent on needle gauge and may take up to one hour. The volume of the infusate will go up gradually with the size of the fetus. Trials that have performed amnioinfusion in a randomized fashion have infused up to 1.5 liters, as tolerated to minimize the number of procedures. Each amnioinfusion has approximately 2-4% rate of premature preterm rupture of membranes (PPROM) (see section 2.3.1 for detailed risks of amnioinfusion). Larger volume infusions decrease the need for more frequent infusions and also increase the probability of residual fluid being present at the next opportunity for amnioinfusion. This residual fluid provides a target and facilitates amnioinfusion. We will aim for a MVP of 4-5 cm measured by transabdominal ultrasound and normal amniotic fluid index (AFI) for GA after each amnioinfusion.

Participants in the expectant management arm will undergo standard of care and monitoring approximately every 1-3 weeks without the intervention. Post-procedure office visits may be requested at the discretion of the medical provider if it is the institution's standard of care but are not scheduled protocol-mandated study visits.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study when the neonate survives for four years after receiving a renal transplant or at time of death, whichever occurs first. The end of the study is defined as when 35 neonates reach study completion in each of the CoBRA and FRF intervention groups

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

We plan to study three vulnerable populations: pregnant women, fetuses, and neonates. Our rationale for studying these populations is that this is a fetal disease that is thought to be lethal, and therefore the only way to address it is by performing a procedure on the gravid mother. Participants who enroll in the study will have the option to pursue interventional therapy with serial amniotomies or expectant management. In line with federal regulations, both the mother and the father will need to consent.

Enrollment in the study will require confirmation of an isolated EPRA diagnosis and confirmation that the participant does not wish to undergo termination of the pregnancy. This diagnosis can be made as early as 16 weeks GA when amniotic fluid is no longer derived from a maternal trans-placental source but rather from fetal urine production. After a patient is referred to a RAFT center, the diagnosis of isolated EPRA will be confirmed in the following way:

- 1) Anhydramnios in the absence of ruptured membranes (ROM) on ultrasound
- 2) No significant bladder filling on ultrasound

As part of routine care the mother will undergo a diagnostic amniocentesis (this amniocentesis may be waived in mothers who wish to pursue expectant management) at this stage to enable confirmation that there is no ROM and allow for a more detailed sonographic fetal evaluation to characterize whether there is any renal tissue or vasculature, as well as to look for extra-renal anomalies. A schematic of the protocol is provided in Section 1.2. Other significant anomalies will make the patient ineligible for enrollment. Establishing the presence or absence of renal tissue will determine whether the patient has CoBRA or FRF. Approximately one week after this visit, the mother will return per routine for a follow-up ultrasound. Confirmation of the diagnosis will be made by loss of infused fluid without rupture of membranes and again demonstration of diagnostic criteria 1 and 2. After confirmation of the diagnosis, the following inclusion criteria will be applied to the candidates:

- 1) Confirmed anhydramnios before 22 weeks 0 days GA for patients with FRF or confirmed diagnosis of CoBRA. As the intervention arm for CoBRA has been closed since July 2022, patients with confirmed fetal CoBRA will be able to join the expectant management arm only.
Independent confirmation of isolated FRF will be made by coordinating center PI central review of screening diagnostic imaging before the participant is enrolled.
- 2) Consent is signed and first therapeutic amniocentesis can and does occur before 26 weeks and 0 days GA
- 3) Confirmation that the expectant mother does not wish to undergo termination of the pregnancy
- 4) Age ≥ 18 years for expectant mothers
- 5) Willingness to be followed and deliver at a RAFT center if in the intervention arm. Expectant management arm has the option to be followed and deliver at a local hospital/hospital other than a RAFT site, and allow medical records to be sent to the RAFT site.
- 6) Willingness for postnatal care to be performed at a RAFT center until discharge if in the intervention arm
- 7) Completed consults with pediatric nephrology, neonatology, transplant surgery, pediatric surgery, maternal-fetal medicine, a licensed clinical social worker, and a genetic counselor

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

- 1) Cervix less than 2.5 cm in length
- 2) Any significant pathogenic or likely significant pathogenic findings on Karyotype or Microarray
- 3) Other clinically relevant congenital anomalies in the fetus
- 4) Autosomal recessive polycystic kidney disease in the fetus
- 5) Evidence of chorioamnionitis or abruptio placentae
- 6) Evidence of rupture of membranes or clinically relevant chorioamniotic separation
- 7) Clinically relevant evidence of preterm labor
- 8) Multiple gestation
- 9) Severe maternal medical condition in pregnancy
- 10) Maternal depression, as assessed by a Beck Depression Inventory score ≥ 17 , that is refractory to treatment
- 11) Technical limitations precluding amnioinfusion

5.3 LIFESTYLE CONSIDERATIONS

Participants enrolled in the intervention arm of the study will be asked to transfer care to the RAFT center for all prenatal care and relocate near the RAFT center (within 30 minutes) for the duration of the pregnancy after 26 weeks GA. Participants will be given standard pregnancy guidelines and precautions for anhydramnios.

5.4 SCREEN FAILURES

Participants will only be offered enrollment after all inclusion criteria have been met, including confirmation of isolated ERPA. If any additional, clinically relevant congenital anomaly is identified in a fetus after the mother has been enrolled in the amnioinfusion arm of the trial, the participant will be given the option to withdraw from the study, to discontinue the study interventions but continue with study observations, or to continue with amnioinfusions if desired. However, given that any additional clinically relevant congenital anomaly may confound the assessment of the primary efficacy endpoint of the study (proportion of neonates who demonstrate respiratory survival and survival to use of a dialysis catheter for >14 days), the participant will be moved to a new analysis category for participants with additional significant anomalies. This will allow us to maintain a uniform cohort of patients with isolated EPRA, while also utilizing data from all participants enrolled in the study. Participants moved to the additional clinically relevant anomaly category due to discovery of an additional clinically relevant congenital anomaly will continue the intervention per the RAFT protocol with all normal necessary precautions. All events will be recorded and reported on all required future audits and reviews.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

One hundred percent of the target study sample will be pregnant women with an affected fetus. Ethnicity and race have no bearing on the scientific question of safety and efficacy for RAFT. These variables will be recorded but will not be part of inclusion or exclusion criteria of the study. We do not expect difficulty in recruiting any specific ethnic groups or races.

Anticipated accrual rate is based on historical referral patterns and counseling. We estimate that 5-10 patients per year will undergo RAFT and survive to dialysis to recruit a total sample size of 35 maternal/fetal pairs in the intervention arm. We anticipate 7-15 sites participating in the trial, all within the United States. Participants will be recruited at maternal fetal medicine and fetal therapy RAFT centers where they are referred for treatment. All referred patients with EPRA will be screened for enrollment. Participants in the intervention arm will be required to transfer all prenatal care to the RAFT center and to return on-site for serial amniotomies and ultrasounds according to the institution's standard of care practice. Participants in the expectant management arm will have the option to be followed locally, but will be requested to return to the RAFT Center for the echocardiogram with hyperoxygenation and fetal MRI at about 32 wks GA. Delivery and neonatal care will also occur at the RAFT center if participant is in intervention arm. A licensed clinical social worker (LCSW) will work with the participant to ensure the appropriate arrangements can be made so that the participant will be retained for the entirety of the study

We plan to study three vulnerable populations: pregnant women over the age of 18, fetuses, and neonates. Our rationale for studying these populations is that this is a fetal disease that is thought to be lethal to the fetus and, therefore, the only way to address it is by performing a procedure on the gravid mother. No experimental interventions will take place on neonates but they will be followed through infancy and until four years after transplant. This population must be included in order to assess the efficacy of RAFT. Patients who enroll in the study will have the option to pursue interventional therapy with frequent amniotomies or expectant management. In line with federal regulations, both the mother and the father will need to consent. If the father is not reasonably available (incarcerated, incapacitated, deceased or unknown) or the pregnancy is a result of a rape or incest, the requirement for his consent will be waived and herein a note to file. If there are any questions about obtaining a paternal consent, direct contact with the trial lead PI is mandatory.

This protocol meets all federal requirements for the study of pregnant women and fetuses. Amniotomies have been performed in pre-clinical and clinical settings before.⁹ The risk profile to mother and fetus for this procedure is clearly established in similar patients.⁹ The risk to the fetus from amniotomies is far outweighed by the possible salutary effects of amniotomy. Percutaneous amniotomies are the most well studied and least risky way to deliver amniotic fluid to an EPRA fetus. Individuals engaged in this research will have no part in any decision as to the timing, method or procedures to terminate pregnancy.

Participants will not be compensated for their participation. However, a travel stipend will be offered to all participants for the 32 wk GA research visit for the echocardiogram with hyperoxygenation and the fetal MRI.

Prior to enrollment, counseling provided by a neonatologist, pediatric nephrologist, pediatric surgeon, transplant surgery specialist, clinical licensed social worker, genetic counselor, and maternal fetal medicine specialist will take place in a nondirective and supportive environment in the RAFT center's fetal center. Independent confirmation of isolated FRF will be made by coordinating center PI central review of screening diagnostic imaging before the participant is enrolled. Only the principal investigator or his or her physician consent designee(s) will obtain informed consent. They will ensure that all of the appropriate consultants listed above have met and counseled the participants. Consent will be obtained in a private room away from patient care areas. During the consenting process, maternal, fetal, neonatal and childhood risks will be fully explained. The uncertainty of serial amniotomy success will be discussed. Furthermore, the postnatal challenges if survival is achieved will be discussed in detail. These challenges will be discussed by the many consultants, but will also be discussed by the investigators obtaining informed consent. These challenges include the need for dialysis, the likelihood of complications from dialysis access, the need for supplemental

nutrition likely by gastrostomy tube, the risk of sepsis, recurrent hospitalizations, the eventual need for

kidney transplant, the requirement for immunosuppression after a kidney transplant and the likely need for urinary tract reconstruction. Discussion with parents will include that in the case of a neonate who survives delivery but subsequently dies, parents may be asked to consent to an autopsy for the infant, in order to gather more information about cause of death.

In the case of participants in the expectant management arm only, and after all in-person prenatal counseling is provided at the on-site screening visit, potential participants may elect to leave the RAFT center to consider participation; in this case, participants may provide informed consent to enroll in the expectant management arm of the trial via teleconference.

Furthermore, the financial and logistical challenges of insurance copays, time away from work, and the need for temporary relocation to a RAFT center area will be discussed. The parents will receive the consent form at the first visit giving the patient 1-2 weeks to review it. The patient will be asked to repeat back the critical counseling points to ensure understanding. A licensed clinical social worker will also counsel the patients and ensure understanding. Enrollment will be open to non-English speakers at all participating RAFT centers. An official hospital interpreter will be used for non-English speakers and those with language/hearing impairments. An approved short form consent and procedure will be used to assess participant understanding throughout the entire screening process and obtain informed consent. This will require the interpreter to read the entire consent form and accompanying documents to the participant in their native language. Upon completion of this process, the participant will sign the short form consent and the interpreter will sign the short form as a witness. The consent designee will sign the English long consent form, the interpreter will sign as a witness, and the participant will sign the English version. A copy of the short form and English form will be given to the participant and a second and third copy of each will be included in the participants research folder and medical chart. Both prenatal consents will allow data to be collected on the neonate for up to 15 days of life.

Once the child is born, we will again use the same methodology to ensure understanding and obtain consent to follow the child, contact the family and to continue prospectively following the outcomes of the neonate. This consent will be obtained by the principal investigator or his or her consent designee(s). A private space away from the neonates' care will be utilized. There is no risk to the child from the study after birth. Assent from the child will also be sought once they reach the age of six. The clinical variables and our data security plan will be described. We will also explain the NAPRTCS registry and its use to keep track of outcomes after the participant leaves the hospital. NAPRTCS has its own consent form and IRB supervision by the Johns Hopkins IRB.

5.6 STATE LAWS

New RAFT centers that join this study may be located in states that have specific regulations about research involving pregnant women/fetuses as well as research with neonates of uncertain viability. As new centers join the trial, we will actively solicit information about state regulations and ensure that the protocol is in compliance with all applicable regulations. An addendum to the protocol specific to those RAFT centers in states with different regulatory requirements will be made before the trial can begin there. The Johns Hopkins IRB and the Johns Hopkins RAFT investigators will ensure that compliance with these regulations does not negatively impact the scientific integrity of the trial. If compliance will negatively impact the scientific integrity, centers in those affected states will not be able to participate in the study.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

The study intervention is ultrasound-guided amnioinfusion of isotonic fluid including oxacillin, nafcillin, or clindamycin using only a 20-gauge or 22-gauge spinal needle. These needles are already FDA approved for uterine access and are commercially available for the purpose of amnioinfusion. The needle will need to remain in the amniotic cavity until the appropriate amount of isotonic fluid is delivered. This may take up to one hour. The isotonic fluid will gradually be absorbed by the fetal-placental unit over the course of several days. Repeat amnioinfusions will occur before the fluid is completely gone from the amniotic cavity. This interval will be tailored for each participant depending on the rate of absorption.

6.1.2 DOSING AND ADMINISTRATION

Amnioinfusions, both diagnostic and therapeutic, will not be offered until 18+0 weeks gestation. Therapeutic amnioinfusions will be performed until delivery as clinically indicated. After 34 weeks 6 days of gestation, infusions may continue if in the clinical judgement of the fetal therapist continued infusions will benefit the participant and/or the fetus. The amount of amnioinfusate will be determined by the volume of isotonic fluid required to achieve normal MVP as measured by ultrasound. This will fluctuate by week and by participant due to difference in biological absorption rates. The amnioinfusions will be performed under local anesthesia.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 PRODUCT STORAGE AND STABILITY

Amnioinfusion needles and isotonic fluid bags will be stored in standard clinical areas at room temperature and will not be used if expired or if seals have been broken. Needles and bags of isotonic fluid will only be used once.

6.2.2 PREPARATION

Isotonic fluid will be warmed to body temperature by the fetal therapy physician in a standard clinical warmer in preparation for the amnioinfusion.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

This study will rigorously examine the efficacy of serial amnioinfusions in treating EPRA. It will not be randomized, and therefore no blinding is required. Bias will be minimized by ensuring critical decision-making parts of the neonatal care team are independent from the PI and other study team members.

6.4 STUDY INTERVENTION COMPLIANCE

Adherence to the protocol will be required for continued participation in the study. If a participant is

unable to return for two consecutive follow-up surveillance or amnioinfusions as part of the intervention group, they will be excluded from continuing in the study. Participation will be assessed by

monitoring participant attendance to scheduled appointments and delivery. This will occur at a RAFT center if participant is in intervention arm, and if participant is in expectant management arm it may occur at their local hospital. The expectant management participants being followed locally will only be required to return to the RAFT center at about 32 wk GA for the protocol specified echocardiogram with hyperoxygenation and the fetal MRI. These studies will be paid for by the study. A travel stipend will be offered to encourage attendance to the 32 wk research visit. Please see Section 7 for further detail.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from the interventional group of serial amnioinfusions does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol for the expectant management group. These follow-up visits will be captured in the periodic follow-up case report form (CRF) for expectant management.

If a clinically relevant fetal finding is detected in a participant, such as a separate lethal or clinically relevant congenital anomaly that was not detected on pre-enrollment imaging, the participant will have the following options as detailed above in section 5.4: withdraw from the study, discontinue the study interventions but continue with study observations, or continue with amnioinfusions. Any new clinically relevant finding after enrollment will be reported as an adverse event (AE), serious adverse event (SAE), or unanticipated problem (UP) as appropriate.

If the participant experiences any unresolved AE or SAE, the study intervention will be discontinued as outlined in Section 7.2 of this protocol.

The data to be collected at the time of study intervention discontinuation will include the following:

- Gestational age at discontinuation
- All fluid measurements
- Fetal biometry
- Reason for discontinuation
- Maternal perception of contractions, bleeding, fetal movement, and fluid leakage
- Status of placental health and if there is chorioamnion separation
- Umbilical Dopplers
- Maternal vital signs

To avoid losing these participants to follow-up after discharge from the hospital post-delivery, we will ensure that all participants are enrolled in the NAPRTCS registry, which has a secure internet-based data collection, management and storage facility that has been operational since 1987. The DCC for NAPRTCS is located at the Emmes Corporation, which uses two internet service providers to minimize the risk of internet connectivity failures: Qwest Communications and AboveNet Communications. The Emmes Corporation is a privately owned small business located in Rockville, Maryland, 7 miles from the National Institutes of Health campus. Emmes Services Private Limited (Emmes India) is a subsidiary of the corporation and was incorporated in April 2006 in Bangalore, India. The India office provides research and development capabilities and services for the Emmes electronic data capture (EDC) product suite,

Advantage EDCSM, as well as local clinical site support including data management, protocol monitoring, and technical site support. Project data reside in master databases located on the Emmes

local area network. Its network security ensures that only authorized personnel have access to the master databases. These mechanisms protect the files from inadvertent deletion or modification. Confidentiality is maintained by the use of subject ID number designations, rather than names, social security numbers or hospital numbers. Emmes limits the collection of personal health information (PHI) to only those identifiers that are absolutely required for a study (for example, birth dates) and are approved by the study sponsor; any PHI that is required to be collected is subject to the corporate standard operating procedures (SOPs) that define the protection of PHI and confidentiality.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

All RAFT centers will adhere to the following stopping rules:

In the event of preterm contractions after a trial amnioinfusion visit, the participant will be administered tocolytics and admitted if indicated. If contractions are unresolved in seven days and the participant is in the intervention group, they will discontinue serial amnioinfusions until tocolytics can be stopped for 3-5 days according to the judgment of the local team. If the tocolytics cannot be stopped, amnioinfusions will not be reinitiated, but the patient will continue participation in the study. For the purpose of analysis, they will be considered part of the amnioinfusion interventional group.

In the event of frank PPRM, all amnioinfusions will stop, and the participant will be admitted to the participating RAFT center's Labor and Delivery Unit. If the membranes reseal, amnioinfusions may be restarted. If there is uncertainty about resealing, a diagnostic amnioinfusion may be performed. If rupture persists, appropriate treatment will be provided, and delivery timing will be determined by standard obstetric indications. All future interventions will be discontinued at this point. The participant will be followed as an intervention group participant. If intractable rupture is before 32 weeks, delivery will be induced between 32-34 weeks.

In the event of minor leakage due to suspected chorion-amnion separation, the participant will be evaluated and observed to ensure that the membranes are not ruptured. If there is no PPRM, the participant will continue with amnioinfusions.

In the event that a participant cannot tolerate amnioinfusions after the first diagnostic amnioinfusion, the mother will be removed from the interventional group of the study and offered enrollment into the observational expectant management group of the study. If amnioinfusion intolerance occurs later in the study, interventions will cease but the mother will be followed as an intervention group participant.

In the event that the participant does not adhere to necessary intervention appointments and necessary follow-up, the participant will be removed from study protocol after two missed visits/no shows.

In the event of acute placental abruption, intervention will be discontinued, and an obstetrical decision will be made immediately about treatment or timing of delivery.

In the event of uterine rupture, intervention will be discontinued, and emergent delivery will occur according to the standard of care.

In the event of amniotic fluid embolism, intervention will be discontinued, and an obstetrical decision

will be made immediately about the need for urgent delivery.

In the event of uterine bleeding in the absence of placental abruption, a determination will be made about the need for blood transfusions. If more than two blood transfusions are required during a 24-hour period, intervention will be discontinued, and the need for delivery will immediately be considered.

Participants are free to withdraw from participation in the study at any time.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance (missing two scheduled follow-up visits, transferring care to a non-RAFT participating center).
- Safety concerns, as listed above in this section.

The reason for participant discontinuation or withdrawal from the study will be recorded on the Final Status Case Report Form.

7.3 LOSS TO FOLLOW-UP

A participant will be considered lost to follow-up if she fails to return for two scheduled visits and is unable to be contacted by the study site staff.

As part of this study, participants will be required to receive all their prenatal care at a RAFT center. Participants will be issued a Fetal Center alert card to ensure rapid understanding of their care by other providers. As with any patient with a fetal anomaly being followed at a fetal center, extensive contact information for the patient and the RAFT center will be obtained. In the event that a participant is difficult to contact, we will make every effort to contact them or their designees by phone, email, certified mail, and all other identified means of contact.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 1-2 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record and study file.
- Should the participant continue to be unreachable, she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

To be enrolled, participants have until 26 weeks 0 days gestation to complete all screening evaluations to determine eligibility, unless special dispensation is given by the Johns Hopkins IRB. Patients will be referred to a RAFT center by their primary care providers upon learning of an EPRA diagnosis. Once referred, patients will be scheduled for an urgent evaluation at a RAFT center. During this evaluation,

the diagnosis will be confirmed by ultrasound and diagnostic amnioinfusion. All inclusion and exclusion criteria will be applied to determine eligibility. This process should be as expeditious as possible. If a patient would like to participate in the expectant management group, the requirement for a diagnostic amnioinfusion may be waived.

8.1.1 POSTNATAL VIABILITY

This study will examine the efficacy of RAFT and will necessarily include study of non-viable neonates/neonates of uncertain viability. After delivery, neonatologists who are not part of the study team will participate in determining viability of the neonate. Viability, as in all human research, will be defined as being able, after delivery, to survive (given the benefit of available medical therapy to the point of independently maintaining a heartbeat and respiration). For non-viable neonates, an autopsy will be highly recommended.

All participants in the intervention arm will deliver at a RAFT center. Participants in the expectant management arm will have the option of delivering at a RAFT center, or at a hospital other than a RAFT center including at their local hospital. A neonatal team with providers that are independent of the study will resuscitate the fetus and provide respiratory support. Extracorporeal membranous oxygenation (ECMO) is thought to not be indicated in these patients during initial resuscitation and is discouraged; however, final determination of ECMO use for a patient is ultimately the decision of the clinical care team. Survival to successful peritoneal dialysis or hemodialysis will be recorded. The neonatal care team, in consultation with independent neonatology, nephrology and pediatric surgery providers, will determine the feasibility of placing a dialysis catheter. Survival to ≥ 14 days and placement of dialysis access is the defined primary outcome variable. Other perinatal variables such as gestational age at delivery, delivery mode, Apgar scores, and umbilical cord blood gases will be recorded. First arterial blood gas, ventilator support and settings, and use of other supplemental therapies (e.g., surfactant, nitric oxide) will be recorded. Procedures to initiate dialysis and their success will be recorded (peritoneal dialysis [PD] or hemodialysis [HD]). Episodes of peritonitis will also be defined as antibiotics started for an intra-abdominal infection. Need for a gastrostomy tube, tunneled central line and tracheostomy will be recorded.

Neurologic injury or dysfunction will be recorded, and in addition to neuroimaging reports images of pre- and post-natal neuro imaging studies will be uploaded via REDCap. All participants in the RAFT trial undergo fetal MRI (mothers) and an initial screening head ultrasound after delivery (infants). Over the course of the trial, we have observed that many of the infants have had strokes and other neurologic sequelae that has been identified on both head ultrasound and MRI, and may or may not have been clinically apparent. Interestingly, the pattern of injury observed on neuroimaging is unlike patterns that have been described previously in the literature, and may represent an entirely new pattern of injury unique to infants with fetal renal failure receiving neonatal dialysis. Some of the infants have had multiple strokes, which is very atypical for neonates who have a stroke within the first month of life, and the timing of the strokes has ranged from <1 month to toddlerhood, representing timepoints both during the initial birth hospitalization as well as during long-term follow-up. Given the atypical timing, presentation and recurrence of the neurologic sequelae, it is imperative that the study team have access to the primary neuroimaging for further assessment and characterization of neurologic injury, as the written reports currently included in RedCap do not adequately capture the nuance, novelty and temporality of the findings. Thus, for each infant enrolled in the RAFT trial, we will provide a mechanism for study teams to upload the primary images from any neuroimaging that fetuses and surviving

neonates undergo, including fetal MRI, head ultrasounds and MRIs of the brain or spine (including long-term follow-up to age 4 years). This will allow detailed analysis of neurologic imaging outcomes.

Survival to discharge is one of the secondary outcome variables and will be recorded. Medications at discharge, respiratory support at discharge, and nutritional support at discharge will be recorded. Death before discharge will be recorded. Age and weight at discharge will be recorded. Discharge to home or another facility will be recorded.

In order to follow the outcomes of these patients longer term, we plan to use the infrastructure and data gathering mechanisms of the NAPRTCS registry. This registry is an ongoing research effort that began in 1987 and includes follow-up on over 20,000 patients. Greater than 500 pediatric patients with chronic kidney disease, dialysis, and renal transplant are entered into the registry each year. There are over 60 active NAPRTCS centers and 100 centers that have data sharing agreements. All of these centers have established protocols for capturing data from pediatric patients with renal failure. A study team member at each RAFT center will ensure that upon discharge all participants are offered enrollment in NAPRTCS and arrange for the follow-up to take place at a NAPRTCS center. NAPRTCS uses an EDC system that allows data to be securely entered and retrieved on the internet. Validated electronic data forms for each patient visit have been developed. There are data forms for entry into the registry, each dialysis visit, transplant, and follow-up after transplant.

In order to evaluate our hypotheses about quality of life in EPRA patients, the validated PEDs QLTM measurement model for the pediatric quality of life inventory will be performed at least every 6 months for each patient at their follow-up visit in concert with NAPRTCS data acquisition. PEDs QLTM instrument is the premier multilingual tool for assessing quality of life in pediatric patients with chronic disease.¹² It takes 4 minutes to administer and has excellent reliability and flexibility. This tool is already being administered by NAPRTCS to all chronic renal failure patients. We have arranged to create a contemporaneous control group from within the NAPRTCS registry of patients with neonatal renal failure requiring neonatal dialysis for reasons other than EPRA.

Consults with specialists in pediatric nephrology, neonatology, transplant surgery, pediatric surgery, maternal-fetal medicine, and genetics, plus a licensed clinical social worker, will take place in a nondirective and supportive environment at each RAFT center. Only the local principal investigator or his or her physician consent designee(s) will obtain informed consent. They will ensure that all of the appropriate consultants listed above have met and counseled the patient. Consent will be obtained in a private room away from patient care areas. During the consenting process, maternal, fetal, neonatal and childhood risks will be fully explained. The uncertainty of serial amnioinfusion success will be discussed. Furthermore, the postnatal challenges if survival is achieved will be discussed in detail. These challenges will be discussed by the many consultants involved before consent, as listed above, but will also be discussed by the investigators obtaining informed consent. These challenges include the need for dialysis, the likelihood of complications from dialysis access, the need for supplemental nutrition likely by gastrostomy tube, the risk of sepsis, recurrent hospitalizations, the eventual need for kidney transplant, the requirement for immunosuppression after a kidney transplant, and the likely need for urinary tract reconstruction. Furthermore, the financial and logistical challenges of insurance co-pays, time away from work, and the need for temporary relocation near a RAFT center will be discussed. We have explored the ethical framework for offering this therapy in a national ethics symposium that reported its findings in *Obstetrics and Gynecology*.¹⁴ This trial was designed in accordance with the key issues identified at the symposium and published in our manuscript.

For participants that may discontinue or withdraw early, see Section 7 for details.

Biological specimen collection:

- Results of genetic analysis of fluid removed during diagnostic amnioinfusion/ultrasound for karyotype and possibly microarray depending on patients' insurance as per standard of care will be collected.
- Fluids collected during the study will be biobanked in a de-identified manner. Amniotic fluid samples will be obtained in patients enrolled in the intervention arm at each infusion. Samples will be collected both pre- and post-infusion if enough fluid is available to sample.
- A substudy examining whole exome sequencing in affected fetuses and the parents and more specimens may be acquired with a separate consent for that study.

Full ultrasound and genetic reports will be provided to the participants upon request.

An individual's medical chart or results of diagnostic tests performed as part of an individual's regular medical care will be used for screening or as a part of collection of trial data, and thus Health Insurance Portability and Accountability Act (HIPAA) rules, other relevant federal or state laws, and local institutional requirements will be followed. A HIPAA authorization waiver from the IRB will allow collection of this data.

8.2 SAFETY AND OTHER ASSESSMENTS

Enrollment in the study will require confirmation of an isolated EPRA diagnosis and confirmation that the participant does not wish to undergo termination (Figure 1). This diagnosis can be made as early as 16 weeks GA when amniotic fluid is no longer derived from a maternal transplacental source but rather from fetal urine production. After a patient is referred to a RAFT center, the diagnosis of isolated EPRA will be confirmed in the following way:

- 1) Anhydramnios in the absence of ruptured membranes (ROM) on ultrasound
- 2) No significant bladder filling on ultrasound

As part of routine care, the mother will undergo a diagnostic amnioinfusion at this stage to enable confirmation that there is no ROM and allow for a more detailed sonographic fetal evaluation to characterize whether there any renal tissue or vasculature as well as to look for extra-renal anomalies. This diagnostic amnioinfusion may be waived for patients wishing to join the expectant management group. A fetal MRI will be obtained during the screening period to look for extra-renal anomalies not apparent on ultrasound. A schematic of the protocol is provided in Figure 1. Other significant anomalies will make the patient ineligible for enrollment. Establishing the presence or absence of renal tissue will determine whether the patient has CoBRA or FRF. Approximately one week after this visit, the mother will return per routine for a follow-up ultrasound. Confirmation of the diagnosis will be made by loss of infused fluid without rupture of membranes and again demonstration of diagnostic criteria 1-2.

For patients who are eligible and wish to join the study, the options of expectant management or serial amnioinfusions will be offered. Consults provided by pediatric nephrology, neonatology, transplant surgery, pediatric surgery, maternal-fetal medicine, a genetic counselor, and a licensed clinical social worker will take place in a nondirective and supportive environment at each RAFT center. After counseling, patients who are greater than 20 weeks GA but less than 26 weeks 0 days GA who wish to continue the pregnancy and undergo intervention will be eligible for experimental therapy; they will return approximately every 2-12 days for amnioinfusions with warmed isotonic fluid until delivery or frequently enough so that there is residual fluid to act as a target for the next amnioinfusion. The interval of amnioinfusions will be tailored for each patient with the goals of a target of normal maximal vertical pocket and that some amniotic fluid remain to facilitate the next amnioinfusion. Chest size, lung-to-head ratios for both lungs, and pulmonary artery Doppler waveforms will be measured at each

amnioinfusion visit. A fetal MRI will be performed during the screening period to assess for additional anomalies not visible on ultrasound imaging, with a second fetal MRI performed after enrollment and at around 32 weeks gestation. For potential participants who desire to enroll in the expectant management group, a fetal MRI is required during the screening period and also will be performed

around 32 and by 33 weeks. A fetal echocardiogram will be performed at enrollment and at around 32 weeks not to exceed 33 weeks.

In the expectant management groups, chest size, lung-to-head ratios for both lungs, and pulmonary artery Doppler waveforms will be measured at each ultrasound visit. A fetal MRI will be performed before 26 weeks gestation during the study screening period, and near 32 weeks GA. A fetal echocardiogram will be performed during enrollment and near 32 weeks GA. Participants may also receive standard steroid therapy depending on parental wishes.

After 34 weeks 6 days GA, the fetal therapist will determine the risk/benefit ratio of further infusions in light of the likelihood of inducing preterm labor. Amnioinfusion will be performed under local anesthesia with a 22-gauge or 20-gauge needle guided by the clinical circumstances. The maximal flow rates will be dependent on needle gauge and may take up to one hour. The volume of the infusate will go up gradually with the size of the fetus. Each amnioinfusion has an approximately 5% rate of PPROM. If frank PPROM does occur, the patient will be admitted (see Section 7.2). Delivery will be guided by obstetric indications and local guidelines with a target of after 32 weeks GA and estimated fetal weight of >1500 grams. Importantly, enrolled mothers may withdraw from the intervention group (or the study entirely) at any time.

Inclusion and exclusion criteria will be applied. To be enrolled, participants have until 26 weeks 0 days gestation to complete all screening evaluations to determine eligibility. As part of those evaluations, the following examinations will be made to ensure the safety of participants:

- *Physical examination by a physician:* auscultation of the heart and lungs, assessment for peripheral edema.
- *Vital signs:* temperature, pulse, respirations, blood.
- *Comprehensive fetal ultrasound:* a standard level II ultrasound will be performed by a board-certified maternal fetal medicine doctor. High-resolution ultrasound machines with electronic real-time 4D probes will be utilized. Standard of care imaging techniques will be performed.
- *Biological specimen collection and laboratory evaluations.* Infused amniotic fluid will be collected and assessed for karyotype and/or microarray. This will be done at each RAFT center according to standard laboratory guidelines for these routine prenatal tests. Samples may also be biobanked in a de-identified fashion.
- *Assessment of adverse events.* The investigators, DSMB, and IRB will review AEs and SAEs to ensure that patient safety is optimized on a continuing basis.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related. The following events are considered expected for this patient population and should be reported as an AE:

- Amniotic fluid embolism
- Bleeding with hemoperitoneum

- Bleeding in the absence of placental abruption
- Chorioamnionitis
- Hydrops fetalis
- Placental abruption
- Preterm premature rupture of membranes
- Preterm labor (after 20 but before 37 weeks gestation)
- Severe maternal illness
- Uterine rupture
- Amniotic band syndrome
- Chorioamniotic separation
- Fetal injury
- Local wound infection
- Failure of procedure
- Other complication as determined by provider

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the DSMB, it results in any of the following outcomes: death, a life-threatening event, inpatient hospitalization (>7 days), or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Events that should be reported as a SAE include:

- Maternal death
- Fetal death
- Life-threatening event
- Inpatient hospitalization > 7 days/Prolongation of hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

For AEs not listed as possible anticipated events as per the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild – Events require minimal or no treatment and do not interfere with daily activities.
- Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious."

All events will be reported according to reporting guidelines listed in Table 4.

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to amnioinfusion and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after amnioinfusion is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after the amnioinfusion). However, other factors may have contributed to the event.
- Although an AE may rate only as “possibly related” soon after discovery, it can be flagged as requiring more information and later be upgraded to “probably related” or “definitely related”, as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to amnioinfusion makes a causal relationship improbable and in which other drugs or chemicals or underlying disease provides plausible explanations.
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

8.3.3.3 EXPECTEDNESS

The RAFT principal investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the serial amnioinfusions within the protocol.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs and SAEs will be captured on the appropriate CRFs. Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by the fetal therapy physician experts), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution. The events will be reported according to the timelines listed in Table 4.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The site principal investigator will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

This eIRB protocol is for Renal Anhydramnios Fetal Therapy (RAFT), a multi-site clinical trial for which the Department of Pediatrics and the Division of Brain Injury Outcomes (BIOS) at the Johns Hopkins School of Medicine will serve as the study Coordinating Center (CC). The Johns Hopkins School of Medicine Institutional Review Board (IRB) has agreed to act as the IRB of record for this collaborative study.

We will submit one application for the coordinating center submission (IRB Protocol #00164021; Co-PIs: Meredith Atkinson and Jena Miller), which includes study template materials, such as the study protocol, study consent, and other supplemental materials. The Johns Hopkins clinical site will serve as the lead enrolling site, with additional relying sites to be added to this protocol.

All participating sites are expected to use the single IRB to submit documentation of responsible IRB approval and copies of their approved consent forms to the RAFT Coordinating Center as a requirement to initiating recruitment and other participant activities.

RAFT Center Inclusion Criteria

1. Board-certified Maternal-Fetal Medicine (MFM) specialist who has performed greater than 15 amnioinfusions in fetuses with anhydramnios or equivalent needle-based ultrasound-guided procedures as judged by the lead PI.
2. Pediatric surgeons with experience and willingness to place dialysis catheters in neonates 1.8 kg or smaller.
3. Pediatric nephrologist with experience and willingness to perform dialysis in neonates 1.8 kg or smaller.
4. Neonatologist with experience and willingness to care for a neonate as small as 1.8 kg requiring dialysis.
5. Multidisciplinary meeting of local PI, Study Coordinator, MFM, Pediatric Surgeon, Neonatology, Nephrology, and hospital administration to ensure buy-in.
6. Review of standard slide deck that describes critical study policies and procedures.
7. Availability of all required subspecialties and counselors for consent.
8. If any of these are in question, the lead PI will travel to local site to verify #1-7.

Coordinating Center Role

The study is being conducted by a multicenter group that does fetal disease research. As the data coordinating center, we design and maintain the data collection documents (forms and questionnaires), data and project management system (REDCap) for the study, monitor clinical and resource sites, analyze

study data, and oversee the overall conduct of the study. Study collaborators recruit participants for the study, obtain participant consent, and collect and submit data for the study. The site principal investigator at each participating site is responsible for ensuring IRB approval throughout the study for the protocol and consent statement (see below for details regarding IRB structure for this study).

Study Procedure/Document Development and Oversight

The primary decision-making body of the Renal Anhydramnios Fetal Therapy (RAFT) is the Executive Committee (EC), which is composed of the PIs from the Johns Hopkins Children's Center and Center for Fetal Therapy, and the Coordinating Center. The Johns Hopkins Children's Center is the "core" clinical site for design and conduct of the RAFT study. Other members of the EC include Drs. Daniel Hanley, Jonathan Davis, and Dan Warren. The Executive Committee (EC) will provide day-to-day leadership of the trial and make recommendations on issues such as study documents, protocol revisions, and issue resolution. The CC works with the EC to implement any decisions of the EC.

Clinic IRB with an FWA/Collecting IRB Approvals and Renewals

The CC has collected the Federal Wide Assurance (FWA) from the Office of Human Research Protections and the contact information on file for all participating S-CitAD institutions. All RAFT sites are expected to participate with the single IRB (sIRB) at Johns Hopkins. In all cases, the CC will require documentation of IRB approval for each site's activities and will retain copies of all approval letters and approved consent statements. The CC will review all consent statements to assure the presence of all required elements. Participating sites will not be authorized to begin recruitment activities or given access to the data and project management system until certified by the CC. Clinic certification will require CC review of the IRB approval, documentation of staff training in general clinical research methods, assessment of staff knowledge about RAFT practices and policies, and other activities considered study prerequisites by the EC.

Procedures for Providing Local Sites with Current Protocol and for Requesting IRB Approval of Updates

The CC communicates using memoranda called Policy and Procedure Memoranda (PPMs) that originate from the DCC. All memos emanating from RAFT that deal with study documents, policy, or design issues will be numbered sequentially and distributed as a PPM. The intent of this system is to facilitate clear, documented communications about important issues that arise during the course of the study and to help keep track of RAFT memoranda that communicate issues of special importance, including protocol changes and important study procedures. All protocol and study document changes will be communicated via a PPM.

All PPMs have a core email distribution list. For clinical centers, the core distribution list includes the site Director and the site lead study coordinator. The Coordinator is responsible for distributing the PPM to any other study personnel who are not included in the distribution list, as appropriate. PPMs will also be posted on the RAFT study a cloud file sharing and storage website (Dropbox) for later reference. The first PPM sent by RAFT will detail the PPM system and the responsibilities of site lead coordinators to distribute PPMs to all study staff.

Plan for Collecting, Managing, and Protecting Data at the Coordinating Center

Below is the plan for data collection:

- Distributed data entry will be conducted by the study sites via a web-based data and project management system (REDCap).
- Study staff will be able to enter and edit data from any computer connected to the internet.
- Error and consistency checks will be built into the data entry application.
- The data entry application and clinical data will be secured with a password. Username, dates, and times are stored for all entries and edits to implement complete data element attribution stored in the database.
- The data and project management system includes numerous security features to prevent unauthorized access to data and maintains a full audit trail (including a system through which data are never deleted).
- Data will be stored in redundant databases residing on a web server in a password-protected area. Data will be archived daily to an off-site computer.
- The CC will conduct audits of clinical center data during the course of the trial.

Study sites will collect information only from consented participants. Data will be entered into an EDC system. Data will be keyed into the web-based data management system using TLS encryption during transmission of data from the study clinics to the system and employing numerous security controls on the server, including staff-specific passwords for system access. The study data are identified by patient code only; names, social security numbers and addresses are not listed on any forms that are entered into the database. The link between study identifiers and personal identifiers such as name and address will be available only at the enrolling study clinic and not stored in any RAFT database.

Data are stored on a secure server accessible to study clinics through the study web page controlled by password protection. Access to the web page will be controlled by methods only study personnel being granted access to the study web page, limiting access to the website itself, and an individual username/password combination will be required for access to the data management system. The data management system automatically logs a user out of the system after a period of inactivity (usually 20 minutes). Study clinic staff will be trained on the proper creation and use of passwords and the importance of security procedures. Only authorized personnel will be granted access to the physical and electronic study data. Electronic data are not stored on any local computer storage devices at the study clinics, including laptops, CD, USB keys, etc. REDCap is HIPAA-compliant. The CC will train staff on proper procedures for maintaining the security of the study data such as proper password creation and handling, physical security of documents, and common security and confidentiality vulnerabilities.

Brain Injury Outcomes Neuroimaging Center (BIONIC) will be responsible for collecting MRI and ultrasound images that are uploaded to the REDCap EDC. BIONIC staff will download patient images from the EDC, strip all PHI through a standardized anonymization process, and archive the de-identified scans on a locally-housed and secured data-repository hard drive only accessible to BIONIC staff. Once downloaded and archived, the images will then be deleted from the REDCap EDC. The images will be maintained in BIONIC's secured repository for the remainder of the trial. The details of this de-identification and storage workflow are outlined in Section 5.4 of the RAFT Manual of Operations and Procedures (MOP).

Process for Reporting and Evaluating Events

Study personnel will have frequent contact with participants both by phone and in-person. Patients will be monitored regularly for signs or symptoms of adverse events. All adverse events will be handled by the study staff or by consultation with the study chair. Serious adverse events will be reported to the CC within 24 hours. The CC will be responsible for coordinating communication about adverse events in

compliance with study policies and each site will submit information about serious adverse events to their IRBs according to the local IRB requirements. The CC will also track the serious adverse event reporting and query the study clinics for clarification or follow-up information regarding specific reports.

Principles of Data Collection and Reporting of Adverse Events

- Data are to be collected for all serious (according to FDA definition) adverse events, including deaths.
- Data collection on adverse events will take two forms:
 1. The primary mode of adverse event data collection will be via systematically asked, close-ended questions regarding known or expected side effects or complications of the study treatment. These data are to serve as the primary safety data presented to the DSMB and to responsible IRBs/research ethics boards (REBs) of participating institutions for the study population as a whole (not broken down by treatment).
 2. The second mode of data collection will be report forms for individual serious adverse events, including deaths; these are to serve as the documentation for reporting to responsible IRBs/REBs and to the sponsor, and will be provided to the DSMB as well.
 - Reports are to be completed for all serious adverse events, including deaths, regardless of presumed association with study treatment.
 - Reports are to be submitted to the Coordinating Center within 24 hours of knowledge of the adverse event.
- Events meeting the FDA guidelines for expedited reporting will be reported by the Coordinating Center using information on the FDA MedWatch voluntary form. The following website provides information on adverse event reporting via MedWatch for FDA:
<http://www.fda.gov/Safety/MedWatch/HowToReport/ucm085568.htm>.

Responsibilities

Clinical investigator responsibilities include:

- Accurate and systematic data collection for potential side effects and complications contained in data collection forms;
- Determination of severity of events and of presumed association with study treatment;
- Prompt completion and submission to the Coordinating Center of report forms for serious adverse events, including serious adverse events that led to a death;
- Investigation and follow-up of study-related adverse events as needed;
- Knowledge of the investigator's responsible IRB policies and adherence to them;
- Submission to the responsible IRB/REB of reports for deaths and serious adverse events that occur at the investigator's institution, unless instructed to do otherwise by the responsible IRB;
- Annual reporting of all adverse events to the responsible IRB/REB as per local policies; and
- Maintenance of study documentation regarding data collection and reporting.

Medical monitor responsibilities include:

- Consultation on medical management of participants, including the proper completion of SAE reports.
- Provide trial safety reports to the DSMB.

Coordinating Center responsibilities include:

- Design of data collection forms in accordance with principles stated above;

- Analysis of data and preparation of reports by treatment group for the DSMB;
- Preparation of reports of aggregate data (not broken down by treatment) for dissemination to clinical centers and ultimately to IRBs/REBs, following DSMB meetings; and
- Timely forwarding of reports for serious adverse events, including deaths, to clinical centers, the DSMB, regulatory agencies, IRBs when appropriate, and NIH.

Procedures for Reporting Protocol Deviations

- Protocol deviations originating from the clinics are usually documented on our Unusual Event form (UE); we request that the clinics send these to the CC within 2 working days.
- Protocol deviations will be reported to the responsible IRB per the JHU reporting requirements (see Table 4).
- Summary tables of deviations will be included in performance reviews to the DSMB and the EC.

We will monitor protocol deviations for purposes of patient safety, quality control, training, and reduction of future errors. In addition to those submitted on UE forms, protocol deviations may also be detected from submitted data, data queries and audits, and site visit findings. The Coordinating Center will report serious protocol deviations to the sIRB and DSMB. The study clinics will also be asked to report protocol deviations their responsible IRBs, if applicable. Protocol deviation reports will be included in study meeting books and reviewed by the EC as a measure of study clinic performance. At the Coordinating Center we would evaluate the seriousness of the event in consultation with the EC.

Compliance with the Study Protocol: Procedures on Monitoring, Review, and Remediation

A site may be terminated due to poor recruitment, poor performance, or evidence of scientific misconduct. Performance monitoring will be continuous throughout the trial via both remote monitoring and site monitoring. AEs will be monitored by the DCC remotely as will the other clinical data. The DCC has the option of performing additional site monitoring visits if indicated by concerns raised through other monitoring mechanisms. The CC will also hold periodic coordinator conference calls to share information about the study, to provide additional training as needed, and to foster communication about best practices and problems that have been noted. Site performance (including but not limited to recruitment, follow-up visit completion, data entry progress, protocol deviations, site visit summaries) will be presented to the DSMB and the EC for review and comment. Either committee can recommend further investigation into performance at a particular site by the EC. The CC will gather information needed (including additional audits or site visits, if necessary) to prepare summary reports upon request for review by the EC. The EC may suggest no further action, a plan for remediation and re-evaluation (coordinated with the site in question), or termination of the site after review.

8.3.6 REPORTING EVENTS TO PARTICIPANTS

Participants are informed of the level of amniotic fluid at each serial amnioinfusion and ultrasound measures. Because they are also being clinically followed for their high-risk pregnancy, any concerning symptom or sign will be evaluated immediately, any AE or SAE will require possible admission to the hospital, the participant will be directly informed of the status of all events during her pregnancy and events occurring during study-related procedures.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems (UPs) involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in IRB-approved research protocol and informed consent document(s); and (b) the characteristics of the participant population being studied.
- Related or possibly related to participation in the research.
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

All unanticipated and anticipated events will be recorded on the appropriate CRFs. The participating RAFT site investigator will report UPs to the DCC and Co-PIs Meredith Atkinson, MD, MHS and Jena Miller, MD, per the timelines listed in Table 4. The DCC will report UPs to the Johns Hopkins Medicine Institutional Review Board (IRB) and to the DSMB as outlined in Table 4. The UP reports will include the following information:

- Protocol identifying information: protocol title and number, PI name, and IRB project number.
- A detailed description of the event, incident, experience, or outcome.
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP.
- A description of any corrective actions taken or proposed in response to the UP.

Table 4. Reporting of SAEs, AEs, and unanticipated research-related problems

Problem Type	IRB Reporting deadline	Multi-center participating sites	What to submit
Death of research participant (mother) (within 30 days of study intervention)	Unexpected: within 3 working days after PI is aware of death Expected: as soon as possible within 10 working days	Inform leading site PI as soon as participating PI is aware of the death	IRB protocol event report DSMB meeting
Death of research participant more than 30 days after stopping study	At time of continuing review	Inform leading site PI within 6 weeks of continuing review deadline	IRB continuing review protocol event summary sheet

Emergency deviation – required to immediately protect the life or physical well-being of a participant	As soon as possible, no later than 5 days after the emergency situation occurs	Inform leading site PI at the time of emergency deviation	IRB protocol event report DSMB meeting
Unanticipated Research problems (UPs) that affect risk to participant or others	As soon as possible, no later than within 10 working days	Inform leading site PI at the time of the unanticipated event	IRB protocol event report DSMB meeting
Anticipated events	At time of continuing review	Inform leading site PI within 6 weeks of continuing review deadline	event report summary sheet DSMB meeting
Minor protocol and administrative departures	At time of continuing review	Inform leading site PI within 6 weeks of continuing review deadline	protocol deviation summary sheet
Major – non-emergent deviation	IRB approval must be obtained before change is implemented	Leading site will be notified of suggested change; all changes will be made through leading site	IRB Change in Research DSMB meeting
Use of device/intervention without informed consent	Within 5 working days of event	Participating site informs leading PI at time of event	IRB Protocol event report DSMB meeting
Minor – non-emergent deviation – one that does not affect the scientific soundness of research plan, rights, safety, or welfare of human subject	Submitted at time of continuing review to the IRB	All changes will be made through leading site	IRB annual continuing review event report
Event of non-compliance	Submit at time of non-compliance event	Participating site report at time of non-compliance event to leading site PI	IRB written report (informal or formal submission)
Withdrawal of IRB approval	Within 5 working days	Leading site PI will notify all participating sites at time of withdrawal notice	Notice to all participating sites

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Participants will be contacted about unanticipated problems that affect safety to participants so that they may be fully informed to determine if they wish to continue or not continue their consent to participate in RAFT study-related procedures.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Primary Objective:

- 1) To determine the proportion of neonates surviving to ≥ 14 days and placement of dialysis access after serial amnioinfusions.

Secondary Objectives:

- 2) To determine the feasibility of serial amnioinfusions for EPRA.
- 3) To perform an exploratory study of the in-utero natural history of untreated EPRA.
- 4) To examine correlations between fetal ultrasound, echocardiogram MRI biomarkers as well as amniotic fluid biomarkers, and the success of RAFT for EPRA.
- 5) To determine short-term and long-term quality of life of patients and families that undergo RAFT.

9.2 SAMPLE SIZE DETERMINATION

Sample size determination for this prospective study is based on the primary outcome variable: survival rate to successful dialysis in the intervened upon CoBRA and FRF groups, respectively. Survival to successful dialysis will be a single endpoint defined as effective use of a dialysis catheter for greater than 14 days. For efficacy of serial amnioinfusions we used exact methods to calculate 95% confidence intervals around survival proportions from 0.2-0.8 (Table 5).

Table 5. Sample size calculations at survival proportions from 0.2-0.8 and associated 95% confidence intervals

Sample Size	Survival Proportion	95% Confidence Interval
35	0.2	0.05-0.35
35	0.3	0.14-0.46
35	0.4	0.24-0.56
35	0.5	0.33-0.67
35	0.6	0.43-0.77
35	0.7	0.54-0.86
35	0.8	0.66-0.96

For example, based on these calculations, a sample size of 35 maternal/fetal pairs in the intervened upon CoBRA and FRF groups, respectively, is sufficient to show the true proportion of infants with successful dialysis is greater than 30% if the observed rate in the study is 50% or greater.

Participant dropout rates are expected to be low, but increased recruitment will be necessary to compensate for those that dropout, or for participants in the intervention group who are noted to have an additional clinically relevant fetal anomaly identified after enrollment in the study. We anticipate that recruitment will be assisted by the North American Fetal Therapy Network's support of this protocol and prevent non-study regulated intervention for this disease. Of the patients screened, we expect 75% of

patients to be eligible for the trial. After appropriate counseling, we expect 50% of patients to elect termination. Patients lost to follow-up before delivery will be excluded from the study and not included in any analysis of the primary outcome variable.

Our target recruitment for the entire study is 70 maternal/fetal pairs in the intervention group (35 CoBRA and 35 FRF). This number is based on the needed 35 pairs in both **intervention** groups to determine success rate of the primary outcome variable from 20-80%. Therefore 70 pairs are required for the intervention group. Our goal is to have an additional pairs enroll in the expectant management group for both CoBRA and FRF. Enrollment in the expectant management group does not have a target N based on our primary outcome variable, but rather will provide a prospective detailed descriptive analysis of this group.

9.3 POPULATIONS FOR ANALYSES

- **Intention-to-Treat (ITT) Analysis Dataset:** All patients who enroll and elect intervention and undergo at least one therapeutic amnioinfusion.
- **Isolated ERPA Analysis Dataset:** All patients who elect intervention and undergo at least one therapeutic amnioinfusion, and excluding any participants diagnosed with any additional clinically relevant fetal congenital anomalies after enrollment in the study.
- **Safety Analysis Dataset:** All patients who receive any amount of amnioinfusion will be included in this data set.
- **Per-Protocol Analysis Dataset:** All patients who received amnioinfusions per the protocol described intervention.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Descriptive statistics will be used to present the data and to summarize the results. Categorical variables will be presented using counts and percentages. Proportions will be calculated using known non-missing values. Continuous variables will be summarized by presenting the number of observations (N), mean, 2-sided 95% confidence interval of the mean, standard deviation, median, minimum and maximum values. Unless otherwise indicated, all statistical tests and/or confidence intervals will be performed at $\alpha = 0.05$ (2-sided).

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary efficacy endpoint in this study is the proportion of neonates who survive to ≥ 14 days and placement of dialysis access.

The number and percentage of neonates who survive to the primary outcome as well as the exact confidence interval will be presented for CoBRA and FRF patients separately in the intervention group. Patients (mothers) lost to follow-up will be excluded from the study and not included in any analysis of the primary efficacy outcome variable.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

The feasibility of serial amnioinfusions for EPRA will be determined using the number and timing of infusions as well as the interval after the initiation of amnioinfusions before rupture of membrane. Descriptive statistics will be used to summarize this information for the CoBRA and FRF groups separately.

The data collected from the nonintervention group will not be used for direct comparison of the survival rates. These contemporaneous control groups will be critical to understanding the natural history of this disease in-utero. For patients that switch to the expectant management group of the trial we will use an intention to treat paradigm for analysis.

The number and percentage of neonates who survive to the primary outcome as well as the exact confidence interval will be presented for the expectant management group.

Time to discharge and time to kidney transplant will be summarized using survival methods. Data will also be presented as mean survival with confidence intervals. Missing survival data will eliminate patients from appropriate secondary outcome analysis.

The correlations between fetal ultrasound, echocardiogram, and MRI biomarkers as well as amniotic fluid biomarkers and the success of RAFT for EPRA will be assessed.

Data on quality of life of patients and families that undergo RAFT will be collected using the PEDs QL™ Pediatric Measurement Model for the Pediatric Quality of Life Inventory Scores™ (Lyon, France).

9.4.4 SAFETY ANALYSES

AEs will be coded according to the Medical Dictionary for Regulatory Activities. Each AE will be counted once for a given participant. The relationship of AEs to serial amnioinfusions will be reported in relationship to timing of amnioinfusion, start date, stop date, severity, expectedness and outcome. AEs that would lead to premature discontinuation from the study intervention are:

- Chorioamnionitis
- Severe maternal hemorrhage
- Amniotic fluid embolism
- Fetal death
- Hydrops Fetalis
- Placental abruption
- Severe maternal illness
- Maternal death

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Baseline information will be collected in the intervention and expectant management arm including age, gestational age at diagnosis, number of other children, and demographics. Descriptive statistics, as described in Section 9.4.1, will be generated for baseline demographics, procedural characteristics and follow-up data collected.

9.4.6 PLANNED INTERIM ANALYSES

We will perform two interim analyses of the primary endpoint of neonate survival to successful dialysis defined as survival to ≥ 14 days of and placement of dialysis access. The proposed interim analyses will be after approximately 9 FRF and 17 CoBRA infants are enrolled, which is roughly 25% and 50% of the total expected sample size of 35, respectively. An additional interim analysis will be performed once 50% of the expected FRF sample size has been enrolled.

Based on an alpha spending function determined by the method of Lan and DeMets (1983), the boundaries for early stopping of the trial due to efficacy at the first 'data look' are ± 4.3326 (corresponding to an $\alpha = 0.00001473$), and the boundaries for early stopping of the trial at the second 'data look' are ± 2.9631 (corresponding to an $\alpha = 0.003051$). Finally, the boundaries for the final analysis of the data when the FRF enrollment is 35 are ± 1.9686 (corresponding to an $\alpha = 0.05$).

At each look, we will compare the proportion of survival ≥ 14 days and placement of dialysis access between either the FRF or CoBRA group (whichever is lower) against an expected survival of 30%.

9.4.7 DATA AND SAFETY MONITORING BOARD

The centralized DSMB will monitor the accumulating safety, feasibility, and efficacy data. The DCC will make a limited dataset available to the DSMB for review. The DSMB will also be consulted for any maternal or fetal safety concerns that arise during the study. We expect approximately 20-50% success for our primary outcome variable in the intervention group. In the patients who do not undergo interventions, we expect a 0% success rate for our primary outcome variable. If survival to successful dialysis is less than 20% in the intervention group (0 out of 5), the DSMB will consider recommending study termination. If survival to successful dialysis in the nonintervention group is 20% or higher (1 or more neonates), study modification will be considered. Regarding maternal safety, in the case of a single maternal death or significant medical or surgical complication, although that is extremely unlikely, recruitment will be suspended until the DSMB has reviewed the case. Regarding fetal safety, in the case of peri-procedural intrauterine fetal demise, recruitment will be suspended until the DSMB has reviewed the case. See Section 10.1.2 for further details about the DSMB.

9.4.8 SUB-GROUP ANALYSES

Confidence intervals, survival curves and summary descriptive statistics will be presented separately within each group by CoBRA versus FRF.

9.4.9 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual participant data will be tabulated.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

Only the local principal investigator or his or her physician consent designee(s) will obtain informed consent. They will ensure that all of the appropriate consultants listed in the inclusion criteria have met and counseled the patient. Consent will be obtained in a private room away from patient care areas. During the consenting process, maternal, fetal, neonatal and childhood risks will be fully explained. The uncertainty of serial amnioinfusion success will be discussed. Furthermore, the postnatal challenges if survival is achieved will be discussed in detail. These challenges will be discussed by the many consultants who will be involved before consent, but will also be discussed by the investigators obtaining informed consent. These challenges include the need for dialysis, the likelihood of complications from dialysis access, the need for supplemental nutrition likely by gastrostomy tube, the risk of sepsis, recurrent hospitalizations, the eventual need for kidney transplant, the requirement for immunosuppression after a kidney transplant and the likely need for urinary tract reconstruction. Furthermore, the financial and logistical challenges of insurance copays, time away from work, and the need for temporary relocation near a RAFT center will be discussed. We have explored the ethical framework for offering this therapy in a national ethics symposium that reported its findings in *Obstetrics and Gynecology*.¹⁴ This trial was designed in accordance with the key issues identified at the symposium and published in our manuscript.

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

The Johns Hopkins central IRB will approve all consent forms used at all participating RAFT centers. The consent forms will describe in detail the study intervention, study procedures, and risks. These forms will be given to all participants and signed before any study related amnioinfusions are begun. There will be a study site specific page for local contact information and regulations. Once the baby is delivered, the consent process will be repeated on a separate newborn consent form to collect study data after birth until four years after transplantation. Both prenatal consents will provide for the ability to collect data on the mother and the neonate until day of life 15. Appropriate interpreters will be provided to non-English speaking patients so that full informed consent can be obtained.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Consent forms will be approved by the Johns Hopkins Institutional Review Board. Consults provided by Pediatric Nephrology, Neonatology, Transplant Surgery, Pediatric Surgery, Maternal-Fetal Medicine Specialist, Genetic Counselor, and a Licensed Clinical Social Worker will have taken place in a nondirective and supportive environment in the fetal clinic at the RAFT center. Only the principal investigators or their physician designees will obtain informed consent. They will ensure that all of the appropriate consultants listed above have met and counseled the patient. Consent will be obtained in a private room away from patient care areas. During the consenting process, maternal, fetal, neonatal and childhood risks will be fully explained. The uncertainty of serial amnioinfusion success will be discussed. Furthermore, the postnatal challenges if survival is achieved will be discussed in detail. These challenges

will be discussed by the many consultants who will be involved before consent, as listed above, but will also be discussed by the investigators obtaining informed consent. These challenges include the need for dialysis, the likelihood of complications from dialysis access, the need for supplemental nutrition likely by gastrostomy tube, the risk of sepsis, recurrent hospitalizations, the eventual need for kidney transplant, the requirement for immunosuppression after a kidney transplant and the likely need for urinary tract reconstruction. Furthermore, the financial and logistical challenges of insurance copays, time away from work, and the need for possible temporary relocation near the RAFT center will be discussed. Both prenatal consents will allow the study team to capture data up to 15 days after the delivery of the baby. The parents will receive the consent form during screening and will have ample time to review it and ask questions. The patient will be asked to repeat back the critical counseling points to ensure understanding. A social worker will also counsel the patients and ensure understanding. Interpreters will be used for non-English speakers and those with language/hearing impairments. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date and time), and the form signed, before the participant undergoes any study-specific procedures.

Once the child is born and determined to be viable, we will again use the same methodology to ensure understanding and obtain consent from the mother to follow the child beyond the initial 15 days of life and contact the family, to continue prospectively following the outcomes of the neonate. The principal investigator or his or her physician consent designee(s) will obtain this consent. A private space away from the neonates' care will be utilized.

Study Discontinuation and Closure

If the study is prematurely terminated or suspended, the PI will promptly inform all participating RAFT sites, all study participants, and the Johns Hopkins Institutional Review Board (IRB), and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

10.1.2 DATA AND SAFETY MONITORING BOARD

Three independent physicians familiar with the conduct of fetal trials, but not involved in this study, will comprise a data and safety management board (DSMB) at one or more NAFTNet centers that are not participating in the trial. Additionally, a statistician and ethicist that are not from a participating center will be appointed to the DSMB. The DSMB will report to the JHU IRB as well as the RAFT study PIs Drs. Atkinson and Miller.

Our primary outcome variable is neonatal survival to successful dialysis. This data will be examined by following the care of neonates with EPRA born at a RAFT center. The DSMB will meet and review the data after five neonates are enrolled in the intervention group and reach the primary endpoint. Thereafter, the DSMB will meet within 10 business days after every cohort of 10 participants reaches the primary outcome variable. If 10 participants are not enrolled within a year, the DSMB will meet at least biannually.

The DSMB will also be consulted for any adverse events (AE) as well as maternal or fetal safety concerns that arise during the study at any time. AEs are defined in the IRB protocol and will be reported by the local PI to the lead PI and the DCC. The lead PI will communicate the AEs to the DSMB and the JHU IRB for review. We expect approximately 50% success for our primary outcome variable for participants that undergo RAFT. If there are no survivors in either group after serial amnioinfusions at the first interim analysis, the DSMB will be empowered to alter the protocol or halt the trial. Regarding maternal safety, in the case of a single maternal death or significant medical or surgical complication, although that is extremely unlikely, recruitment will be suspended until the DSMB has reviewed the case. Regarding fetal safety, in the case of peri-procedural intrauterine fetal demise, recruitment will be suspended until the DSMB has reviewed the case. Additionally, if recruitment is low, the DSMB will be empowered to recommend modification of the sample size, the study design, or terminate the study for inadequate accrual or data quality. The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the PI, DSMB, and IRB.

10.1.3 CONFIDENTIALITY AND PRIVACY

All research activities will be conducted in as private a setting as possible. Clinical data about interventions during pregnancy, ultrasound findings, adverse events, survival, dialysis, respiratory support, nutritional support, complications, medicines, kidney transplant and long-term survival will be collected. Prenatal and in-hospital data, which will contain individually identifiable private information, will be kept on the principal investigators' secure computers in a locked research office at the RAFT center. The study ID will be the link to the participant. These computers will be behind a firewall, are encrypted, and are password protected. Only principal investigators and the research coordinator will have access to the data. Post-hospital data will be tracked using the NAPRTCS registry infrastructure, which utilizes Emmes. Emmes is experienced in the implementation of privacy and security safeguards that require centers to satisfy the anticipated conditions of acquiring and transferring confidential information. This activity includes the protection of subject privacy, as well as data integrity and security during electronic transmission. Policies and procedures are in place to address these needs. Authorized users can access only directories and data files that have been specified by the network supervisor or the file's owner, according to project responsibilities. Emmes' network administrators tightly control access to the file system. New users are given access gradually to project areas as their skill levels are demonstrated and as work needs dictate. Using trustee security, users are given full access to data, restricted from knowing the data exist, or granted some level of access between these extremes. Database systems provide dynamic data encryption for sensitive data. When required, sophisticated encryption techniques have been coded directly into software applications to encrypt/decrypt data, as they are being entered and displayed. This technique is used frequently for confidential reports and study participant data. All virtual access to the network is restricted to authorized personnel only and is accomplished through a combination of hardware, software, and process security controls that have been implemented at various levels in the network. For internal security, Microsoft Windows Active Directory provides multiple levels of logical data security. Secure Computing token authentication in conjunction with login/password security controls access to the network components. The user's login name, password and token challenge code must all be valid or access is denied.

In addition, Emmes enforces strict physical security for application and data servers, paper files, and backup tapes. All confidential material is maintained in separately locked rooms. Access to the network equipment and storage media is restricted to personnel requiring access to perform contract work.

No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, and representatives of the Johns Hopkins Institutional Review Board (IRB) may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The participating RAFT study site will permit access to such records.

At the end of the study, all records will continue to be kept in a secure location for at least seven years dictated by the reviewing Johns Hopkins IRB and institutional policies.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored in REDCap, a mature, secure web application for building and managing online surveys and databases, at the Johns Hopkins University. This data will be stored for seven years after the completion of the study.

With the participant's approval and as approved by the Johns Hopkins sIRB, biological samples will be collected with each amniocentesis for protein and lipid analysis. De-identified biological samples will be stored in a laboratory at a Johns Hopkins Medicine facility. These samples could be used to research the causes of congenital renal agenesis, its complications, and to improve treatment. Each sample will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, thereby maintaining the blinding of the identity of the participant.

At any point during the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to bio-sample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the Johns Hopkins Hospital.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Lead Principal Investigator	Medical Monitor	Lead of DCC
Meredith Atkinson, MD, MHS	Arthur Vaught, MD	Dan Hanley, MD
Johns Hopkins Hospital	Johns Hopkins Hospital	Johns Hopkins University Trial Innovation Center
200 N. Wolfe St., Room 3064 Baltimore, MD 21287	600 N. Wolfe Street Baltimore MD, 21287	750 East Pratt St. Baltimore, MD 21202
410-955-2467		410-614-6996
matkins3@jhmi.edu	avaught2@jhmi.edu	dhanley@jhmi.edu

The internal monitor will oversee and follow participant clinical course and trigger required alerts to the IRB and DSMB as required. The data coordinator will ensure all data is accurately and completely

entered into the database. The research coordinator will ensure all required forms and reports are accurately completed and submitted in a timely manner.

10.1.6 SAFETY OVERSIGHT

The DSMB will review trial results and will be consulted at any time if safety concerns arise during the conduct of the research study. All RAFT cases will be discussed at interdisciplinary meetings at multiple time points throughout study participation. An independent broad range of specialists will address the clinical status of participants (before first amnioinfusion), the clinical progression (during amnioinfusion intervention period and after amnioinfusions are completed) and clinical outcome of the participant (post-delivery, at time of dialysis and transplantation). Along with other clinical cases of note, the team will review the current imaging, laboratory notes, and particularly the treatment of all adverse events of the patient at all points during their care. The dates on which the meetings take place, the main points addressed, and any suggested actions will be recorded.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, and that the reported trial data are accurate, complete, and verifiable.

- Monitoring for this study will be performed by the local PI at each RAFT site, the lead PI, the JHH institutional compliance team, the medical monitor, and the DSMB.
- Monitoring at each RAFT center will be the responsibility of the local PI. The site will communicate any SAE to the lead PI who will in turn report to the medical monitor and DSMB.
- The principal investigator and research coordinator at each RAFT center will provide logs of all research events and deviations to the lead RAFT center. Each RAFT site will be provided copies of centralized monitoring reports that are written by the DSMB, IRB, and medical monitor.
- Details of clinical site monitoring are documented in a Clinical Monitoring Plan. The plan describes in detail who will conduct monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and how monitoring reports will be distributed.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

The designated PI at the participating RAFT center will delegate review of participant research folders by the data coordinator and research coordinator to ensure file completion and accuracy. The internal monitor will oversee and follow participant clinical course and trigger required alerts to the DCC, IRB, and DSMB as required. The PI and research coordinator and assigned administrative coordinator will ensure all study members have completed required research training modules, HIPAA training, and compliance/conflict of interest training. All CVs and applicable licensing will be stored on electronic file prior to participating in any study activities. The principal investigator will assign responsibilities to other study team members as necessary and within their scope of practice.

The Johns Hopkins School of Medicine Institutional Review Board's Compliance Monitoring Program in the Office of Human Subjects Research will begin monitoring activities for this study before the first participant is enrolled into the study and at six months after the start of enrollment. This may be

amended, and a tapered approach to monitoring may be employed, if conduct and documentation of the study reaches a level of reliability that would permit valid conclusions based upon a sampling of data. Monitoring reports will be provided to the principal investigator. The DSMB and/or Johns Hopkins IRB will be notified if required.

Each additional participating RAFT site will perform internal quality management of study conduct, data and biological specimen collection, documentation, and completion. Each RAFT site will be responsible for developing its internal monitoring plan. The participating RAFT sites will provide a report of their internal monitoring to the leading RAFT site (JHH) and to lead PI Meredith Atkinson, MD, MHS, if requested.

The DSMB will convene after five participants have been enrolled across all sites and reach the primary endpoint. The DSMB will review trial results and will be consulted at any time if safety concerns arise during the conduct of the research study.

All JHH RAFT cases will be discussed at interdisciplinary meetings at multiple time points throughout study participation. A broad range of specialists will address the clinical status of participants (before first amnioinfusion) the clinical progression (during amnioinfusion intervention period) and clinical outcome of the participant (post-delivery, at time of dialysis and transplantation). Along with other clinical cases of note, the team reviews the current imaging, laboratory notes, and particularly the treatment of all adverse events of the patient at all points during their care. The dates on which the meetings take place, the main points addressed, and any suggested actions will be recorded. All participating RAFT sites will be encouraged to develop a clinical panel review, as well, as part of safety and data quality management.

The leading RAFT site (JHH) will be provided direct access to all trial-related participating RAFT sites, source data/documents, and reports for the purpose of monitoring and auditing by the lead PI, sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Prenatal data will be collected by each RAFT site study team using standardized intake forms, a screening log, and standardized CRFs for each patient visit. Postnatal hospital data will similarly be recorded on CRFs by the study team at time of discharge or death. These data points will be securely uploaded to a REDCap database hosted by Johns Hopkins University, which will serve as the DCC. AEs will be reported on standardized AE forms at each center and sent to the lead PI who will share them with the DSMB and IRB.

Data collection is the responsibility of the approved clinical study staff at each RAFT site under the supervision of the RAFT site principal investigator. Each RAFT site principal investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All physical study related documents will be stored in a secure locked research office. All electronic data and documents will be stored on a restricted access, password-protected, secure REDCap database housed at the Johns Hopkins University. All source documents will come from the medical chart of the participants. All clinical procedures, observations, and decisions will be recorded in the participants' medical chart. A copy of approved and signed consent forms will be uploaded to the participants' electronic medical chart. Clinical data and clinical laboratory data will be entered into the secure REDCap EDC system. The system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Data will be entered directly from the source documents. Participating RAFT sites, after IRB approval, will be given appropriate access to the REDCap database to enter data. Only the lead PI and IRB-approved study staff will have access to the

study documents and data. Each site will have access to its own dataset. Each RAFT site will be assigned unique number, and all RAFT participants will be assigned a separate unique study ID.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of two years after the completion of the study. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the principal investigator of the designated coordinating RAFT center.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical study protocol. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

All deviations must be addressed in study source documents to the lead RAFT center at JHH.

Each RAFT site's internal monitor (the site PI) will delegate review of participant research folders by the data coordinator and research coordinator to ensure file completion and accuracy. The internal monitor will monitor and follow participant clinical course and trigger required alerts to the DCC, IRB, and DSMB, as requested. Protocol deviations and all adverse events descriptions that would trigger a report and review are listed in Section 8.3 and 8.4 (see Table 4). All actions and events will be recorded in the protocol deviation and adverse event logs.

The DSMB will convene after five participants reach the primary endpoint. The DSMB will review trial results and will be consulted at any time if safety concerns arise during the conduct of the research study. The outcome summary after five patient procedures was submitted to the Johns Hopkins IRB for review, and approval was given to continue enrollment. Subsequent fetal oversight meetings after the initial review of the protocol will be at the discretion of the committee.

10.1.11 PUBLICATION AND DATA SHARING POLICY

The study is registered at ClinicalTrials.gov, and the results from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers up to seven years after the completion of the primary endpoint by contacting Dr. Meredith Atkinson, the lead PI.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the IRB has established policies and procedures for study group members to disclose conflicts of interest and will establish a mechanism for the management of all reported dualities of interest. All conflicts of interest of participating RAFT centers will be managed locally. Any identified financial conflicts of interest will be communicated to the IRB for review.

10.2 ABBREVIATIONS

AE	Adverse Event
AFI	Amniotic Fluid Index
CoBRA	Congenital Bilateral Renal Agenesis
CRF	Case Report Form
DCC	Data Coordinating Center
DSMB	Data and Safety Monitoring Board
EC	Executive Committee
EDC	Electronic Data Capture
EPRA	Early Pregnancy Renal Anhydramnios
FDA	Food and Drug Administration
FRF	Fetal Renal Failure
GA	Gestational Age
HD	Hemodialysis
HIPAA	Health Insurance Portability and Accountability Act
IRB	Institutional Review Board
MRI	Magnetic Resonance Imaging
MVP	Maximum Vertical Pocket (in cm)
NAFTNet	North American Fetal Therapy Network
NAPRTCS	North American Pediatric Renal Trials and Collaborative Studies
NCT	National Clinical Trial
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PD	Peritoneal Dialysis
PEDs QL	Pediatric Quality of Life Inventory
PHI	Patient Health Information
PI	Principal Investigator
PPM	Policy and Procedure Memoranda
PPROM	Premature Preterm Rupture of Membranes
RAFT	Renal Anhydramnios Fetal Therapy
REB	Research Ethics Board
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
UP	Unanticipated Problem

11 REFERENCES

1. Hislop A, Hey E, Reid L. The lungs in congenital bilateral renal agenesis and dysplasia. *Arch Dis Child*. 1979;54(1):32-38.
2. Whitehouse W, Mountrose U. Renal agenesis in nontwin siblings. *Am J Obstet Gynecol*. 1973;116(6):880-882.
3. Novikova N, Hofmeyr GJ, Essilfie-Appiah G. Prophylactic versus therapeutic amnioinfusion for oligohydramnios in labour. *Cochrane Database Syst Rev*. 2012;(9):CD000176. doi(9):CD000176.
4. Bienstock JL, Birsner ML, Coleman F, Hueppchen NA. Successful in utero intervention for bilateral renal agenesis. *Obstet Gynecol*. 2014;124(2 Pt 2 Suppl 1):413-415.
5. Cameron D, Lupton BA, Farquharson D, Hiruki T. Amnioinfusions in renal agenesis. *Obstet Gynecol*. 1994;83(5 Pt 2):872-876.
6. Polzin WJ, Lim FY, Habli M, et al. Use of an amniopore to maintain amniotic fluid volume in fetuses with oligohydramnios secondary to lower urinary tract obstruction or fetal renal anomalies. *Fetal Diagn Ther*. 2016.
7. van Stralen KJ, Borzych-Duzalka D, Hataya H, et al. Survival and clinical outcomes of children starting renal replacement therapy in the neonatal period. *Kidney Int*. 2014;86(1):168-174.
8. Carey WA, Martz KL, Warady BA. Outcome of patients initiating chronic peritoneal dialysis during the first year of life. *Pediatrics*. 2015;136(3):e615-22.
9. Mitsnefes MM, Laskin BL, Dahhou M, Zhang X, Foster BJ. Mortality risk among children initially treated with dialysis for end-stage kidney disease, 1990-2010. *JAMA*. 2013;309(18):1921-1929.
10. Roberts D, Vause S, Martin W, Green P, Walkinshaw S, Bricker L, Beardsmore C, Shaw BN, McKay A, Skotny G, Williamson P, Alfirevic Z. Amnioinfusion in preterm premature rupture of membranes (AMIPROM): a randomised controlled trial of amnioinfusion versus expectant management in very early preterm premature rupture of membranes--a pilot study. *Health Technol Assess*. 2014 Apr;18(21):1-135.
11. Haeri S, Simon DH, Pillutla. Serial amnioinfusions for fetal pulmonary palliation in fetuses with renal failure. *J Matern Fetal Neonatal Med*. 2017 Jan; 30(2):174-176
12. Moessinger AC, Collins MH, Blanc WA, Rey HR, James LS. Oligohydramnios-induced lung hypoplasia: The influence of timing and duration in gestation. *Pediatr Res*. 1986;20(10):951-954.
13. Yoshimura S, Masuzaki H, Miura K, Hayashi H, Gotoh H, Ishimaru T. The effects of oligohydramnios and cervical cord transection on lung growth in experimental pulmonary hypoplasia in rabbits. *Am J Obstet Gynecol*. 1997 Jul;177(1):72-7.
14. Jelin E, Lee H. Tracheal occlusion for fetal congenital diaphragmatic hernia: the US experience. *Clin Perinatol*. 2009 Jun;36(2):349,61, ix.
15. Segev DL, Muzaale AD, Caffo BS, et al. Perioperative mortality and long-term survival following live kidney donation. *JAMA*. 2010;303(10):959-966.
16. Thomas AN, McCullough LB, Chervenak FA, Placencia FX. Evidence-based, ethically justified counseling for fetal bilateral renal agenesis. *J Perinat Med*. 2017 Jul 26;45(5):585-594.
17. Cremeens J, Eiser C, Blades M. Factors influencing agreement between child self-report and parent proxy-reports on the Pediatric Quality of Life Inventory 4.0 (PedsQL) generic core scales. *Health Qual Life Outcomes*. 2006 Aug 30;4:58.