Complement Regulation to Undo Systemic Harm in Preeclampsia: The CRUSH Study

Protocol Number: STUDY 00000039

National Clinical Trial (NCT) Identified Number: pending

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Summary of Changes from Previous Version:

Affected Sections	Summary of Revisions Made	Rationale
1.1. Synopsis 2.3.2. Known Potential Benefits 3. Objectives and Endpoints 4.1. Overall Design 4.2. Scientific Rationale for Study Design 5.1. Inclusion Criteria 5.5. Strategies for Recruitment and Retention	Reduced upper end of gestational age inclusion criteria to 29w6d (from 33w6d) based on FDA guidance The anticipated accrual rate was reduced from 1 subject per month to 1 subject every 2 month. Enrollment period extended from 12 to 24 months.	FDA recommended this change based on the risk of severe infection with eculizumab and limited data to support a benefit in the study population. FDA stated that women at 23w0d-29w6d were more likely to demonstrate a positive benefit:risk profile with eculizumab treatment. Expected accrual rate was modified due to the reduced gestational age criteria recommended by FDA. Due to the rarity of preeclampsia <30 weeks, enrollment is now anticipated over 24 months instead of 12 months.

8.1. Efficacy Assessments **8.2. Safety and Other Assessments** **9.1. Statistical Hypotheses**		
5.2. Exclusion Criteria	**Added the following exclusion criteria:** **1. Neutropenia (<1500/mm3)** **2. Gonorrhea, chlamydia, or syphilis in current pregnancy** **3. Illicit substance use in current pregnancy** **4. Currently homeless or incarcerated** **5. Alcoholism** **6. Liver cirrhosis** **7. Insulin dependent diabetes** **8. Active use of immunosuppressive therapies, other than use of corticosteroids for fetal lung maturity**	**The FDA recommended additional patient exclusions to reduce the participation of women who may be at risk for** **acquiring Neisseria meningitidis, and the development of invasive meningococcal** **disease, as well as Neisseria gonorrhoeae, Neisseria sicca/subflava, and Neisseria spp unspecified and Aspergillus infections**
5.2. Exclusion Criteria	**Added the following exclusion criteria:** **Use of prophylactic or therapeutic heparin, or low molecular weight heparin, in pregnancy for hypercoagulable condition**	**The FDA recommended exclusion of women with increased risk of VTE**
5.2. Exclusion Criteria	**Added the following exclusion criteria:** **Body weight <40kg**	**Eculizumab dosing regimen varies for pediatric patients with body weight <40kg. To maintain dosing consistency for this pilot study of 12 patients, we excluded those with weight <40kg.**
2.3.1. Known Potential Risks	**To minimize risk of infection, our protocol will require meningococcal vaccination with the first dose of eculizumab as well as prophylactic**	**FDA recommended administration of prophylactic antibiotics for 4 weeks after**

2.33. Assessment of Potential risks and benefits	**antibiotics for 4 weeks following the last dose of eculizumab. Finally, subjects will be closely monitored for signs of infection and all participants will be provided a patient safety information card regarding the risk of meningococcal infection. The participant safety card will describe signs and symptoms of meningitis for the patient (e.g., fever, headache and a fever, headache and a stiff neck, etc.) and will provide information for healthcare providers regarding risk of meningococcal infection following C5 blockade with eculizumab. The study drug will be stopped immediately if an infection, other than simple urinary or yeast infection, is suspected. Treatment decisions for known or suspected meningococcal infection will be made in conjunction with infectious disease specialists.**	**the last dose of eculizumab, due to the risk of meningococcal infection.** **FDA recommended additional plans to monitor and address the risk for Neisseria infections, including Neisseria meningitis**
8.3.6. Serious Adverse Event Reporting	**Neisseria meningitidis infection will be reported as an adverse event of special interest (AESI) and will be reported to the FDA and the Data Safety Monitoring Board (DSMB).**	**Revised per FDA recommendation**
8.2. Safety and other assessments	**We added paragraphs for:** **- maternal and fetal assessments** **- duration of treatment** **- indications for delivery** **- criteria for magnesium sulfate therapy** **- management of headache** **- criteria for betamethasone therapy** **- prevention of venous thromboembolism**	**For safety purposes, FDA recommended additional wording regarding:** **- maternal and fetal assessments** **- duration of treatment** **- indications for delivery** **- criteria for magnesium sulfate therapy** **- management of headache** **- criteria for betamethasone therapy** **- prevention of venous thromboembolism**

	- criteria for discontinuation of study drug	**- criteria for discontinuation of study drug**
1.3 Schedule of Activities **8.1 Efficacy assessments**	**Modified schedule of activities and efficacy assessments. In addition to the study visits designated in the table, maternal and fetal assessments will be performed daily by a member of the study team to rule out adverse events or worsening disease**	**FDA recommended daily assessment of maternal and fetal status by a member of the study team**
1.1. Synopsis **4.1. Overall design** **5.1. Inclusion criteria** **10.1.1.2 Informed consent procedures and documentation**	**Maternal age criteria modified. Previously women <18 years old were excluded. In revision, only minors <13 years old were excluded.** **We added a paragraph regarding informed consent among emancipated minors between 13-18 years old.**	**FDA recommended inclusion of adolescents given that they could potentially benefit from this therapy and are not likely to differ substantially from the adult population in relation to safety risks. We agreed and modified the inclusion criteria to include adolescents.**
1.1. Synopsis. **3.0. Objectives and Endpoints**	**Added secondary objective:** **Safety of eculizumab in the setting of early onset preeclampsia between 23+0/7 and 29+6/7 weeks gestation.** **Added secondary endpoint:** **Serious adverse events, including Neisseria Meningitidis infection, and graded clinical and laboratory abnormalities, according to the U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of**	**FDA recommended that we list safety of eculizumab as a secondary objective.**

	AIDS; DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. [July 2017].	
1.1. Synopsis **3.0. Objectives and endpoints**	**Added secondary objective:** **To assess eculizumab pharmacokinetics and pharmacodynamics in pregnant women with early-onset preeclampsia between 23+ 0/7 and 29+ 6/7 weeks gestation.** **Added secondary endpoint:** **Pharmacokinetics and pharmacodynamic studies, utilizing peak and trough eculizumab levels.**	**FDA recommended assessment of eculizumab pharmacokinetics in pregnant women with early onset preeclampsia**
10.1.5 **Key Roles and Study Governance, Data Safety Monitoring Board** **Appendix C.** **Data Safety and Monitoring Board**	**Elizabeth Lemoine and Dr. Ravi Thadhani removed from study team (left institution). Added Martha Bautista as Clinical Research Coordinator. Changed DSMB chairperson to Dr. Bobbie J. Rimel. Added Dr. John Williams III to the DSMB team. DSMB updated to comprise on clinical trials expert, 2 Ob-gyn with expertise in Maternal-Fetal Medicine, and 1 Pediatrician.**	**Updated DSMB team due to prior members leaving institution and due to change in DSMB leadership role.**

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and applicable United States (US) Code of Federal Regulations (CFR). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND), funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the 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: Complement Regulation to Undo Systemic Harm in Preeclampsia: The CRUSH Study.

Study Description:
This is a Phase II, single-arm, open-label, case-control study to determine if treatment with eculizumab prolongs pregnancy compared to historical controls in women with preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation. Based on the substantial evidence that preeclampsia is a complement-mediated disease and on case reports of the successful, safe treatment of preeclampsia with eculizumab, we believe that treatment with eculizumab will prolong gestation in women with preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation compared with historical controls receiving expectant management (standard of care).

Objectives:
Primary Objective: To determine if treatment with eculizumab prolongs pregnancy, compared to historical controls, in women with preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation.

Secondary Objectives:
1. To determine if treatment with eculizumab for early-onset preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation decreases adverse maternal and neonatal outcomes, compared to historical controls.

2. To determine if treatment with eculizumab reduces terminal complement activation in women with preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation.

3. To determine if treatment with eculizumab improves laboratory measures of end-organ injury and improves anti-angiogenic imbalance in women with preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation.

4. To determine the safety of eculizumab in the setting of early-onset preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation.

5. To assess eculizumab pharmacokinetics and pharmacodynamics in pregnant women with early-onset preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation.

Endpoints:
Primary endpoint: Latency (in days) from enrollment to delivery

Secondary endpoints:

1. Composite measures of adverse maternal and neonatal outcomes.
    a. Composite maternal outcome: Syndrome of hemolysis, elevated liver enzyme, and low platelet count (HELLP), eclampsia, placental abruption, stroke, venous thrombosis or pulmonary embolism, pulmonary edema, posterior reversible encephalopathy syndrome, postpartum hemorrhage (>1000 cc), blood transfusion, admission to the intensive care unit, acute kidney injury, acute tubular necrosis, dialysis, or death
    b. Composite neonatal outcome: Respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, retinopathy of prematurity, necrotizing enterocolitis, seizure, hypoxic-ischemic encephalopathy, metabolic acidosis, infection, sepsis, previously unrecognized major malformation detected at birth, patent ductus arteriosus requiring indomethacin, or death
2. Blood and urine concentrations of terminal complement proteins C5a and C5b-9, before and after each treatment.
3. Laboratory measures of end-organ injury and anti-angiogenic imbalance, before and after each treatment.
    a. Measures of end-organ injury: Serum creatinine, aspartate transaminase, alanine transaminase, hemoglobin, platelet count, lactate dehydrogenase, urine protein/creatinine ratio, and CD59
    b. Measures of anti-angiogenic imbalance: soluble fms-like tyrosine kinase 1 (sFLT-1), placental growth factor (PlGF).

4. Serious adverse events:
    a. Neisseria meningitidis infection or development of invasive meningococcal disease, Neisseria gonorrhoeae, Neisseria sicca/subflava, and Neisseria spp unspecified and Aspergillus infections.
    b. Graded clinical and laboratory abnormalities, according to the U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS; DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. [July 2017].

5. Pharmacokinetics and pharmacodynamic studies, utilizing peak and trough eculizumab levels.

Study Population: 12 female patients $\geq$ 13 years old (with body weight ≥40kg), between $23^{0/7}$ and $29^{6/7}$ weeks gestation diagnosed with preeclampsia per the American College of Obstetricians and Gynecologists (ACOG) guidelines and undergoing expectant management at Cedars-Sinai Medical Center (CSMC). 36 patients between $23^{0/7}$ and $29^{6/7}$weeks gestation previously diagnosed with preeclampsia per ACOG guidelines who underwent expectant management at CSMC between October 1, 2014 and September 30, 2019 will be used as historical controls.

Phase: 2

Description of Sites/Facilities Enrolling Participants: Single-center, single-arm trial at CSMC.

Description of Study Intervention: Eculizumab per standard dosing for atypical hemolytic uremic syndrome (aHUS): 900 mg IV every 7 days for 4 weeks, 1200 mg IV on week 5, followed by 1200 mg IV every 14 days until 48 hours postpartum

Study Duration: 24 months

Participant Duration:
Participants will be enrolled until 6 weeks postpartum. Enrollment periods will be variable depending on delivery latency from enrollment and gestational age at enrollment. Maximum time enrolled will be 20 weeks.

1.2. SCHEMA

1.3. SCHEDULE OF ACTIVITIES (SOA)

	Visit 1 Day 1	Visit 2 Day 4	Visit 3 Day 7	Visit 4 Day 10	Visit 5 Day 14	Visit 6 Day 17	Visit 7 Day 21	… ***	… ***	Visit … PP 1-2 weeks	Visit… PP 6 weeks	Visit… *
Consent, screening tests, medical history and exam, enrollment	X											
Meningococcal vaccines*	X											X
Prophylactic Antibiotics**	X	X	X	X	X	X	X	X	X	X	X	
Eculizumab 900 mg IV	X		X		X		X					
Eculizumab 1200 mg IV									X			
Blood draw Prior to study drug on treatment visits.	X	X	X	X	X	X	X	X	X			
Urine collection Prior to study drug on treatment visits.	X	X	X	X	X	X	X	X	X			
Medical record review	X	X	X	X	X	X	X	X	X	X	X	
Assessment for adverse maternal or fetal outcomes†	X	X	X	X	X	X	X	X	X	X	X	
Newborn assessment for adverse outcomes										X	X	
Total time	60 min	20 min	60 min	20 min	60 min	20 min	60 min	20 min	60 min	20 min	20 min	

Sample and Procedure Table:

PP, postpartum

* For subjects without prior meningococcal vaccination, administer Quadrivalent meningococcal conjugate vaccine (MenACWY): 2 doses of Menactra or Menveo at least 2 months apart, and; Meningococcus serogroup B vaccine Bexsero (2 doses administered at least one month apart). For subjects with prior vaccination, revaccinate in accordance with the recommendation of the Advisory Committee on Immunization Practices, considering the duration of eculizumab therapy. Second dose of meningococcal vaccine may be administered up to 8 weeks postpartum.

** Penicillin 500 mg PO bid, or Azithromcyin 250 mg PO daily (if allergy to penicillin), until 4 weeks after the last dose of eculizumab

*** Following induction dose of eculizumab (900 mg IV every 7 days x 4 weeks), eculizumab will be given at maintenance dose of 1200 mg IV on week 5, followed by 1200 mg every 2 weeks. Weekly administration of Eculizumab (induction dose of 900 mg IV for 4 weeks, 1200 mg IV on week 5, followed by maintenance dose of 1200 mg IV every 2 weeks). Alternating study visits for specimen collection & medical record review will continue until delivery.

† In addition to the study visits designated in the table, maternal and fetal assessments will be performed daily by a member of the study team

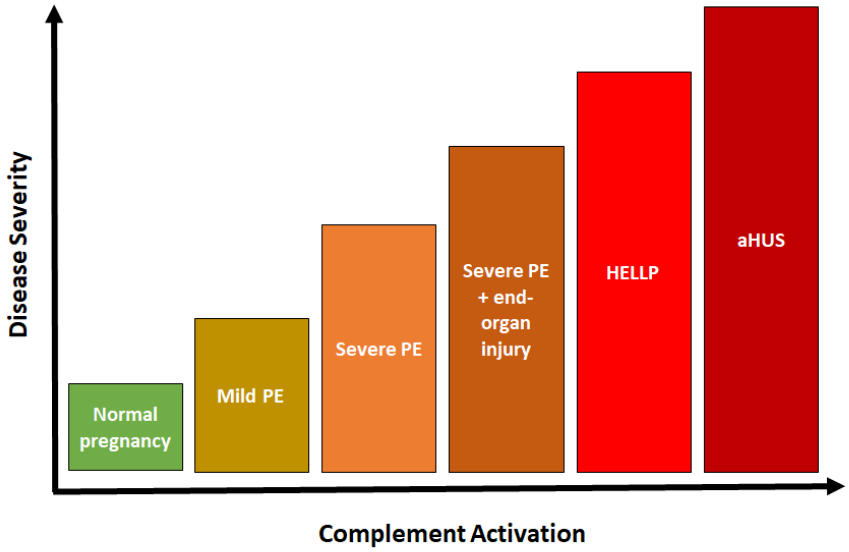
2. INTRODUCTION

2.1. STUDY RATIONALE

Preeclampsia occurs in 3-4% of pregnancies and is characterized by hypertension and proteinuria or end-organ injury (1-2). Severe forms of the disease may be life-threatening, and complications include kidney and liver failure, seizure and stroke. Unfortunately, the only treatment for preeclampsia is interruption of the pregnancy (delivery), often resulting in a premature neonate. To improve neonatal outcomes, providers attempt to prolong pregnancy when preeclampsia is diagnosed <34 weeks gestation (3-4). As no treatment is available for preeclampsia, this approach is termed “expectant management,” which means to watch and wait. Given the unrelenting and unpredictable nature of preeclampsia, expectant management places mother and child at significant risk until delivery occurs (5-6). There is a compelling need for therapeutic measures, in lieu of expectant management, for women with early-onset preeclampsia <34 weeks gestation. Prolongation of pregnancy for 7 days or more, in the setting of preterm preeclampsia, is expected to greatly improve neonatal outcomes. This potential benefit is greatest in women <30 weeks gestation. Moreover, days gained for the fetus prior to delivery usually translate directly to days saved in the neonatal intensive care unit.

There is increasing evidence that preeclampsia is a complement-mediated disorder, which closely resembles atypical hemolytic uremic syndrome (aHUS), a complement-mediated thrombotic microangiopathy (TMA) disorder (7-9). Like aHUS, preeclampsia predisposes to microangiopathic hemolytic anemia, thrombocytopenia, and kidney injury, usually in the form of HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome (10). There is strong evidence that preeclampsia and HELLP syndrome are propagated by increased complement activation or decreased complement regulation (11-17). Though no treatment exists for preeclampsia, eculizumab is a terminal (C5) complement blocker and an FDA-approved treatment for complement mediated disorders (28). Eculizumab has been used safely and efficaciously to treat aHUS and paroxysmal nocturnal hemoglobinuria (PNH) in pregnancy (22-25). Given that preeclampsia is also a complement-mediated disorder, there is clinical equipoise regarding the use of eculizumab in the treatment of preeclampsia.

Figure 1. Complement activation in pregnancy and disease severity



aHUS, atypical hemolytic uremic syndrome; GHTN, gestational hypertension; HELLP, hemolysis, elevated liver enzymes, low platelet count; PE, preeclampsia; SF, severe features

2.2. BACKGROUND

The safety of eculizumab for treatment of preeclampsia is supported by its use in pregnant women with paroxysmal nocturnal hemoglobinuria (PNH) (22). There is also an increasing number of reports describing eculizumab treatment for pregnancy-associated aHUS prior to delivery (23-25). Importantly, while effective as a maternal terminal complement blocker, eculizumab treatment during pregnancy does not impair the complement system activity of the newborn (26). A human case study described successful off-label use of eculizumab for treatment of early-onset preeclampsia and HELLP syndrome (18). To our knowledge, it is the first and only published case of its kind. In that case, eculizumab treatment led to cessation of red cell hemolysis, normalization of platelet count, and reduction in soluble C5b-9 (terminal complement complex) in plasma and urine. Furthermore, eculizumab treatment led to the prolongation of pregnancy by 17 days, compared to a historical average of less than 7 days using expectant management. Eculizumab not only benefited the neonate, by allowing extra time for fetal maturation, but also benefited maternal health by reversing HELLP syndrome and avoiding major sequelae, such as disseminated intravascular coagulation, acute kidney injury, and admission to the intensive care unit. Eculizumab also proved safe for the neonate, who had an uncomplicated neonatal course. Moreover, there were insufficient levels of eculizumab observed in the cord blood to block complement in the neonate (18).

In addition to the human case report, there is strong evidence in the literature that complement mediates severe preeclampsia (11, 18-20). Urinary MAC (C5b-9) levels correlate strongly with high levels of sFLT-1 and low levels of PlGF (i.e. the anti-angiogenic state), a characteristic feature of preeclampsia (20). Recently, utilizing a large cohort of patients in Colombia, urinary C5b-9 levels were found to distinguish severe preeclampsia from mild preeclampsia, and alterations in plasma C5b-9 levels were found to correlate with features of severe disease, including low platelet count, elevated lactate dehydrogenase, and elevated serum creatinine. Furthermore, urinary excretion of the terminal complement complex (MAC) has been shown to be specific to severe preeclampsia, suggesting it might be a useful biomarker for distinguishing severe disease from chronic hypertension (11).

Given the mounting data of preeclampsia as a complement-mediated disorder, we hypothesize that disease severity correlates with increasing complement activation, with HELLP syndrome and pregnancy associated aHUS at the most extreme (Figure 1). This is emphasized by the fact that women entering pregnancy with aHUS have severe complications including early-onset preeclampsia (21). Given the similarity between aHUS, HELLP and severe preeclampsia as complement-mediated TMA disorders, we hypothesize that the two conditions will respond similarly to eculizumab treatment. Therefore, we aim to treat women with early-onset preeclampsia <34 weeks with eculizumab to prolong pregnancy, as compared to standard management without eculizumab (historical controls).

In the documented human case report of eculizumab-treated HELLP syndrome (18), eculizumab was dosed at 1200 mg weekly, which was enough to prolong pregnancy in the setting of active hemolysis. However, the 1200 mg induction dose of eculizumab is not well studied in pregnancy. The current FDA-approved dose of eculizumab for aHUS is 900 mg IV weekly, 1200 mg IV on week 5, and 1200 mg IV every 14 days. This regimen has been used effectively in women with aHUS in pregnancy and postpartum, and we believe it will be an effective regimen to treat preeclampsia (a less severe form of disease compared to HELLP syndrome). Thus, we hypothesize that eculizumab, dosed in accordance with FDA-approved regimen for aHUS, will prolong pregnancy in women with preeclampsia. In the existing human preeclampsia case report, eculizumab was dosed at 1200 mg IV weekly, above the FDA package label, and there was no evidence of fetal or neonatal harm. Furthermore, cord blood levels of eculizumab were insufficient to block complement (18). This suggests that a lower dose of eculizumab, given in accordance with the FDA label for treatment of aHUS, will also be safe for use in preeclampsia.

In summary, there is strong evidence that preeclampsia is a complement-mediated disorder, which may progress to end-organ injury and hemolytic anemia, similar to aHUS. The published case report of eculizumab to treat preeclampsia gives proof-of-concept for a pilot study to evaluate the efficacy of eculizumab for treatment of preeclampsia. Given the serious and life-threatening nature of HELLP syndrome and eclampsia, and the significant maternal risk of prolonging pregnancy in the setting of these conditions, we will exclude women with HELLP syndrome and eclampsia from our study.

Finally, the use of eculizumab in treatment of severe preeclampsia has significant potential as a cost-saving therapy, particularly as a salvage therapy at early gestational ages. Delivery for preeclampsia <28 weeks gestation results in an average maternal cost of $29,131 and infant cost of $282,570 per birth (27). In 2012, the total health care cost in the United States attributed to preeclampsia deliveries <34 weeks was $1.2-2.0 billion. At early gestational age, prolongation of pregnancy by 7 days reduces adverse outcomes such as intracranial hemorrhage, respiratory failure, seizure, and death. Due to these benefits, prolongation of pregnancy by 1-2 weeks results in cost savings of $50-150,000 per infant delivered for preeclampsia <28 weeks and $25-50,000 per infant delivered at 28-34 weeks. There is also a large global market for preeclampsia therapy as it is a leading cause of maternal morbidity and mortality worldwide. Therefore, the use of eculizumab as a preeclampsia therapeutic has the potential to reduce healthcare costs in the United States and in developing countries, where burden of disease is greatest.

2.3. RISK/BENEFIT ASSESSMENT

2.3.1. KNOWN POTENTIAL RISKS

There is a known risk of meningococcal infection with eculizumab (28). In clinical trials of patients with PNH, aHUS, and non-PNH treated with eculizumab, <1% of subjects developed meningococcal infection. The risk of meningococcal infection is mitigated with immunization with meningococcal vaccines,

prophylactic antibiotics, and close monitoring of patients for early signs of infection. As eculizumab blocks terminal complement activation, there is also some unknown risk for infection with encapsulated bacteria (28). To minimize risk of infection, our protocol will require meningococcal vaccination with the first dose of eculizumab as well as prophylactic antibiotics through for 4 weeks following the last dose of eculizumab. Finally, subjects will be closely monitored for signs of infection and all participants will be provided a patient safety information card regarding the risk of meningococcal infection. The participant safety card will describe signs and symptoms of meningitis for the patient (e.g., fever, headache and a fever, headache and a stiff neck, etc.) and will provide information for healthcare providers regarding risk of meningococcal infection following C5 blockade with eculizumab. The study drug will be stopped immediately if an infection, other than simple urinary or yeast infection, is suspected. Treatment decisions for known or suspected meningococcal infection will be made in conjunction with infectious disease specialists. Neisseria meningitidis infection will be reported as an adverse event of special interest (AESI) and will be reported to the FDA and the Data Safety Monitoring Board (DSMB).

As a biologic, eculizumab carries some unknown risk of infusion reaction. However, in clinical trials of eculizumab, no subject experienced an infusion reaction requiring discontinuation of treatment (28). Subjects in this trial will be closely monitored in an inpatient setting throughout infusion, and treatment will be stopped immediately with any signs of anaphylaxis or hypersensitivity.

Safety measures and adverse maternal / neonatal events will also be assessed daily throughout the trial. There are no available data to inform drug-associated risk of major birth defects and miscarriage. However, there is an abundance of safety data from on-label use of eculizumab in PNH and aHUS (22-25). In a study of 10 women with PNH who breastfed their infants while on eculizumab, the drug was not detected in any of the 10 breastmilk samples (22). In that same study, 64 children exposed to eculizumab in pregnancy had long-term follow up, with form development assessments performed at an average of 31 months (range 4-94). Of those, 64 of 64 (100%) met developmental milestones for vision, hearing, locomotion, fine-motor skills, behavior and physical health; 63 out of 64 (98.4%) achieved milestones for speech and language (1 child was referred to a speech and language specialist for delayed speech), which is below the national prevalence of speech and language delays (41). Taken together, this data suggests that eculizumab is safe for use in pregnant and breastfeeding women (22,28). As fetal and neonatal risks from eculizumab appear to be minimal, there is a compelling rationale to treat preeclampsia and prevent severe maternal adverse outcomes, in lieu of expectant management, in women with early-onset disease.

There is minimal risk associated with venipuncture for a research blood draw, including bleeding, bruising or infection at the puncture site. There is also a small risk of fainting. Subjects will be closely monitored for any signs of venipuncture-related injury at the time of study blood draw.

2.3.2. KNOWN POTENTIAL BENEFITS

As demonstrated in the human case report (18), there is significant potential for benefit in the treatment of preeclampsia with eculizumab. Current management of preeclampsia prior to 30 weeks gestation consists of watchful waiting, which carries the risk of maternal end-organ damage— including seizures and death — and prolongation of gestation of less than 1 week. The reported use of eculizumab in severe preeclampsia/HELLP syndrome mitigated end-organ damage with cessation of hemolysis, normalization of platelet counts, and prolongation of pregnancy by 17 days (17). Additionally, given the abundance of data suggesting preeclampsia is a complement-mediated disorder, complement blockade

with eculizumab — in lieu of expectant management— is expected to improve outcomes for women with severe, early-onset (<30 weeks) preeclampsia.

In the short term, potential benefits include mitigation of severe disease, including maternal end-organ damage, and prolongation of gestation and increased fetal maturation with less risk of the complications of prematurity. However, it is well established in the literature that women with a history of preeclampsia are at long-term risk of cardiovascular and renal complications (29). Potential intermediate and long-term risks include acute kidney injury, cardiomyopathy, vascular dementia, end-stage renal disease, acute myocardial infarction, and hypertension (29-33). Given that this constellation of complications is secondary to vascular injury, attenuation of vascular damage through complement blockade may decrease the long-term risk of cardiovascular and renal disease. However, the follow-up time-frame of this study (6 weeks post-partum) does not allow for assessment of these long-term risks.

2.3.3. ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Currently no therapy exists for the treatment of early-onset preeclampsia. Expectant management of these patients until the late preterm period carries the risk of maternal end-organ damage, diseases of prematurity of the newborn, and maternal and fetal mortality. Therefore, the possibilities of mitigating progression of end-organ damage in the mother and improving fetal maturation with the treatment of eculizumab suggest significant benefit over current management. Though treatment with eculizumab does carry some risk of meningococcal infection, <1% of subjects treated with eculizumab developed meningococcal infection in clinical trials (28). To minimize risk of possible infection with encapsulated bacteria (such as Neisseria meningitidis), our protocol will include meningococcal vaccination and prophylactic antibiotics until 4 weeks after the last dose of eculizumab, a sufficient amount of time for the effects of complement blockade to wane and sufficient time for subjects to develop adaptive immunity following meningococcal vaccine. Furthermore, subjects will be closely monitored for infection. Should any signs or symptoms suspicious for infection develop in a study subject, all study interventions will be stopped immediately for the subject. Given the potential for maternal and fetal benefit and the mitigating steps of vaccination and prophylactic antibiotics, we believe that treatment with eculizumab outweighs the risk of infection for this population.

The risk of infusion reaction is minimal with eculizumab and has not necessitated discontinuation of treatment. Therefore, we believe that treatment of eculizumab for early-onset preeclampsia outweighs the risk of infusion reaction. However, given the known risk, subjects will be monitored for anaphylaxis and hypersensitivity reactions in an inpatient setting while the subject is undergoing infusion.

Though no clinical trial evidence exists for the study of eculizumab in pregnancy, an abundance of case reports documenting the use of eculizumab in pregnancy suggests that eculizumab is safe and does not affect the complement system of the fetus/neonate (18, 22-26). Given the risk of severe neonatal morbidity and mortality in early-onset preeclampsia and the minimal risk of neonatal complement blockade, we believe the potential benefit of prolonged gestation with treatment of eculizumab outweighs the risks of prematurity for the neonate. The risks of transmission of eculizumab through breast-milk is minimal, as drug levels are undetectable in the breastmilk of nursing mothers on eculizumab for on-label indications (22).

3. OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine if treatment with eculizumab prolongs pregnancy, compared to historical controls, in women with preeclampsia between 23+0/7 - 29 +6/7 weeks gestation.	Latency (in days) from enrollment to delivery.	When preeclampsia develops between 23+0/7 - 29 +6/7 weeks gestation, the primary aim is to prolong pregnancy for additional fetal maturation, without compromising maternal health. Latency (in days) from enrollment to delivery is the best marker to gauge clinical response to treatment because pregnancy is only prolonged when both maternal and fetal health are reassuring. Days gained for the fetus prior to delivery, usually translate directly to days saved in the neonatal intensive care unit.
Secondary		

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
To determine if treatment with eculizumab for early-onset preeclampsia <30 weeks gestation decreases adverse maternal and neonatal outcomes, compared to historical controls.	Composite maternal outcome: Syndrome of hemolysis, elevated liver enzyme, and low platelet count (HELLP), eclampsia, placental abruption, stroke, venous thrombosis or pulmonary embolism, pulmonary edema, posterior reversible encephalopathy syndrome, postpartum hemorrhage (>1000 cc), blood transfusion, admission to the intensive care unit, acute kidney injury, acute tubular necrosis, dialysis, or death Composite neonatal outcome: Respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, retinopathy of prematurity, necrotizing enterocolitis, seizure, hypoxic-ischemic encephalopathy, metabolic acidosis, infection, sepsis, previously unrecognized major malformation detected at birth, patent ductus arteriosus requiring indomethacin, or death	Composite adverse maternal and neonatal outcomes were chosen because of the rarity of individual outcomes. In addition, preeclampsia predisposes to a range of maternal complications due to its heterogeneous nature. The maternal outcomes listed are all established complications from preeclampsia (42). Neonatal outcomes are related to preeclampsia indirectly, because they are primarily complications secondary to premature delivery. The outcomes listed are those directly relating to premature delivery.
To determine if treatment with eculizumab reduces terminal complement activation in women with preeclampsia <30 weeks gestation	Blood and urine concentrations of terminal complement proteins C5a and C5b-9, before and after each treatment.	Terminal complement proteins C5a and C5b-9 are increased in women with preeclampsia, and eculizumab is a C5 inhibitor designed to reduce concentrations of C5a and C5b-9. Thus, we aim to show that C5a and C5b-9 concentrations are effectively decreased in women with preeclampsia treated with eculizumab.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
To determine if treatment with eculizumab improves laboratory measures of end-organ injury and reduces anti-angiogenic imbalance in women with preeclampsia <30 weeks gestation.	Measures of end-organ injury: serum creatinine, aspartate transaminase, alanine transaminase, hemoglobin, platelet count, lactate dehydrogenase, urine protein/creatinine ratio, and CD59 Measures of anti-angiogenic imbalance: soluble fms-like tyrosine kinase 1 (sFLT-1), placental growth factor (PlGF), and their ratio.	Preeclampsia is associated with end-organ injury, but effects on end-organs are heterogeneous. Thus, we will evaluate the impact of eculizumab treatment on various markers of kidney, liver, and hematologic injury. We selected laboratory markers that are commonly deranged in the setting of preeclampsia, particularly those associated with severe or progressive disease. CD59 is a specific marker for complement-mediated end-organ injury. Preeclampsia is also associated with the anti-angiogenic state, with increased sFLT1 and decreased PlGF concentrations, and an increased sFLT1/PlGF ratio (42). Complement activation correlates with the anti-angiogenic state. We will assess whether sFLT1 and PlGF concentrations improve in conjunction with complement blockade.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
To determine safety of eculizumab in the setting of early-onset preeclampsia between 23-30 weeks gestation	Neisseria meningitidis infection or development of invasive meningococcal disease, Neisseria gonorrhoeae, Neisseria sicca/subflava, and Neisseria spp unspecified and Aspergillus infections Graded clinical and laboratory abnormalities, according to the U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS; DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. [July 2017]. Available at: https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf	We will monitor the occurrence of Neisseria or Aspergillus infections due to the increased risk in the setting of complement blockade with the study drug. In addition, to assess safety of the study drug we will monitor graded clinical and laboratory abnormalities. This will help determine if the eculizumab is safe in the study population.
To assess eculizumab pharmacokinetics in pregnant women with early-onset preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation.	Peak and trough serum eculizumab levels for pharmacokinetic and pharmacodynamic assessment.	Pharmacokinetic and pharmacodynamic modeling will help determine if eculizumab dosing regimen is adequate in pregnant women, considering increased volume of distribution and other physiologic changes of pregnancy.

4. STUDY DESIGN

4.1. OVERALL DESIGN

Hypothesis: Complement blockade with eculizumab to treat early-onset preeclampsia (< 30 weeks gestation) will prolong pregnancy and decrease adverse maternal and neonatal outcomes, compared to historical controls not treated with eculizumab.

Phase: 2

Design: Single-center, single-arm trial at Cedars-Sinai Medical Center

Bias: To minimize bias, all eligible subjects meeting inclusion and exclusion criteria will be approached for enrollment in the study, until the enrollment target of 12 subjects is met.

Study Arms: 12 female patients ≥ 13 years old (body weight ≥40kg) between $23^{0/7}$ and $29^{6/7}$ weeks gestation diagnosed with preeclampsia per the American College of Obstetricians and Gynecologists (ACOG) guidelines undergoing expectant management at Cedars-Sinai Medical Center (CSMC). 36 patients between $23^{0/7}$ and $29^{6/7}$ weeks gestation previously diagnosed with preeclampsia per ACOG guidelines who underwent expectant management at CSMC between October 1, 2014 and September 30, 2019 will be used as historical controls.

Study Intervention: Eculizumab per standard dosing for atypical hemolytic uremic syndrome (aHUS): 900 mg IV every 7 days for 4 weeks, 1200 mg IV on week 5, followed by 1200 mg IV every 14 days until 48 hours postpartum

Participant duration: Participants will be enrolled until 6 weeks postpartum. Enrollment periods will be variable depending on delivery latency from enrollment. Maximum time enrolled will be 20 weeks.

1. Selection of controls: Historical controls will be those women with early-onset preeclampsia (between $23^{0/7}$ and $29^{6/7}$ weeks gestation), managed at Cedars-Sinai Medical Center but not treated with eculizumab between October 1, 2014 and September 30, 2019. Following inclusion/exclusion criteria, 36 controls will be matched 3:1 to 12 cases by

   i) parity (nulliparous / multiparous)

   ii) gestational age at diagnosis (± 7 days)

   iii) fetal count of current gestation

iv) race (white/non-white)

The designated team member selecting control subjects will otherwise be blinded to details of the corresponding case, including clinical outcome.

4.2. SCIENTIFIC RATIONALE FOR STUDY DESIGN

This is a phase 2, open-label study of eculizumab for treatment of early-onset preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation. To determine whether eculizumab prolongs pregnancy in women with preeclampsia, we will compare pregnancy latency in cases treated with eculizumab to pregnancy latency in matched historical controls not treated with eculizumab. It is well-established that pregnancy latency in early-onset preeclampsia (<30 weeks) is 7-10 days or less. However, there is variation in latency based on patient population and clinical characteristics. It is critical to evaluate historical controls from within our own institution, rather than historical controls from the medical literature. In addition, practice patterns change over time and we will therefore select a contemporaneous group of controls from the last 5 years at our institution. To limit differences between the control group and the case group, which might confound our results, we will match controls (3:1) to cases by gestational age, parity, and race. Such clinical characteristics are major factors associated with pregnancy latency in preeclampsia. By matching for these characteristics we will strengthen the argument that any change in latency in the intervention group is due to study drug. Controls will be matched to cases in a 3:1 ratio to increase our statistical power to detect a difference in the primary outcome between groups and to minimize the number of cases that need to be enrolled.

4.3. JUSTIFICATION FOR DOSE

All 12 subjects in the intervention group will receive the study drug, eculizumab, at an induction dose of 900 mg IV (every 7 days for 4 weeks), followed by a maintenance dose of 1200 mg IV on week 5 and then 1200 mg every 14 days until 48 hours postpartum. Eculizumab is only available as an intravenous infusion. The dosing regimen is identical to the FDA-approved eculizumab dosing schedule for non-pregnant adults (≥18 years old), as well as pediatric and adolescent patients aged 13 to 18 years with body weight ≥40kg, with atypical hemolytic uremic syndrome (aHUS). The safety profile observed in pediatric and adolescent patients (aged 13 to 18 years with body weight ≥40kg) is similar to that observed in the adult population.

In adults with aHUS, as well as pediatric and adolescent patients (aged 13 to 18 years with body weight ≥40kg), eculizumab is dosed at 900 mg IV weekly for 4 weeks, then 1200mg for the 5th dose, then 1200mg every 2 weeks. We are proposing an identical dosing regimen for treatment of preeclampsia. The FDA-approved regimen for aHUS has been used effectively to treat aHUS arising during pregnancy (21, 24). Higher doses of eculizumab have also been used, with the rationale that pregnancy is characterized by increased complement activation and increased volume of distribution (21). In a human case report, eculizumab was given at induction dose of 1200 mg IV weekly, above FDA-label, for treatment of preeclampsia and HELLP syndrome (18, 35). While higher doses have been used successfully in pregnancy, without evidence of fetal or neonatal harm, there is very limited safety data at this higher dose. The FDA-approved dosing regimen of eculizumab for aHUS has been well studied, with numerous case reports and case studies in pregnancy and postpartum, and we believe that the

same dosing regimen will be efficacious in preeclampsia (a less severe form of disease compared to HELLP syndrome).

4.4. END OF STUDY DEFINITION

A participant is considered to have completed the study if she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

5. STUDY POPULATION

5.1. INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form
2. Stated willingness to comply with all study procedures & availability for study duration
3. Biologically female, aged ≥13, body weight ≥40 kg
4. Diagnosed with preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation, by following criteria:
    a. Blood pressure ≥160 mmHg systolic or ≥110 mmHg diastolic OR
    b. Blood pressure ≥140 mmHg systolic or ≥90 mmHg diastolic and at least one of the following
        i. Proteinuria (spot protein/creatinine ≥0.3mg/mg or 24Hr protein ≥300 mg)
        ii. Platelet count <100,000/µl
        iii. Aspartate or alanine transaminase >2x upper limit of normal
        iv. Creatinine >1.1 mg/dl or oliguria
        v. Pulmonary edema
5. Ability to take intravenous medication and be willing to adhere to the eculizumab regimen
6. Ability to receive meningococcal vaccine and be willing to adhere to antibiotic regimen

5.2. EXCLUSION CRITERIA

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

1. Body weight <40kg
2. Known allergic reactions eculizumab or meningococcal vaccine
3. Febrile illness within prior 2 weeks
4. Treatment with another investigational drug within *previous 6 months*
5. Inpatient expectant management for preeclampsia >72 hours prior to enrollment
6. Fetal contraindication to expectant management of pregnancy
7. Platelet count <50,000/µl
8. Diagnosis of hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome
9. Must meet all of the following criteria to be excluded: LDH >600 U/L, platelet count < 100,000/µl, AST >2x upper limit of normal, ALT >2x upper limit of normal

10. Diagnosis of Eclampsia
11. Diagnosis of Placental abruption
12. Intrauterine fetal demise
13. Coagulopathy (INR ≥ 1.5)
14. Fibrinogen <200 mg/dl
15. Persistent, severe headache unresponsive to medications
16. Persistent, severe visual disturbances
17. Persistent, severe epigastric or RUQ pain unresponsive to medications
18. Diagnosis of Systemic lupus erythematosus
19. Diagnosis of Anti-phospholipid antibody syndrome
20. Diagnosis of Atypical hemolytic uremic syndrome
21. Diagnosis of Paroxysmal nocturnal hemoglobinuria
22. Known complement deficiency
23. Diagnosis of Venous thromboembolism active or within 6 months of enrollment
24. Diagnosis of Human immunodeficiency virus (HIV)
25. Diagnosis of Hepatitis C virus (active viremia)
26. Diagnosis of Cancer (not in remission)
27. History of Solid organ transplant
28. Systemic viral or bacterial infection (active, untreated)
29. Active use of eculizumab at time of enrollment
30. Contraindication to eculizumab treatment or complement system blockade
31. Contraindication to meningococcal vaccine
32. Neutropenia (<1500/mm^3)
33. Gonorrhea, chlamydia, or syphilis in current pregnancy
34. Illicit substance use in current pregnancy
35. Currently homeless or incarcerated
36. Alcoholism
37. Liver cirrhosis
38. Insulin dependent diabetes
39. Active use of immunosuppressive therapies, other than use of corticosteroids for fetal lung maturity
40. Use of prophylactic or therapeutic heparin, or low molecular weight heparin, in pregnancy for hypercoagulable condition

5.3. LIFESTYLE CONSIDERATIONS

There will be no lifestyle and/or diet restrictions required by this protocol. Participants may be subject to lifestyle restrictions (e.g. bedrest) at the discretion of their treating physicians.

5.4. SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individual who do not meet the criteria for participation in this trial (screen failure) because of a misdiagnosis may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5. STRATEGIES FOR RECRUITMENT AND RETENTION

Sample size: For the prospective, treatment arm, we are targeting enrollment of 12 pregnant women, age ≥18, with preeclampsia between $23^{0/7}$ and $29^{6/7}$ weeks gestation. Our disease of interest – early onset preeclampsia – is unique to pregnant women, a vulnerable population. We will screen all women presenting to the labor and delivery triage unit, regardless of race/ethnicity until enrollment targets are met.

Anticipated accrual rate: We anticipate enrollment of 1 subject every 2 months, with completion of enrollment over a 24-month period.

All subjects will be enrolled at Cedars-Sinai Medical Center. All study interventions (i.e. eculizumab, antibiotic, and vaccine administration) will be administered while the study subject is in an inpatient or hospital-like setting. Given the severity of disease of this population, we anticipate that all subjects will require inpatient management. Therefore, we anticipate a high retention rate.

All subjects will be enrolled from the inpatient hospital setting, including Labor and Delivery ward or antepartum unit.

Subjects will not receive payment for study participation.

For the retrospective, matched-controlled arm, 36 historical controls who previously underwent expectant management for preeclampsia between October 1, 2014 and September 30, 2019 will be matched 3:1 to cases in the prospective, treatment arm by gestational age (± 7 days), parity (nulliparous/multiparous), fetal count of current gestation, and race/ethnicity (white/non-white). Controls will be selected in reverse chronological order as they appear in the database by research staff blinded to the clinical outcomes of the case group.

6. STUDY INTERVENTION

6.1. STUDY INTERVENTION(S) ADMINISTRATION

6.1.1. STUDY INTERVENTION DESCRIPTION

Eculizumab (Soliris) is a C5 inhibitor biological product that is administered intravenously and is currently FDA-approved for treatment of paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), and anti-acetylcholine receptor antibody-positive myasthenia gravis. We propose the off-label use of eculizumab for the treatment of early-onset preeclampsia, a complement-mediated disease. Eculizumab will be administered intravenously in accordance with its approved administration method. Dosing will be within the on-label use range as described in section 6.1.2.

This is a non-blinded single-arm study with historical controls, therefore there is no control product.

6.1.2. DOSING AND ADMINISTRATION

As described in section 4.3, all subjects in the prospective, interventional arm will receive the study drug, eculizumab, at an induction dose of 900 mg IV weekly (q7 days) for 4 weeks, followed by a maintenance dose of 1200 mg IV on week 5 and then 1200 mg IV every 14 days. The last dose will be administered up to 48 hours postpartum. The specific dose (900 mg vs. 1200 mg) will depend on where the subject is on the dosing schedule (i.e. post-partum dose will be 1200 mg if it is the 5th dose or greater). The dosing regimen is identical to the FDA-approved package insert for dosing in adults (≥18 years old) with atypical hemolytic uremic syndrome (aHUS). In adults with aHUS, eculizumab is dosed at 900 mg IV weekly for 4 weeks, then 1200 mg for the 5th dose, then 1200mg every 2 weeks. We are proposing an identical dosing regimen for treatment of preeclampsia.

We propose dosing in accordance with the maintenance schedule outlined by the FDA-approved package insert, as clinical stability of the subject allows. Given the elevated levels of complement activation immediately post-partum, the last dose may be administered up to 48 hours post-partum.

Eculizumab will be administered intravenously, the only currently FDA-approved method of administration. In accordance with the FDA-approved package insert administration guidelines, the infusion should be administered via intravenous infusion over 35 minutes in adults and 1 to 4 hours in pediatric patients via gravity feed, a syringe-type pump, or an infusion pump. Patients will be monitored for signs/symptoms of infusion reaction.

6.2. PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1. ACQUISITION AND ACCOUNTABILITY

The Cedars-Sinai Medical Center investigational pharmacy will be used for study drug storage, reconstitution, and preparation. Eculizumab vials will be stored under light-protected, refrigerated conditions at 2-8°C per the FDA-approved package insert. Investigators will obtain study drug directly from the investigational pharmacy. Study drug will be administered by the antepartum or L&D nursing staff. Expired or unused study drug will be return to Alexion Pharmaceuticals, provider of the study drug, for disposal.

6.2.2. FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Eculizumab (*Soliris*) is supplied as 300 mg single-dose vials containing 30 mL of 10 mg/mL sterile, preservative-free solution. 90-120 mL of normal saline (diluent) will be used to constitute three-four 300 mg vials, depending on dosing schedule (900 mg or 1200 mg). Each vial will be labeled per FDA-approved package insert instructions:

Alexion Pharmaceuticals, Inc.
100 College Street
New Haven, CT 06510 USA

US License Number 1743

Infusion bag will be labeled with study protocol number and ID, study drug name (eculizumab), dose (900mg or 1200 mg), and subject number.

Information in the FDA-approved package insert medication guide and expected side effects as elaborated in section 8.3.3 will be included in the patient informed consent for this trial.

There is no control product being used in this study. Instead, matched, historical controls will be used.

6.2.3. PRODUCT STORAGE AND STABILITY

Eculizumab (study drug) will be stored under light-protected, refrigerated (2-8°C) conditions in the Cedars-Sinai Medical Center investigational pharmacy. Vials will not be shaken or frozen. Study drug will not be used beyond the expiration date stamped on the vial carton. If the vial seal is broken for reconstitution but not used, the vial will be considered expired. If the vial seal is broken or there is particulate matter or discoloration visible in the vial, the vial will be considered expired and will be marked and returned to Alexion Pharmaceuticals. Expired vials will be stored in a separate location from the un-expired study drug per investigational pharmacy standards.

Admixed solutions of eculizumab are stable for 24 hours at room temperature.

6.2.4. PREPARATION

900 mg (3 vials of 300 mg) diluted in 90 mL of normal saline for a final volume of 180 mL or 1200 mg (4 vials of 300 mg) of eculizumab will be diluted in 120 mL of normal saline by pharmacy staff to achieve a final volume of 240 mL. Any unused portion left in the vials will be discarded. The infusion bag will be gently inverted to ensure thorough mixing and the admixture will be allowed to adjust to room temperature prior to administration. No heat source will be used to accelerate adjustment. Once adjusted to room temperature, the study drug admixture will be administered by IV infusion over 35 minutes in adults and 1 to 4 hours in pediatric patients via gravity feed, a syringe-type pump, or an infusion pump.

6.3. MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Randomization not-applicable: The interventional phase of this trial is a single-arm non-blinded study. Matched, historical cases treated with expectant management will be used for control groups.

Historical controls with preeclampsia at our institution between October 1, 2014 and September 30, 2019 will be identified, and data abstracted, according to Cedars-Sinai IRB Study #00052119. To avoid selection bias, research staff blinded to the clinical outcomes of the case group will be utilized to select controls. Controls will be selected in reverse chronological order by delivery date. Research staff will be asked to select two controls to match each case, based on gestational age (±1 week), parity (multiparous or nulliparous), fetal count in current gestation, and race/ethnicity.

6.4. STUDY INTERVENTION COMPLIANCE

All study interventions (i.e. infusions of study drug, prophylactic antibiotics, and vaccines) will be performed while the study subject is in a hospitalized setting. A participant drug log will be maintained by the investigational pharmacy and periodically reviewed by study staff. Therefore, subjects will be closely monitored by research staff and by treating physicians for completion of study interventions.

Study samples will be collected at the timepoints detailed in the schema (section 1.2) and the schedule of activities above. Samples will be centrifuged within 6 hours of collection, transferred into cryovials, and stored in -80°C freezers to ensure protein stability.

Other data points will be collected from the electronic medical record and transcribed onto electronic case report forms maintained behind the firewall. Enrolled subjects' electronic medical records will be reviewed daily while inpatient and weekly after hospital discharge until 6 weeks post-partum.

6.5. CONCOMITANT THERAPY

Not applicable: No concomitant therapy for preeclampsia is currently FDA approved. Therefore study subjects should not be on concomitant therapy for preeclampsia. Patients on other interventional trials will be excluded. Standard clinical care, such as treatment with anti-hypertensives, low-dose aspirin, magnesium for seizure prophylaxis, and steroids for fetal lung development, is permitted.

6.5.1. RESCUE MEDICINE

Not-applicable: There are no rescue treatments for eculizumab. Prophylactic antibiotics and meningococcal vaccines will be administered prior to the first dose of study drug to mitigate risk of infection of encapsulated bacteria.

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1. DISCONTINUATION OF STUDY INTERVENTION

Subjects will be discontinued from eculizumab treatment if they develop any signs or symptoms of infection, including but not limited to fever (≥100.4°F). The subject's primary physician may elect to discontinue the subject's eculizumab treatment at any time.

Discontinuation from eculizumab treatment does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. The data collected in this study is observational and does not affect clinical management. Therefore, continued data collection should not affect the study subject's ongoing clinical care. Any observed events that qualify as an adverse event (AE) will be reported as such.

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

Hospital laboratory tests
- Hemoglobin (g/dl)
- Platelet count (k/µl)
- Lactate dehydrogenase (U/L)
- Creatinine (mg/dl)
- Aspartate transaminase (U/L)
- Alanine transaminase (U/L)
- Haptoglobin (mg/dl)
- C3, C4 (mg/dl)
- CH50 (U/ml)
- Urine protein / creatinine ratio (mg/mg)
- 24-hour urine protein (mg)
- Urinalysis

Specimen Samples
-Urine
-Serum
-Plasma

7.2. PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Signs or symptoms of meningococcal or invasive fungal infection.
- Signs or symptoms of hypersensitivity or anaphylaxis to study drug.
- If participant is discharged prior to being given study drug.
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression does not preclude further study participation.
- Participant unable to receive study drug for >8 days from previous dose.

The reason for participant discontinuation or withdrawal from the study will be recorded on the electronic Case Report Form (eCRF). Subjects who sign the informed consent form but do not receive the study drug – even if they undergo a research blood draw – will be counted as a screen failure may be replaced. Subjects who sign the informed consent form and receive at least one dose of the study drug, and subsequently withdraw, or are withdrawn or discontinued from the study. Subjects who receive at least one dose of the study drug but decline any further doses or are unable to receive further doses (i.e. development of hypersensitivity/anaphylaxis or severe infection) may be followed for maternal and neonatal adverse outcomes.

7.3. LOST TO FOLLOW-UP

All subjects will receive the study drug in a hospital-like setting. Therefore, it is unlikely that subjects will be lost to follow-up antepartum. Subjects who are discharged prior to receiving study drug will be considered screen failures for misdiagnosis.

A participant may be lost to follow-up in the post-partum period if she fails to return for any scheduled visits > 6 weeks post-partum. Given that all data collected in the post-partum period is acquired from the electronic medical record from the participant's regular post-partum visits, no efforts will be made to contact the subject. Revision of the participant's medical record will be reviewed until 20 weeks post-enrollment if the subject fails to attend her 1-2 week or 6 week post-partum visits.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1. EFFICACY ASSESSMENTS

1. Pre-screening:
- Potential subjects will be pre-screened for study eligibility by a member of the research study team (research assistant or co-investigator)
- Subjects will be pre-screened from labor and delivery (L&D) or maternal fetal care unit (MFCU) at Cedars-Sinai Medical Center
- Eligible subjects will meet the following criteria:
    Admitted for inpatient care
    Gestational age ≥23.0 AND <30.0 weeks
    Clinical diagnosis of gestational hypertension or preeclampsia

2. Subject consent:

- Subjects will be approached for enrollment in the study after they are identified as eligible candidates.
- The research study team will contact the primary provider of the eligible subject, to obtain approval to approach the patient for written informed consent
- Once approval is obtained, the research study team will consent the patient for enrollment in the study.

3. Inclusion / Exclusion criteria:

- After obtaining informed consent, the research team will perform detailed chart review to assess the full list of inclusion / exclusion criteria as detailed in this study protocol.

4. Laboratory evaluation- Subjects will be excluded if they have a platelet count <50,000/µl prior to enrollment, or a diagnosis of HELLP syndrome defined by the constellation of three findings: platelet count <100,000/µl, lactate dehydrogenase >600 U/L, and aspartate or alanine transaminase >2x upper limit of normal. Subjects will also be excluded if they have a fibrinogen level <200 mg/dl or INR ≥1.5.

5. Physical examination and symptom evaluation- In addition to chart review, subjects will be evaluated by the research team to determine final study eligibility. Subjects will be excluded if they have persistent and severe headache, visual disturbances, or right upper quadrant abdominal pain. Subjects will also be excluded if they are incapacitated, or have an altered mental status, and are unable to provide written, informed consent.

6. Meningococcal vaccine- Subjects must agree to, and have no contraindication, to receiving the meningococcal vaccine. Subjects without prior history of meningococcal vaccination will be given the vaccine prior to administering the first dose of eculizumab. For subjects with prior vaccination, revaccinate in accordance with the recommendation of the Advisory Committee on Immunization Practices, considering the duration of eculizumab therapy.

Efficacy

Laboratory measures in blood and urine
Subjects will have blood and urine samples collected prior to the first dose of the study drug (eculizumab) on day 1. Measurements from these samples will represent baseline, or pre-treatment, values. Subsequent blood and urine collections will be performed every 3-4 days to monitor efficacy of the study drug, by assessing the laboratory response over time. To assess peak and trough effects of the study drug, subjects will have blood and urine collected every 3 days following administration (peak), and every 7 days prior to administration of the subsequent dose (trough). At every study visit which includes a blood draw and urine collection, specimens will also be collected for research purposes to test secondary study objectives.

- Blood and urine will be collected by a member of the research team, the patient's nurse, or inpatient phlebotomist at the next scheduled rounds (AM or PM).

Blood samples:

Tube types
- Purple top: plasma separator tube (EDTA)
- Red top: serum separator tube (SST)
- Green top: lithium or sodium heparin

- **Hospital laboratory testing**
    - Complete blood count (purple top)
    - Complete metabolic panel (red or green top)
    - Lactate dehydrogenase (red top)
    - Haptoglobin (red top)
    - C3, C4, CH50 (red top)

- **Research samples**
    - 10 cc Serum (red top)
    - 10 cc Plasma (purple top)

Urine samples:
- Hospital laboratory testing
    - Urine protein/creatinine ratio
    - Urinalysis
    - 24-hour urine collection for total protein and creatinine

- Research sample
    - 20 cc into sterile plastic cup

Testing from blood and urine specimens for determination of efficacy, as outlined as secondary objectives in this protocol:

1. Blood and urine concentrations of terminal complement proteins C5a and C5b-9, before and after treatment with eculizumab.
2. Laboratory measures of end-organ injury and anti-angiogenic imbalance, before and after treatment with eculizumab.
3. Measures of end-organ injury: Serum creatinine, aspartate transaminase, alanine transaminase, hemoglobin, platelet count, lactate dehydrogenase, urine protein/creatinine ratio, and CD59.
4. Measures of anti-angiogenic imbalance: soluble fms-like tyrosine kinase 1 (sFLT-1), placental growth factor (PlGF)

Blood and urine collection and processing methods for research samples

- See detailed blood and urine collection MOP

Maternal assessment for adverse outcomes for determination of efficacy, as outlined as secondary objectives in this protocol:

All subjects will have a baseline assessment for adverse outcomes on Visit Day 1, which will establish baseline clinical status. Adverse outcomes will be assessed daily by a member of the study team, with unsettled diagnoses reviewed by the study PI for final classification. Subsequent assessments for adverse maternal or fetal outcomes will be performed daily. Maternal and fetal assessments will enable clinical determination of drug efficacy over time, in comparison to historical controls. In addition, daily assessments of maternal and fetal health will ensure the safety of study continuation by allowing early detection of adverse events or worsened disease.

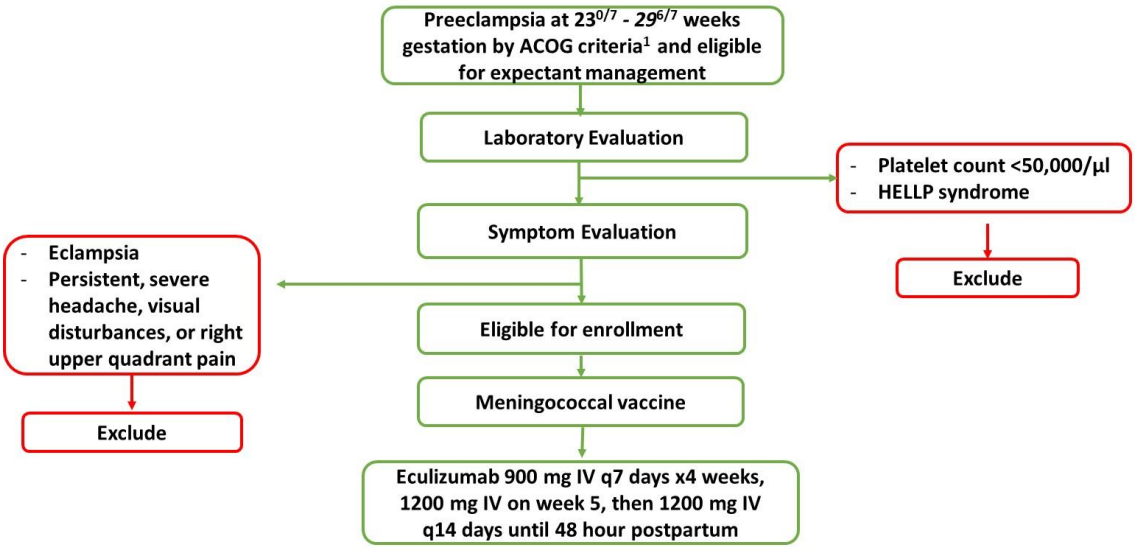
Newborn assessment for adverse outcomes for determination of efficacy, as outlined as secondary objectives in this protocol:

All subjects will have a formal assessment for adverse neonatal outcomes at 1-2 weeks and 6 weeks postpartum. These assessments will be performed by a member of the study team, with unsettled diagnoses reviewed by the study PI for final classification. Neonatal assessments will be used to determine the safety of drug administration in pregnancy, by comparing neonatal outcomes in those exposed to study drug to matched controls not exposed to study drug.

8.2. SAFETY AND OTHER ASSESSMENTS

Screening

Screening as described in section 8.1 will be a rigorous process to include subjects with preeclampsia at $23^{0/7}$ and $29^{6/7}$ weeks gestations and to exclude any subjects with contraindication to expectant management or any risk from meningococcal vaccination. Subjects will be identified by gestational age and diagnosis of preeclampsia or gestational hypertension. After informed consent, a thorough review of the subject's electronic medical record using central laboratory assessment and treating physician clinical assessment will be performed to identify any exclusions for expectant management or study enrollment as outlined in section 8.1 and to identify risk to meningococcal vaccination or infusion reaction. The risk of receiving the study drug (i.e. history of infusion reaction with eculizumab), antibiotics, or meningococcal vaccination will exclude the subject from the study. This safety screening will be performed on the day of enrollment prior to administration of the study drug.



1. Gestational hypertension and preeclampsia. ACOG Practice Bulletin No. 202. American College of Obstetricians and Gynecologists. Obstet Gynecol 2019; 133:e1-25.

Laboratory assessment for safety

To assure the safety of subjects to receive study drug, all subjects must have a baseline laboratory assessment of their condition, to include complete blood count, liver enzymes, and kidney function. Those with severe thrombocytopenia <50 k/ul or HELLP syndrome will be excluded for safety, as expedited delivery is recommended in this scenario. In addition, subjects with evidence of coagulopathy, with fibrinogen <200 mg/dl or INR ≥1.5 will be excluded due to their advanced disease requiring urgent intervention. Subjects with other forms of laboratory abnormalities, such as liver enzyme elevation, mild thrombocytopenia (platelet count 50-150 k/ul), or mild kidney injury, will be eligible for inclusion if the primary provider has deemed the patient a candidate for expectant management of pregnancy.

Physical examination for safety

The research study team will initially consent the study subject and review the chart for inclusion/exclusion criteria. When the subject is deemed eligible for study inclusion, a physical examination and symptom screen via the patient's medical record will be performed to rule out severe

headache, visual disturbances or right upper quadrant abdominal pain, which would preclude the patient from receiving study drug due to safety concerns.

Specimen Collection Drug Monitoring

1 tube (~10 cc.) of serum, 1 tube (~10 cc.) of plasma and 1 urine collection (~20 cc.) will be obtained for research purposes on day 1 prior to administration of study drug (pre-treatment baseline). The same samples will be collected every 3-4 days (q3-4days) through the last dose of eculizumab. The final serum, plasma, and urine specimen collections will be 3 days following the last dose of eculizumab administered. Specimens will be processed and stored in -80 degree Celsius research freezer and will be analyzed after the last study subject has completed the study. Results from these specimen analyses will not contribute to the study subject's clinical care.

All plasma and urine specimens will be assessed for complement levels including C5b-9 (MAC complex) and C5a. CD59, sFLT-1, and PlGF levels will also be assessed in plasma and urine specimens. Serum specimens will be assessed for Eculizumab level (PK) and activity (PD, % hemolysis) to determine peak and trough levels.

Maternal and fetal safety monitoring and duration of treatment

Maternal and fetal safety assessments will be performed daily by the study team, in addition to standard daily evaluations by the primary treatment team. All study patients will remain hospitalized for the duration of the study. Standard inpatient monitoring of preeclampsia will include maternal vital signs at least every 8 hours and fetal heart assessment at least every 12 hours. Subjects will continue study drug until maternal and/or fetal indications for delivery necessitate cessation of expectant management or until 34 0/7 weeks of gestation (for preeclampsia with severe features) or 37 0/7 weeks of gestation (for preeclampsia without severe features). Expectant management is expected to extend pregnancy for approximately 7 days on average. It is assumed that treatment with eculizumab will provide an additional increase in gestational age as compared to standard of care of 5-7 days. Total duration on study drug is therefore estimated to be approximately 7 to 14 days on average. However, it is possible that the duration on study drug could be 13 weeks if a patient enters the trial at 24 0/7 weeks of gestation and reaches 37 0/7 weeks of gestation.

Clinician guide for patient management-

The primary treatment team will guide clinical management of the patient and timing of delivery, but the following may be used as a guide for preeclampsia management among study patients:

I. **Indications for delivery may include one or more of the following**:
- Refractory hypertension despite maximal medical intervention
- HELLP syndrome (defined as all three of the following: AST or ALT >2x upper limit of normal, platelet count <100,000/µL, LDH ≥ 600 U/L)
- Platelet count <50,000/µL
- Eclampsia
- Placental abruption
- Intrauterine fetal demise
- Coagulopathy with INR ≥1.5 or Fibrinogen <200 mg/dl

- Pulmonary Edema
- Reverse end diastolic flow on umbilical Doppler ultrasound
- Biophysical score ≤4/10 on 2 occasions
    - Recurrent or prolonged fetal heart rate decelerations
- Intractable headache unrelieved with analgesia
- Intractable right upper quadrant abdominal pain or vomiting

II. Magnesium sulfate for seizure prophylaxis

- Magnesium sulfate, as a 4-6 gram intravenous (IV) bolus over 20-30 minutes, followed by 1-2 gram IV infusion per hr x24 hours, should be given at initial diagnosis of preeclampsia with severe features, during labor and delivery, and 24 hour postpartum.
- For patients undergoing expectant management of preeclampsia beyond 24 hours, magnesium sulfate may be stopped. However, it should be reinitiated (per dosing regimen above) if new severe features of preeclampsia develop such as severe headache, visual disturbances, eclampsia, or HELLP syndrome.
- All patients should be monitored for signs of magnesium toxicity every 2 hours and concerning signs may include loss of deep tendon reflexes, respiratory depression, blurred vision or slurred speech. If concern for magnesium toxicity, stop magnesium sulfate infusion and check serum magnesium level. Calcium gluconate 1g IV can be given for magnesium toxicity.
-

III. Treatment of headaches

- Persistent, severe headaches should raise suspicion for preeclampsia with severe features. Hyperreflexia and clonus are also suggestive of worsening preeclampsia. If headaches do not respond to analgesics, delivery should be considered.
- Possible analgesics may include:
    - Tylenol up to 1000mg q 6hr
    - Reglan 10mg q 6hr
    - Benadryl 25mg q6hr

IV. Betamethasone for fetal lung maturity

- Betamethasone, at a dose of 12mg IM q24hr x2 doses, should be given for fetal lung maturity when delivery is anticipated between before 37 weeks gestation.
- Among patients receiving a course of betamethasone <32 weeks gestation, a second "rescue" course may be given after 2 weeks have passed (up to 34 weeks gestation) if the patient remains pregnant.
- An alternative to Betamethasone is Dexamethasone (6mg IM every 12hr x 4 doses)

V. Magnesium sulfate for neuroprotection

- Magnesium sulfate, as a 4-6 gram intravenous (IV) bolus over 20-30 minutes, followed by 1-2 gram IV infusion per hr x12 hours, should be given for fetal neuroprotection when delivery is anticipated <32 weeks gestation. If delivery does not occur after 12 hours and delivery is no longer considered, the infusion should be discontinued and resumed when delivery was

deemed imminent again (at any point up to 32 weeks gestation). If at least 6 hours have passed since the discontinuation of the magnesium infusion, another loading dose may be given.

VI. Prevention of venous thromboembolism (VTE)

- Patients undergoing expectant management for preeclampsia are at high risk for VTE and they require mechanical or pharmacologic prophylaxis through delivery and immediate postpartum period.
- Spontaneous compression devices (SCDs) are recommended on both legs while the patient is lying in bed.
- Pharmacologic prophylaxis may be considered for VTE prophylaxis in patients at high risk including nephrotic range proteinuria, prior VTE, known thrombophilia, lupus, heart disease, sickle cell disease, or prolonged immobility >1 week in the hospital. Options include unfractionated heparin (7500mg SC daily <28 weeks and 10,000mg SC daily >28 weeks) or prophylactic dose lovenox 40mg SC daily.
- Pharmacologic prophylaxis should be stopped 24hr prior to epidural or spinal anesthesia. Anesthesiology should be consulted for preeclampsia patients receiving heparin or lovenox.

VII. Discontinuation of study drug

- The study drug may be stopped at any time upon patient request or the discretion of the treating provider.
- Worsening of preeclampsia, as noted above under indications for delivery, is not an indication to stop study drug.
- The study drug will be continued through delivery but will be discontinued at 48 hours postpartum.
- Indications for discontinuation of study drug include severe allergic reaction or bacterial, viral or fungal infection (aside from uncomplicated urinary tract infections or vaginal candidiasis). The study drug should be stopped for any signs of meningococcal infection, and appropriate treatment should be given.
- The data safety monitoring board will monitor all serious adverse events and will establish guidelines to determine if the study can be continued safely or if early termination is required. .
- Study patients will be followed until resolution of any adverse maternal or fetal outcomes.

Additional Safety Monitoring

Given the small number of subjects and the open-label study design, the ongoing safety of the study drug will be assessed after completion of each study subject by the Principal Investigator and the co-investigators. If at any point the study drug is deemed unsafe, all further study procedures will be stopped and safety committee will be assembled for subject case review.

Additional safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of 1 expert in clinical trials research, 1 obstetrician-gynecologist, and 1 pediatrician. Members of the DSMB will be independent from the study conduct and free of conflict of interest, or measures will be taken to minimize perceived conflict of interest. The DSMB will meet at least semiannually to assess safety and efficacy data on each arm of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to Alexion Pharmaceuticals and the Principal Investigator

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 (21 CFR 312.32 (a)). A medical condition that is present when the patient enters the study is not considered an AE unless this medical condition worsens after the patient undergoes a study-related intervention. Clinically significant laboratory abnormalities (for example, abnormal x-rays, ECGs, etc.) that occur or worsen with the intervention also are AEs. AEs in both mother and fetus/neonate will be recorded. Because this population is expected to have abnormal laboratory values, abnormal laboratory values should not be recorded as “Adverse Events” on the CRF unless medical intervention is necessitated (clinically significant). Abnormal laboratory results should be graded for severity by the Investigator only if they are considered AEs.

8.3.2. DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction at any dose in the subject or fetus/newborn is considered "serious" if, in the view of either the investigator or Alexion pharmaceuticals, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization 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. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

The following exceptions from expedited reporting as pertains to categorizing an event as serious, where serious is not the same as severe:

- Prolonged hospitalization due to resolution of signs and/or symptoms of preeclampsia or gestational hypertension as a result of the study therapy, as prolonging gestation is an intended side effect of the therapy and will not be considered an SAE.
- Premature delivery as this is an expected outcome in this patient population.

8.3.3. CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1. SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild (Grade 1)**– Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate (Grade 2)** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe (Grade 3)** – 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".

8.3.3.2. RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **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 study intervention administration 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 administration of the study intervention, 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 administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). 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 study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **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

Expected adverse reactions are AEs that are known to occur for the study intervention being studied and will be collected in a standard, systematic format using a grading scale based on functional assessment or magnitude of reaction. Expected adverse reactions for eculizumab according to the approved labeling package insert (2/2018 update) include headache, diarrhea, abdominal pain, hypertension, upper respiratory infection, nasopharyngitis, back pain, and nausea/vomiting, anemia, cough, peripheral edema, urinary tract infections, and musculoskeletal pain. Percent adverse reactions reported in at least 10% of adults and adolescent patients receiving eculizumab in aHUS clinical studies, include:

- Hypertension or hypotension – 33%
- Bronchitis – 12%
- Nasopharyngitis - 27%
- Gastroenteritis – 12%
- Respiratory tract infection – 19%
- Urinary tract infection – 22%
- Diarrhea – 37%
- Vomiting – 30%
- Nausea – 23%
- Abdominal pain – 19%
- Headache – 41%
- Anemia– 26%
- Leukopenia – 15%
- Insomnia – 14%
- Renal impairment – 18%
- Cough – 23%
- Fatigue – 13%
- Hypokalemia – 12%
- Rash – 14%
- Pruritus – 10%
- Joint pain – 13%
- Back pain – 10%
- Pyrexia - 21%

Other expected serious adverse events include,

- Viral Infection (2%)
- Meningococcal infection (1-2%, in all studies)
- Other infections
    - Streptococcus pneumoniae infection
    - Haemophilus influenza type b (Hib) infection
    - Aspergillus infection
- Infusion reaction, including anaphylaxis or other hypersensitivity reactions.

Eculizumab is administered via IV infusion. In this trial a patient´s vascular access will be obtained using the antecubital veins. Adverse reactions of IV access are infection of puncture site at the worst leading to bacteremia with the requirement of hospitalization and antibiotic therapy, bleeding from puncture site due to dysfunctional coagulation or vascular trauma secondary to traumatic puncture, nerve

damage as well as pain around the needle site or arm discomfort. All IV access sites will be examined each day and removed/changed if redness or pain (early signs of infection) at the site exists.

The Principal Investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. A copy of the package insert for the study drug will be available to guide determination of "expectedness vs. unexpectedness." 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 study intervention.

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

The occurrence of an adverse event (AE) or serious adverse event (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 including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), 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.

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 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 dose of study drug. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Specific, solicited events based on those reported in the eculizumab package insert (2/2018) will be listed in the CRF and will be asked at each visit. Unsolicited events will be captured by recording responses to the question, "Have you noticed anything different since you started the study?" Events will be followed for outcome information until resolution or stabilization.

8.3.5. ADVERSE EVENT REPORTING

Investigators will notify the principal investigator of all adverse events for severity assessment. All adverse events will be recorded in the CRF. Severe (grade 3) or life-threatening (grade 4) adverse events will be immediately reported to the PI, the study sponsor. The PI will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the PI's initial receipt of the information. In addition, the PI must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as

possible, but in no case later than 15 calendar days after the PI determines that the information qualifies for reporting. Unanticipated problems (regardless of severity) will be immediately reported to the PI. Unanticipated problems greater than or equal to grade 3 (severe) will be reported as above. Unanticipated problems less than grade 3 will be reported 10 days after the PI becomes aware of the event or receives an external report. Anticipated, non-serious adverse events will be reported in annual safety monitoring reports.

8.3.6. SERIOUS ADVERSE EVENT REPORTING

The study investigator will immediately report to the principal investigator (PI), the study sponsor, any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the PI.

Neisseria Meningitidis infection will be reported as an adverse event of special interest (AESI) and will be reported to the FDA and DSMB. In addition, all serious adverse events (SAEs) will be followed until satisfactory resolution or until the PI deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested and should be provided as soon as possible.

The PI, the study sponsor, will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the PI's initial receipt of the information. In addition, the PI must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the PI determines that the information qualifies for reporting.

8.3.7. REPORTING EVENTS TO PARTICIPANTS

Common adverse events associated with the study drug identified in the package insert will be summarized in the consent form to inform subjects of the possible risks. If unexpected or serious expected events occur, the IRB will be notified per typical regulatory reporting standards. If an unexpected or serious, expected adverse event occurs, subjects will be notified through their care-givers, who will be notified by the FDA and the Data Safety Monitoring Committee. A change will also be made to the informed consent, which will be submitted to the IRB for review.

No incidental findings are anticipated in this study. Should a reportable incidental finding be detected, the IRB will be notified and the subject will be notified through her care-giver.

9. STATISTICAL CONSIDERATIONS

9.1. STATISTICAL HYPOTHESES

Primary endpoint: Latency (in days) from enrollment to delivery

Hypothesis: Treatment with eculizumab prolongs pregnancy, compared to historical controls, in women with preeclampsia between $23^{0/7}$ - $29^{6/7}$ weeks gestation

Secondary endpoints:
1. Composite adverse maternal and neonatal outcomes
2. Terminal complement activation (C5a, C5b-9) in blood and urine
3. End-organ injury and anti-angiogenic imbalance

> Measures of end-organ injury: Serum creatinine, aspartate transaminase, alanine transaminase, hemoglobin, platelet count, lactate dehydrogenase, urine protein/creatinine ratio, and CD59
>
> Measures of anti-angiogenic imbalance: soluble fms-like tyrosine kinase 1 (sFLT-1), placental growth factor (PlGF)

Hypotheses:
1. Treatment with eculizumab decreases adverse maternal and neonatal outcomes in women with preeclampsia between $23^{0/7}$ - $29^{6/7}$ weeks gestation, compared to historical controls.

2. Treatment with eculizumab reduces terminal complement activation (levels of C5a and C5b-9 in blood and urine) in women with preeclampsia between $23^{0/7}$ - $29^{6/7}$ weeks gestation.

3. Treatment with eculizumab improves laboratory measures of end-organ injury (e.g., serum creatinine, platelet count) and reduces anti-angiogenic imbalance (ratio of sFLT1/PlGF) in women with preeclampsia between $23^{0/7}$ - $29^{6/7}$ weeks gestation.

9.2. SAMPLE SIZE DETERMINATION

In the medical literature, expectant management of early-onset preeclampsia (<34 weeks gestation) allows prolongation of pregnancy by a mean of 5-15 days and median of 5-9 days[43-47]. Median latency is likely shorter than mean latency because some women remain pregnant for weeks, skewing the mean value.

We performed sample size calculations by varying our assumptions for baseline latency with expectant management (7-10 days), varying our assumptions for standard deviation (4-7 days), and varying our effect size with study drug (gain of 3-7 days). See sample size table below.

Assumptions for Latency with Standard of Care Expectant Management (days ± SD)	Anticipated Latency with Eculizumab (days ± SD)	Sample Size Needed in Eculizumab Arm (α=0.05; β=0.80)
7 ± 3	11 ± 3	7
7 ± 3	14 ± 3	2
7 ± 7	11 ± 7	38
7 ± 7	**14 ± 7**	**12**
10 ± 3	14 ± 3	7
10 ± 3	17 ± 3	2
10 ± 7	14 ± 7	38
10 ± 7	**17 ± 7**	**12**

After evaluating this table, we designed the study to show a mean gain of 7 days in latency with study drug (eculizumab) compared to historical controls. Although a gain of 4 days could be shown with a sample size of 2-7 subjects, we believe that a gain of 7 days is more reflective of clinical benefit to the neonate. To show a gain of 7 days between groups (from 7 to 14 days, or 10 to 17 days) with 80% power and alpha=0.05, we require a sample size of 12 subjects in the eculizumab arm, assuming standard deviation of 7 days.

To reach 80% power, our historical control group required 36 subjects (3:1 matching to eculizumab cases), see statistical analysis below. Thus, 36 controls will be matched 3:1 to 12 cases by gestational age (± 7 days), parity (nulliparous/multiparous), fetal count of current gestation, and race/ethnicity (white/non-white).

Sample size calculation based on 3:1 enrollment with 80% power (Stata IC v15.1, Stata Corp, College Station, TX).

Estimated sample sizes for a two-sample means test
Satterthwaite's t test assuming unequal variances
Ho: m2 = m1 versus Ha: m2 != m1

Study parameters:

alpha = 0.0500
power = 0.8000
delta = 7.0000
m1 = 7.0000
m2 = 14.0000
sd1 = 7.0000

sd2 = 7.0000
N2/N1 = 0.3300

Estimated sample sizes:

N = 48
N1 = 36
N2 = 12
N2/N1 = 0.3333

M1= mean latency in control group; M2= mean latency in eculizumab group
N1= number of subjects in control group; N1= number of subjects in eculizumab group
N = total number of subjects
Sd1, sd2 – standard deviation in latency from enrollment until delivery

Summary statement: A sample size of 12 subjects in the treatment arm and 36 subjects in the historical control arm will allow us to detect a prolongation of pregnancy by 7 days in the eculizumab arm compared to standard of care (with 80% power and significance set at P<0.05).

9.3. POPULATIONS FOR ANALYSES

For analysis, we will utilize a modified intention-to-treat dataset. Participants who received at least one dose of the study drug will be included in the treatment group. Participants who were enrolled, but who never received study drug will be excluded from the analysis.

9.4. STATISTICAL ANALYSES

9.4.1. GENERAL APPROACH

Baseline characteristics of subjects in the two arms will be presented with descriptive statistics. Latency from enrollment to delivery, as the primary study endpoint, will be presented as mean days ± standard deviation, and statistical differences assessed by the Student's t-test. Data will be tested for normality. If non-normal distribution, median (interquartile range) values will be compared by a non-parametric test of medians. Secondary endpoints, derived from continuous data, will be assessed by Student t-test or test of medians as appropriate. Changes in lab values before and after treatment will be compared using the paired t-test. Data derived from dichotomous outcomes will be compared by Pearson's chi-squared test. Correlations between complement and laboratory measures will be assessed by Pearson's or Spearman correlation coefficient, depending on data normality. Significance for the primary endpoint will be determined by α=0.05. Significance of secondary endpoints will be determined after Bonferroni correction for multiple comparisons, where α= 0.05/number of hypothesis tested.

9.4.2. ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Primary endpoint: Latency in days from enrollment until delivery.

For participants receiving study drug, the primary endpoint will be latency (in days) from enrollment in the study (day 1) until delivery. For historical controls, we will determine latency from hospital admission for preeclampsia until delivery. For controls diagnosed with preeclampsia following hospitalization for other indication, we will determine latency from initial diagnosis of preeclampsia until delivery. Some cases may have 1-3 days hospital stay for preeclampsia prior to receiving study drug. However, they will not have more than 72 hour hospital stay prior to enrollment, per the exclusion criteria. To appropriately compare expectant management between groups, we will assess the average latency of cases prior to receiving the study drug. These days will be adjusted for when assessing the primary endpoint between groups.

The difference in latency (in days) between cases in the study group and historical controls will be assessed by Student t-test or non-parametric test of medians, depending on data distribution (normal or non-normal, respectively). Each participant receiving study drug will be matched to 3 controls, by gestational age (± 7 days), parity (nulliparous/multiparous), fetal count of current gestation, and race/ethnicity (white/non-white). This matching process was specifically designed to negate the impact of potential confounders, which could have impacted the primary endpoint in this small open-label study of 12 subjects.

9.4.3. ANALYSIS OF THE SECONDARY ENDPOINT(S)

Secondary endpoints:

1. Composite adverse maternal and neonatal outcomes.

Composites were created for both adverse maternal and neonatal outcomes due to the small sample size (12 participants), which is underpowered to detect a difference in individual outcomes between groups. However, while individual outcomes are rare, the range of potential outcomes in the composite measures are designed to capture potential differences between groups. Adverse maternal outcomes were selected in the composite if they could be attributed to preeclampsia (e.g., pulmonary edema, HELLP syndrome, eclampsia). Adverse neonatal outcomes were chosen if they could be attributed to preeclampsia directly (e.g., stillbirth) or indirectly, because of preterm delivery due to preeclampsia (e.g., respiratory distress syndrome, intraventricular hemorrhage).

Differences in the composite (maternal or neonatal) outcome between groups will be assessed by the Chi-squared test. Adjusted analyses will be performed using multivariable logistic regression, to control for potential confounders.

2. Terminal complement activation (C5a, C5b-9) in blood and urine

Concentrations of C5a and C5b-9 in blood and urine will be determined by enzyme linked immunosorbent assays (ELISA), performed in the Karumanchi laboratory at Cedars-Sinai Medical Center. These tests will be run after study completion and will not be made available to the treatment team or the study participant.

Differences in the concentration of C5a and C5b-9 in blood and urine, before treatment compared to after treatment in each participant, will be determined by the paired t-test at each time point. Visit day 1 will serve as the baseline reference measure.

3. End-organ injury and anti-angiogenic imbalance

Measures of end-organ injury: serum creatinine, aspartate transaminase, alanine transaminase, hemoglobin, platelet count, lactate dehydrogenase, urine protein/creatinine ratio, and CD59

Measures of anti-angiogenic imbalance: soluble fms-like tyrosine kinase 1 (sFLT-1), placental growth factor (PlGF)

Concentrations of Serum creatinine, aspartate transaminase, alanine transaminase, hemoglobin, platelet count, lactate dehydrogenase, urine protein/creatinine ratio, will be measured by the central hospital laboratory at Cedars-Sinai Medical Center and results will be available to the treatment team.

Concentrations of CD59, sFLT-1 and PlGF will be determined by enzyme linked immunosorbent assays (ELISA), performed in the Karumanchi laboratory at Cedars-Sinai Medical Center. These tests will be run after study completion and will not be made available to the treatment team or the study participant.

Differences in the concentrations of creatinine, AST, ALT, hemoglobin, platelet count, LDH, urine protein/creatine, CD59, sFLT-1 and PlGF, will be compared before and after treatment in each participant, utilizing the paired t-test at each time point. Visit day 1 will serve as the baseline reference measure.

9.4.4. SAFETY ANALYSES

Safety endpoints include the secondary endpoints 1) To determine if treatment with eculizumab for early-onset preeclampsia <30 weeks gestation decreases adverse maternal and neonatal outcomes, compared to historical controls, and 2) To determine if treatment with eculizumab improves laboratory measures of end-organ injury and reduces anti-angiogenic imbalance in women with preeclampsia <34 weeks gestation. Differences in the composite (maternal or neonatal) outcome between groups will be assessed by the Chi-squared test. Adjusted analyses will be performed using multivariable logistic regression, to control for potential confounders. Differences in the concentrations of creatinine, AST, ALT, hemoglobin, platelet count, LDH, urine protein/creatine, sFLT-1 and PlGF, will be compared before and after treatment in each participant, utilizing the paired t-test at each time point. Visit day 1 will serve as the baseline reference measure. Change scores from baseline will be analyzed as shift tables.

Adverse events will be coded by the Medical Dictionary for Regulatory Activities (MedDRA) and counted once for each given participant. Severity, frequency, and relationship of each AE to the study intervention will be presented by System Organ class and preferred term groupings. For each AE, start date, stop date, location, severity, relationship, expectedness, outcome, and duration will be recorded and reported. Additionally, for SAEs, a brief description of the nature of the SAE, the category (e.g. life-threatening, hospitalization, congenital anomaly, etc.), any intervention or treatments required, any follow-up testing, and whether the study intervention was discontinued will be recorded and reported. SAEs will be presented in a table.

9.4.5. BASELINE DESCRIPTIVE STATISTICS

Not applicable to this study.

9.4.6. PLANNED INTERIM ANALYSES

Given the small number of participants (12) and the pilot nature of this study, no interim analyses will be performed.

9.4.7. SUB-GROUP ANALYSES

The difference in latency (in days) between cases in the study group and historical controls will be assessed by Student t-test or non-parametric test of medians, depending on data distribution (normal or non-normal, respectively). Each participant receiving study drug will be matched to 3 controls, by gestational age (± 7 days), parity (nulliparous/multiparous), fetal count of current gestation, and race/ethnicity (white/non-white). This matching process was specifically designed to negate the impact of potential confounders, which could have impacted the primary endpoint in this small open-label study of 12 subjects. Men will be excluded as the condition – preeclampsia, a pregnancy disorder – only affects biologically female subjects. Adjusted analyses will be performed using multivariable logistic regression, to control for potential confounders.

The 1:3 case-controls matched by gestational age, parity, and race/ethnicity will also be applied to all secondary outcomes to negate the impact of potential confounders. Adjusted analyses will also be performed using multivariable logistic regression, to control for other potential confounders.

9.4.8. TABULATION OF INDIVIDUAL PARTICIPANT DATA

In order to fully characterize this pilot study, individual participant presentation, number of doses received, and outcomes may be described in a table. Baseline presentation and outcomes may also be summarized if the subject develops a serious adverse advent or if the participant's disease progresses to end-organ damage, such as the development of HELLP or eclampsia.

9.4.9. EXPLORATORY ANALYSES

Soluble fms-like tyrosine kinase (sFLT-1) and placental growth factor (PLGF) are well-described markers of progression of preeclampsia (36, 37). Therefore, plasma levels of sFLT-1 and PLGF will be measured to assess how treatment with eculizumab affects biochemical markers of disease and will be compared with clinical progression of disease. Differences in the concentrations of sFLT-1, PLGF, and their ratios, will be compared before and after treatment in each participant, utilizing the paired t-test at each time point. Visit day 1 will serve as the baseline reference measure. Similarly, complement activity (C5a and terminal complex C5b-9) in the plasma and urine will be assessed before and after treatment with eculizumab, using visit 1 C5a and C5b-9 activity in the serum and urine as a baseline. Differences in the concentrations of C5a and C5b-9 will also be compared before and after treatment in each participant, utilizing the paired t-test at each time point.

There is evidence to suggest that terminal complement inhibitor CD59 is upregulated on placental trophoblast cells, micro-vesicles and endothelial cells in preeclampsia (38-40). In response to complement-mediated cellular injury, CD59 may be released into maternal circulation in soluble form. Thus, to assess changes in terminal complement regulation with eculizumab, soluble CD59

concentrations in plasma and urine will be monitored before and after treatment in each patient utilizing the paired t-test at each time point. CD59 levels at visit 1 will be used as baseline values.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1. INFORMED CONSENT PROCESS

10.1.1.1. CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to administering study intervention.

10.1.1.2. CONSENT PROCEDURES AND DOCUMENTATION

Potential subjects will be identified from the labor & delivery unit, triage area, or antepartum wards at Cedars-Sinai Medical Center by a member of the research team. Only patients admitted to the hospital will be included in the study. A member of the research team will pre-screen potential subject charts to determine eligibility. If the patient meets inclusion/exclusion criteria, the patient's primary physician will be approached for active participation in the recruitment and treating process. If the patient's primary physician assents to her participation, the primary physician or a consulting healthcare provider will introduce the study and obtain the patient's verbal permission to be approached by a member of the research team while in the hospital. Study staff will coordinate a study visit with the patient in the hospital to review the consent form and to have a consent discussion. Only physician-investigators will obtain consent.

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants will have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. 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 the form signed, before the participant undergoes any study-specific procedures. A copy of the informed consent will be uploaded into the patient's electronic medical record, and a consent note briefly describing the intervention and the patient's consent will be added to the patient's chart. 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.

All eligible patients regardless of primary language will be approached for enrollment. For non-English speakers a short form will be used in accordance with Cedars-Sinai IRB policies, with forms available in the following languages: Arabic, Armenian, Chinese, Farsi, Korean, Russian and Spanish. An official medical translator will be used to consent non-English speakers, including phone translators.

Subjects that are mentally impaired or physically impaired due to medical illness and unable to give informed consent will not be enrolled in the study. Subjects may consider participation in the study for the duration of the time until they are discharged from the hospital following delivery. Consented subjects may withdraw from the study at any time.

Study participants between ages 13-18 are considered emancipated minors in pregnancy and are eligible to consent as adults for this research study. Pregnant adolescents may potentially benefit from this study therapy and are not likely to differ substantially from the adult population in relation to safety risks. To mitigate potential vulnerabilities among emancipated minors, participants <18 years old will be giving full opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. A verbal explanation will be provided to suit their comprehension of the purposes, procedures, and potential risks of the study and their rights as research participants.

Historical controls with preeclampsia at our institution between October 1, 2014 and September 30, 2019 will be identified, and data abstracted, according to Cedars-Sinai IRB Study #00052119. . To avoid selection bias, research staff blinded to the clinical outcomes of the case group will be utilized to select controls. Research staff will be asked to select two controls to match each case, based on gestational age (±1 week), parity (multiparous or nulliparous), and race/ethnicity.

10.1.2. STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigators, funding agency, the Investigational New Drug (IND) sponsor (the Principal Investigator) and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and Alexion Pharmaceuticals 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.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor (the PI), IRB and/or Food and Drug Administration (FDA).

10.1.3. CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the PI.

All research activities will be conducted in as private a setting as possible.

The study Data Safety and Monitoring Board, other authorized representatives of the PI, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product 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 clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at Cedars-Sinai Medical Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by Cedars-Sinai Medical Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at Cedars-Sinai Medical Center.

10.1.4. FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at Cedars-Sinai Medical Center. After the study is completed, the de-identified, archived data will be transmitted to and stored at Cedars-Sinai Medical Center, for use by other researchers including those outside of the study. Permission to maintain de-identified data at Cedars-Sinai Medical Center will be included in the informed consent.

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at Cedars-Sinai Medical Center. These samples could be used to research the causes of hypertensive disorders of pregnancy such as preeclampsia, its complications and other conditions for which individuals with hypertensive disorders of pregnancy are at increased risk, and to improve treatment. Samples from this study will be stored in the Karumanchi laboratory, and a de-identified code will link the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of 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 biosample 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 Karumanchi laboratory.

10.1.5. KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Data Safety and Monitoring Board Chairperson**
Richard Burwick, MD MPH	*Bobbie J. Rimel, MD*
Cedars-Sinai Medical Center	*Cedars-Sinai Medical Center*
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Reviewing IRB: Cedars-Sinai Medical Center Institutional Review Board

Pharmaceutical Sponsor:
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New Haven, CT 06510 USA

Basic Science Research Consultant:
S. Ananth Karumanchi, MD
Medallion Chair in Vascular Biology & Director of Nephrology Research
Cedars-Sinai Medical Center
Los Angeles, CA

Clinical Research Coordinator:
Martha Bautista
Department of Medicine, Nephrology
Cedars-Sinai Medical Center
Los Angeles, CA

Data Safety & Monitoring Board Members:

Expert in Obstetrics & Gynecology & Maternal Fetal Medicine:
Sarah Kilpatrick, MD PhD
Helping Hand of Los Angeles Chair in Obstetrics & Gynecology
Associate Dean for Faculty Development
Cedars-Sinai Medical Center
Los Angeles, CA

Expert in Pediatrics & Neonatology:
Charles F. Simmons, MD
Ruth & Harry Roman Chair in Neonatology in Honor of Larry Baum
Chair, Department of Pediatrics
Director, Neonatology
Professor of Pediatrics

10.1.6. SAFETY OVERSIGHT

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of 1 expert in clinical trials research, 2 obstetrician-gynecologists with expertise in maternal-fetal medicine, and 1 pediatrician. Members of the DSMB will be independent from the study conduct and free of conflict of interest, or measures will be taken to minimize perceived conflict of interest. The DSMB will meet at least semiannually to assess safety and efficacy data on each arm of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to Alexion Pharmaceuticals and the Principal Investigator.

10.1.7. CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by the Cedars-Sinai Medical Center IRB post-approval monitoring committee.
- Monitoring visits will start within 1 month of approval for training purposes and assure of protocol adherence. Monitoring visits will then be held semi-annually with 100% data verification. Should data by less than 90% accordant, monitoring visits will increase to quarterly with 100% data verification.
- Independent audits may be conducted by agents of the FDA or of Alexion Pharmaceuticals to ensure monitoring practices are performed consistently.

10.1.8. QUALITY ASSURANCE AND QUALITY CONTROL

As a single-center study, Cedars-Sinai Medical Center will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan with study staff will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the 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

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

If source documents are required, hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents. If source documents are not required, the electronic medical record will be considered the source.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into RedCap Database, a 21 CFR Part 11-compliant data capture system provided by Cedars-Sinai Medical Center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2. STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harminosation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. 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 sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10. PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. 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.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within three (3) working days of identification of the protocol deviation, or within three (3) working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to the Cedars-Sinai Medical Center IRB Program Official per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

10.1.11. PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information 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 2 years after the completion of the primary endpoint by contacting Richard Burwick, MD MPH, Cedars-Sinai Medical Center.

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 Cedars-Sinai Medical Center and its IRB have established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest. All members of study staff with

significant conflict of interest will not be eligible to recruit for the study. Furthermore, all significant financial interest will be disclosed on the informed consent.

10.2. ABBREVIATIONS

ACOG	American College of Obstetricians & Gynecologists
AE	Adverse Event
aHUS	Atypical Hemolytic Uremic Syndrome
ANCOVA	Analysis of Covariance
AST	Aspartate Transaminase
ALT	Alanine Transaminase
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRUSH	Complement Regulation to Undo Systemic Harm in Preeclampsia
CSMC	Cedars-Sinai Medical Center
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Forms
ELISA	Enzyme Linked Immunosorbent Assay
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007

FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HELLP	Hemolysis Elevated Liver enzymes Low Platelets
Hib	Haemophilus Influenza Type B
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
INR	International Normalized Ratio
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
IV	Intravenous
L&D	Labor and Delivery
LDH	Lactate Dehydrogenase
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MFCU	Maternal Fetal Care Unit
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center

OHRP	Office for Human Research Protections
PE	Preeclampsia
PI	Principal Investigator
PlGF	Placental Growth Factor
PO	By Mouth
PP	Post-partum
PNH	Paroxysmal Nocturnal Hemoglobinuria
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
sFLT-1	Fms-like Tyrosine Kinase
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
TMA	Thrombotic Microangiopathy
UP	Unanticipated Problem
US	United States
WoA	Waiver of Authorization

10.4. PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
2.0	10/26/20	Modifications per FDA comments in response to IND 146438	Modifications per FDA comments in response to IND 146438

11. PRINCIPAL INVESTIGATOR SIGNATURE PAGE

7-18-19

Principal Investigator

Date

Richard M. Burwick, MD MPH
Assistant Professor
Obstetrics & Gynecology
Division of Maternal Fetal Medicine
Cedars-Sinai Medical Center

Richard Burwick

10/26/20

Principal Investigator

Date

Richard M. Burwick, MD, MPH
Assistant Professor
Obstetrics & Gynecology
Division of Maternal Fetal Medicine
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Appendix A

MONITORING PLAN

IRB STUDY00000039
Protocol Title
Complement Regulation to Undo Systemic Harm in Preeclampsia: The Crush Study

PURPOSE:
The purpose of the monitoring plan is to present the sponsor-investigator's approach to monitoring clinical trials. The plan facilitates compliance with good clinical practices, FDA guidelines and regulations which require monitors to verify the following:

- The rights and well-being of participants are protected
- Reported data are accurate, complete and verifiable from source documents
- Trial conducted in compliance with currently approved protocol and other applicable regulatory requirements

This document identifies key monitoring activities and specifies the data to be reviewed over the course of a clinical trial. The clinical trial monitor will conduct monitoring visits (MVs) in accordance with this plan.

SELECTION AND QUALIFICATION OF MONITORS:
A member of the Cedars-Sinai Medical Center (CSMC) Office of Research Compliance & Quality Improvement will monitor this sponsor-investigator. Documentation of the monitor's qualifications with be maintained by the CSMC IRB. The Monitor will be familiar with the investigational product, protocol, consent form and any other written information given to the participant, sponsor's SOPs, and GCP and the relevant regulatory requirements.

SITE INITIATION
A site initiation visit will be held within 1 month of IRB approval. During this visit, the Site Visitors will review the following with the Sponsor-Investigator– Richard M. Burwick, MD MPH – and their study staff:

- Verify receipt of all documents and supplies needed to conduct study
- Informed consent obtained for each participant
- Source document verification
- Visit checklist and progress note completion
- Investigational product accountability
- Check and review of the regulatory binder and all essential documents
- SAE reporting
- Enrollment issues and targets
- Protocol amendments and their approval by the IRB
- Significant protocol deviations
- Acceptability of facilities
- Personnel changes
- Updated regulatory documentation
- Possible Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs). Two common examples include:
    - Certain AEs that are unexpected, serious, or occur at greater frequency than expected
    - A breach of privacy and/or confidentiality

  A full list of reportable events that are potential UPIRSOs can be found in the IRB policy: Reporting Possible Unanticipated Problems Involving Risks to Subject or Others (UPIRSO).
- Other subject-specific issues such as complaints, withdrawals, unscheduled study visits
- Compliance with relevant regulations and institutional policy and procedure
- Any other issue as deemed important to the conduct of the study

INTERIM MONITORING VISIT
An initial monitoring visit (MV) will be conducted after the first subject has been enrolled and every 6 months thereafter or as required by the Office of Research Compliance & Quality Improvement.

The following issues will be addressed at each interim visit as appropriate:

- Verify receipt of all documents and supplies needed to conduct study
- Informed consent obtained for each participant
- Source document verification
- Visit checklist and progress note completion
- Investigational product accountability
- Check and review of the regulatory binder and all essential documents
- SAE reporting
- Enrollment issues and targets
- Protocol amendments and their approval by the IRB
- Significant protocol deviations
- Acceptability of facilities
- Personnel changes
- Updated regulatory documentation
- Any other issue as deemed important to the conduct of the study

MONITORING COMMUNICATION PLAN

Following each monitoring visit, the Monitor will complete an MV report within 15 working days of the MV date.

The Monitor(s) will meet annually or more frequently (as needed) with the responsible investigator[1], Dr. Richard M. Burwick. Meetings with investigators will be scheduled to coincide with a scheduled monitoring visit and will focus on recurrent findings and subject safety or regulatory compliance concerns. The following topics will be discussed as appropriate:

- Enrollment progress
- Consent process and documentation
- Study conduct and documentation of study activities
- Developing an action plan for identified issues

CLOSE-OUT VISIT

A close-out visit will be conducted to ensure appropriate documentation is present and complete. The visit will occur after the last subject's case report forms have been completed, study has been closed with reviewing IRB/IEC and all regulatory issues have been addressed. The following issues, as applicable to the study, will be addressed at this visit:

- A complete review of the regulatory files, to ensure that all necessary CVs are present and current, all applicable versions of the protocol are present and filed appropriately, all applicable versions of the ICF are present, IRB approval letters are present, all SAEs have been reported to the sponsor and the IRB, and documentation of submission of

[1] The responsible investigator may be a sub-investigator who is taking the lead role in day-to-day study conduct.

protocol deviations to the IRB/Sponsor are present, notification and/or final report to the IRB present.
- A copy of the monitoring log
- A copy of the signature log (with delegation of duties)
- All CRFs have been completed and appropriately filed
- Maintenance and retention of study records are discussed
- Regulatory agency and institutional inspection process is discussed

ESSENTIAL DOCUMENTS
Essential Documents are those documents which individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. These documents serve to demonstrate the compliance of the investigator, sponsor and monitor with the standards of Good Clinical Practice and with all applicable regulatory requirements.

A minimum list of essential documents which has been developed follows. The various documents are grouped in three sections according to the stage of the trial during which they will normally be generated. A description is given below of the purpose of each document. It is acceptable to combine some of the documents, provided the individual elements are readily identifiable.

1) Before the clinical phase of the trial commences:
    - Signed protocol
    - Investigator brochure (if applicable)
    - Consent/HIPAA forms and IRB-approved information for subjects (e.g., recruitment documents)
    - Randomization procedures (if applicable)
    - Investigator, sub-investigators, and study team members' CVs or documentation of qualifications & training
    - Site signature log
    - Delegation of Responsibility Log
    - Lab normal ranges (if applicable)
    - Lab certifications (if applicable)
    - Ancillary Committee approvals (e.g. MRSC, GCS, Risk Management, Biosafety, Clinical Engineering)
    - Contracts/Agreements (i.e., transfer of sponsor responsibilities to other parties) (if applicable)

2) During the clinical conduct of the trial:
    - Equipment certifications, inspection logs, and/or calibration logs (as appropriate)
    - Screening logs
    - Enrollment logs
    - Adverse event logs

- Correspondence
- Subject code lists
- Product (drug or device) accountability logs
- Product handling and storage instructions
- Product shipping records and certificates of analysis (if applicable)
- Record of retained samples (if applicable)

3) After completion or termination of the trial:
    - Decoding procedures for blinded trials (if applicable)
    - Record retention plan
    - Monitoring reports

In summary, the Monitor will serve an important role in the successful conduct of the study. The relationship between the Monitor and the site staff is strengthened by open effective communication with the Monitor providing training and support to ensure participants' rights and safety as well as data quality and compliance with all applicable regulations of the regulatory authorities.

Appendix B

MONITORING SOP

IRB STUDY00000039
Protocol Title
Complement Regulation to Undo Systemic Harm in Preeclampsia: The Crush Study

1. MONITORING VISIT PREPARATION

After scheduling a monitoring visit (MV) (to take place after the first subject is enrolled and every 6 months thereafter), the monitor will:

- Review the current IRB approved protocol and consent/HIPAA versions
- Review previous monitoring reports to identify any unresolved issues

2. MONITOR RESPONSIBILITIES

The monitor's primary responsibilities are to ensure that the trial is conducted and documented properly by carrying out the following activities when relevant and necessary to the clinical trial.

I. INVESTIGATOR QUALIFICATIONS AND RESPONSIBILITIES

a) Verify that the Sponsor-Investigator has adequate qualifications to safely and properly conduct the trial. To accomplish this, the monitor will:

(1) Review the study regulatory file to verify there is curriculum vitae (CV) or other documentation of qualification for each investigator listed in the CSMC IRB application.

(2) Verify that each CV was current at the time of study initiation.

II. STUDY TEAM

a) Verify that the Sponsor-Investigator has a n adequate study team, is supervising study team members, and study team has adequate qualifications to conduct the trial according to the duties and responsibilities the Sponsor-Investigator has delegated. To accomplish this, the monitor will:

(1) Review study regulatory file to verify the names and qualifications of each CSMC study team member is adequately documented.

(2) Adequate documentation can include but not limited to CVs, resumes, licenses, certifications, and training documentation.

III. DELEGATION OF DUTIES

a) Review study regulatory file to verify the Sponsor-Investigator (and PI at non-CSMC sites) has adequately documented the delegations of duties and responsibilities, and that the study team members are qualified for the delegated duties and responsibilities.

b) Ensure trial staff at all sites is adequately informed about the trial and has not delegated responsibilities to unauthorized individuals. To verify this, the monitor will:

(1) Note the identity of all persons involved in study conduct by looking at the Delegation of Responsibility Log and verifying their qualifications.

(2) Check documentation for information about distribution of the currently approved protocol to the study team.

(3) Check documentation of any protocol specific training of authorized individuals

IV. FACILITIES

a) Verify that facilities at all sites, including laboratories and equipment, remain adequate throughout the trial. To accomplish this, the monitor will:

(1) Verify that the regulatory files from all sites contain current licenses and/or certifications, and lab normal ranges for the laboratory performing protocol-required procedures or tests.

V. INVESTIGATIONAL PRODUCT ACCOUNTABILITY

a) Verify storage, dispensing, instructions for use, and disposition of the investigational product complies with regulatory requirements.

(1) *Drugs and Biologics*

The monitor will review the site's IRB application to obtain pertinent information regarding the investigational biologic.

(a) The monitor will:

(i) Review the site's drug accountability log (at least at the time of the first MV and once annually until study closure).

(ii) Consult via email, telephone or otherwise with the Pharmacist as needed throughout study conduct about ongoing study drug management.

VI. PROTOCOL ADHERENCE

a) Verify that the Sponsor-Investigator and all co-investigators follow the IRB approved protocol. To accomplish this, the monitor will:

(1) Verify the (current) IRB approved protocol and the (current) protocol in the regulatory files are the same.

(2) Compare data collected with the IRB approved protocol (data collection should match the limits defined by the protocol).

(3) Verify the number and type of subjects entered into the study was confined to the number and type the protocol defined eligible. Verify eligibility requirements of enrolled subjects were met.

(4) Verify that no deviations from or changes to the protocol have been implemented without prior review and documented approval of the IRB (except where necessary to eliminate an immediate hazard to trial subjects or when the change involves only logistical or administrative aspects of the trial).

(5) Verify that the Sponsor-Investigator maintains records that indicate product has been supplied only to eligible subjects at protocol specified doses.

VII. RECRUITMENT AND ENROLLMENT RATES

a) Report on subject recruiting and enrollment rates. To accomplish this, the monitor will:

(1) Count the number of subjects enrolled (defined by this plan as having signed a consent form) and compare this number to the limit approved by the IRB.

VIII. PARTICIPANT BINDERS/DATA: CONSENT (100%)

a) Verify that written consent was obtained before subjects' participation. To accomplish this, the monitor will:

(1) Verify correct version of IRB-approved consent form was used

(2) Verify the date each consent form was signed and dated

(3) Verify, against subjects' research records, source documentation that the consent was signed before any research test or procedure was performed.

(4) Verify subjects signed and dated the California Research Participants' Bill of Rights (as applicable) and a HIPAA form prior to enrollment.

IX. PARTICIPANT BINDERS/DATA: ELIGIBILITY (50%)

a) Verify that only eligible subjects are enrolled. To accomplish this, the monitor will:

(1) Verify whether the existence of the condition for which the investigational product is being studied is documented by a compatible history.

(2) Compare the protocol inclusion/exclusion criteria against subjects' medical record or other source documentation to determine whether the enrolled subject is eligible for inclusion in the study.

X. PARTICIPANT BINDERS/DATA: COMPLETENESS AND ACCURACY (50%)

a) Verify that trial records are accurate, complete, and current and check the accuracy and completeness of CRF entries, source documents, and other trial- related records against each other. To accomplish this, the monitor will:

(1) Verify that the Sponsor-Investigator or assigned designee has completed visit checklists and progress notes, and that they are signed and dated appropriately.

(2) Verify that source documentation was used to collect study data.

(3) Verify whether clinical laboratory testing, as noted in the case report forms, is/are documented by the presence of completed records within the source documents.

(4) Verify data and source documents in terms of organization, condition, completeness, and legibility.

(5) Verify that required reports and submissions to the IRB have been made.

(6) Verify that required reports to the Sponsor-Investigator have been made.

(7) Verify that the Sponsor-Investigator has made required reports and submissions to the FDA.

(8) Verify that the information in the reports and the information in the Regulatory file and source documents match and are accurate and complete, including reports of any adverse events (AEs).

(9) Verify the data required by the protocol are reported accurately on the visit checklists and progress notes, and are consistent with the source data/documents.

(10) Verify any dose modifications are well documented.

(11) Verify concomitant medications, and underlying illnesses are reported accurately on the visit checklists, in accordance with the protocol.

(12) Verify that visit checklists and progress notes reflect all visits that subjects fail to make and all tests or examinations that are not performed.

(13) Verify, by looking at the visit checklists and progress notes, that all applicable forms are completely filled out; if any subject has withdrawn or dropped out of the study since enrollment, verify that an explanation is provided.

(14) Verify all tests have been completed as stated in the protocol by looking at source documentation, visit checklists and progress notes.

b) If errors are found in greater than 50% of the verified data, verification will escalate to verification of 100% of *new* data for that monitoring visit (i.e. data acquired since last monitoring visit).

XI. ADVERSE EVENT AND PROTOCOL DEVIATION DOCUMENTATION & REPORTING (50%)

a) Determine whether all events, i.e., adverse events, SAEs, deaths, deviations and/or violations, and UPIRSOs are reported appropriately. To accomplish this, the monitor will:

1) Verify that, in accordance with the protocol, adverse events have been documented appropriately and accurately by comparing subject medical records, the visit checklists and progress notes.

2) Verify that serious adverse events have been reported to the IRB, Sponsor-Investigator, and, if applicable, the manufacturer/funding source, by looking at correspondence files and comparing against subject medical records, verifying that they are also recorded on the study AE log.

3) Verify, by reviewing correspondence files and IRB reporting policies and comparing against subject medical records, that the IRB guidelines for reporting have been followed.

4) Verify that all adverse events that are required by FDA regulation to be reported to the FDA have been reported within the specified time frames.

5) Verify if any visit was out of allowable time (deviation) by looking at subject visit schedule.

6) Verify any other protocol deviations by comparing the protocol with source documentation and/or subject visit checklists and progress notes.

7) Verify that all possible Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) have been submitted to the IRB for determination and corresponding action as needed. This is achieved by reviewing subjects' records and noting any unexpected issues. For example, an otherwise not reportable (under institutional policy) AE that is unexpected in the particular research setting, or which is more serious than anticipated, or which has occurred at a higher than anticipated frequency; or a breach of privacy or confidentiality.

8) Verify that subject-specific issues, such as complaints, withdrawals and unscheduled study visits are addressed and resolved within the parameters of the IRB-approved protocol.

9) Verify the study is being conducted in compliance with the IRB-approved protocol, and with respect to relevant regulations and institutional policy and procedure.

XII. ESSENTIAL DOCUMENT MAINTENANCE

a) Verify that all essential documents (as applicable to the study) are maintained. (See study-specific Monitoring Plan)

XIII. COMMUNICATION AND DOCUMENTATION OF DEVIATIONS AND ERRORS

a) Communication of Deviations and Errors

(1) Deviations from the protocol, GCP, or regulatory requirements will be communicated to the sponsor-investigator and appropriate action to prevent recurrence of the deviations will be taken. To accomplish this, the monitor will:

(a) Meet or speak with the investigator or coordinator, at the end of each MV, to go over any findings of the visit.
(b) Provide details of the deviation(s) in the MV report for sponsor-investigator assessment.

(2) Ensure that appropriate corrections, additions or deletions are made, dated, explained (if necessary), and initialed by the sponsor-investigator or their designee authorized to make such changes. (This authorization must be documented on the responsibility log).

XIV. COMMUNICATION OF FINDINGS

a) The monitor will send a monitoring report to the sponsor-investigator. The report will describe the progress of the study, the findings of the visits, unresolved issues, and follow-up required.

b) The monitor will keep an electronic copy of the report and a signed copy will be maintained in the Regulatory file.

c) Follow-up items will be checked and documented at the next MV. The report will include, but will not be limited to, the following:

(1) A list of records reviewed, i.e. subject charts, hospital records, lab slips, etc.

(2) Statement that test article accountability records were or were not sufficient;

(3) Statement regarding whether there was any evidence of under- reporting of adverse events;

(4) Statement regarding protocol adherence.

d) The monitor will meet annually or more frequently (as needed to address recurring or more serious findings) with the responsible investigator named in the monitoring plan. This meeting will occur in conjunction with a scheduled monitoring visit and will review recurrent findings and subject safety or regulatory compliance concerns.

XV. EXTENT OF DATA MONITORING

a) Study Endpoints

(1) Monitors will review clinical data that affect study endpoints defined in the protocol. The extent of subject data monitoring will include verifying:

(a) Initial study consent for 100% of enrolled subjects

(b) Study eligibility for 50% of enrolled subjects

(c) Data to support protocol defined endpoints for 50% of completed subjects. If errors are found in greater than 50% of verified endpoints, monitoring will escalate to verification of 100% of new data acquired since last monitoring visit.

b) Regulatory File

(1) In addition to monitoring subject data, the monitor will review the regulatory file for any additions to GCP-required documents since the last visit. Monitors will, at their first and last MVs, review the regulatory file for the presence, completeness, and accuracy of all GCP-required documents.

Appendix C. Data Safety & Monitoring Board Signature Page

_____ _____

Expert in Clinical Trials Research:: Date
Bobbie J Rimel, MD
Assistant Professor
Department of Obstetrics & Gynecology
Cedars-Sinai Medical Center
Los Angeles, CA

_____ _____

Expert in Obstetrics & Gynecology & Maternal Fetal Medicine: Date
Sarah Kilpatrick, MD PhD
Helping Hand of Los Angeles Chair in Obstetrics & Gynecology
Associate Dean for Faculty Development
Cedars-Sinai Medical Center
Los Angeles, CA

_____ _____

Expert in Obstetrics & Gynecology & Maternal Fetal Medicine: Date
John Williams III, MD
Professor and Director of Reproductive Genetics
Department of Obstetrics & Gynecology
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Los Angeles, CA

_____ _____

Expert in Pediatrics & Neonatology: Date
Charles F. Simmons, MD
Ruth & Harry Roman Chair in Neonatology in Honor of Larry Baum
Chair, Department of Pediatrics
Director, Neonatology
Professor of Pediatrics