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Relationship between Plasma Concentration of 17-hydroxyprogesterone caproate (17-OHPC) and Preterm Birth

Protocol version 3.4

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Obstetric-Fetal Pharmacology Research Centers (OPRC) Network
The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

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Revision History:

Version/Protocol Date	Revision Description
Version 2.3 20 Aug 2017	Original version approved by UPITT IRB
Version 2.4 11 Oct 2017	- Added the option for subjects assigned to the 500 mg dose to either receive one 2 ml injection (500 mg) or two 1 ml injections (250 mg each) administered at the same time - Clarified that screening can occur up to 20 6/7 weeks and that the latest gestational age to start treatment is 21 6/7 weeks - Added information regarding randomization procedures - Added information for removing subjects from the study or stopping treatment, as well as under what scenarios subjects removed from the study will be replaced - Added statement that data from subjects removed from the study will not be used for analyses related to plasma 17-OHPC concentration - Added SAE reporting requirements - Added clarification to the patient management section regarding study non-compliance - Other minor changes were made to improve clarity and ensure consistency among all study documents.
Version 2.5 1 Dec 2017	Addition of information relating to the use of the REDCap Database Revised storage of biological samples to -70° to -80° C.
Version 2.6 11 Jan 2018	- Allow weekly 17-OHPC injections to be administered by research staff, physician's office staff, or a person selected by the subject who can administer the medication in the subject's home. - Research staff will monitor compliance through weekly discussion with the subject and empty vial counts. - Recruited subjects will be seen at MWH every 4-6 weeks to provide additional medication and to perform other aspects of the study. - An additional blood sample will be collected at 6-9 weeks after the 26-30 week specimen.
Version 2.7 26 Feb 2018	- Corrected the size of the placenta sample that will be collected after delivery from 3x3 inch to 3x3 cm. - Clarification that the person administering home injections will attend a study visit conducted prior to the start of home injections for training
Version 2.8 2 Apr 2018	- Section C.2: Enrollment may differ substantially between centers - Additional subsections were added to C.7: criteria for stopping treatment, non-compliant subjects, and subjects that withdraw from the study. - The former section C.9 "Criteria for Removing Subjects from the Study or Stopping Treatment" was deleted, as the content from this section is part of Section C.7
Version 2.9 7 May 2018	- Section E.3: The assay for measuring 17-OHPC is being validated to measure lower concentrations and will be performed using plasma and whole blood samples. ***Note:*** *Analysis of 17-OHPC will be performed using plasma and whole blood samples at the Magee-Womens Hospital site. The other sites will collect plasma samples only.* - Section C.2: Added secondary research question: Does the 17-OHPC concentration in maternal plasma or whole blood correlate better with certain physiological parameters? - Section D.1: Added secondary outcome: The correlation of progesterone and 17-hydroxyprogesterone in maternal plasma and whole blood to clinical outcomes

Version 3.0 29 Aug 2018	- Section C.6.i: study drug "may be administered by...physician's office staff or other qualified medical personnel or a person selected by the subject who can be trained to administer the medication in the subject's home." - Section C.6.iii: examination of the injection sites during the in-person study visits will be performed as needed - Section C.2: revised the last bullet under secondary research questions to replace "certain physiological parameters" with "certain pharmacological and clinical outcomes". - Section D.1: revised the last bullet under secondary outcomes to replace "clinical outcomes" with "pharmacological and clinical outcomes". - Exclusion Criteria: added current or history of thrombosis, liver tumors, liver disease/failure, renal disease/failure, breast cancer, other hormone sensitive cancers, or a history of these conditions, undiagnosed vaginal bleeding unrelated to pregnancy, moderately severe depression (PHQ-9 score of ≥ 15 or suicidal ideation) - Section C.6.iii: Added screening tests (PHQ-2 and PHQ-9) for depression - Section C.7.i: added uncontrolled hypertension and suicidal ideation to the criteria for stopping treatment
Version 3.1 06 Nov 2018	- Removed the list of Steering Committee members from the cover page - Section C.6.i: subject will be given 6-9 weeks' worth of medication to take home with her until the next visit with research staff - Section C.6.iii: frequency of in-person study visits changed to 6-9 weeks - Section C.6.v: added clarification that delivery samples will only be collected if possible - Section C.7.i: removed subjects with hypertension requiring 2 or more agents as criteria for stopping treatment - Section E.1: we will save an aliquot from each lot of the study drug rather than from each patient
Version 3.2 31 July 2019	- Section C.3.1: Added an EPDS score of >13 to the exclusion criteria - Section C.6.iii: Added clarification to depression screening and added EPDS as a test for depression - Section C.6.v: The first maternal blood sample will be drawn prior to the 2nd injection, ideally on day 7 from the first injection. Added clarification that the third maternal blood sample must be drawn no later than 37 weeks
Version 3.3 3 Jan 2020	- Introduction, Rationale, Sections C.2, C.3, C.5, and C.6 were revised to include the addition of the Ancillary Cohort. - Introduction: Added information related to the FDA Advisory Committee decision - Study Design: Added the University of Utah and the University of Texas – Houston as additional study sites
Version 3.3 16 April 2020	- Correction to EPDS question 10: a score of ≥1 indicates suicide risk
Version 3.4 15 July 2020	- Section C.6.1 was revised to allow subjects in the ancillary cohort to receive the Makena study drug

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A. Introduction

Preterm birth (PTB) remains one of the most important problems in obstetrics today, affecting more than 530,000 babies each year in the United States. PTB is the leading cause of newborn death and a major cause of lifelong disability. A recent report by the Institute of Medicine estimated that the annual societal economic burden associated with PTB in the United States to be at least $26.2 billion per year, or $51,600 per infant born preterm. A single preterm birth is associated with a recurrence rate of 14-22%, two prior preterm births with a recurrence of 28-42% and women with more than two prior preterm births face an astounding 67% risk of having another preterm birth. Over the last five decades attempts to reduce the PTB rate have failed despite a wide variety of approaches. Recently, however, administration of intramuscular 17-hydroxyprogesterone caproate (17-OHPC) has proven effective in reducing the recurrence of preterm births in this high-risk group. Currently, the medication is offered to all women with a prior spontaneous PTB. A dose of 250 mg is administered intramuscularly weekly from weeks 16-21 until week 36 of gestation. This dosing regimen does not have a sound pharmacologic basis. Recent data on the pharmacology of 17-OHPC suggest that plasma concentration is closely related to the efficacy of this treatment. As many as half the women receiving this medication achieve an inadequate plasma concentration with the standard 250 mg weekly dose and may not benefit from this treatment.

In this proposed study, we will determine the relationship between plasma concentrations of 17-OHPC and the rate of preterm birth (PTB). The study is a randomized open label study of pregnant women with one or more prior spontaneous preterm births. Subjects will be randomized to a weekly single injection of either 250 or 500 mg 17-OHPC. The randomization will be 2:1 for the 500mg vs the 250 mg dose. The recruited cohort of women will be sufficiently large to describe the association between plasma concentration of 17-OHPC and the rate of preterm birth and to evaluate the impact of several potential covariates on plasma concentration of 17-OHPC and its efficacy.

Primary Objective: To assess the relationship between the plasma concentration of 17-OHPC and rates of preterm (<37 weeks) birth.

B. Background

B.1. Significance of Preterm Birth

Preterm birth (PTB) remains one of the most important problems in obstetrics today, affecting more than 530,000 babies each year in the United States.[1] PTB is the leading cause of newborn death and a major cause of lifelong disability. A recent report by the Institute of Medicine estimated that the annual societal economic burden associated with PTB in the United States to be at least $26.2 billion per year, or $51,600 per infant born preterm.[2] A single preterm birth is associated with a recurrence rate of 14-22%, two prior preterm births with a recurrence of 28-42% and women with more than two prior preterm births face an astounding 67% risk of having another preterm birth.[3]

B.2. Prevention of PTB with 17-OHPC

Over the last five decades attempts to reduce the PTB rate have failed despite a wide variety of approaches. Recently, however, administration of intramuscular 17-hydroxyprogesterone caproate (17-OHPC) has proven effective in reducing preterm births in this high-risk group.[4] The target population of 17-OHPC is the woman with a history of one or more prior spontaneous preterm births. A study from the Maternal-Fetal Medicine Units (MFMU) Network[4] demonstrated a 33% reduction in preterm birth among women with a prior spontaneous PTB who were treated with (17-OHPC) as opposed to placebo. The American College of Obstetricians and Gynecologists (ACOG) supports use of 17-OHPC to reduce the rates of PTB in women with a prior spontaneous PTB.[5] Most insurers endorse the use of 17-OHPC as they see a benefit in controlling rising health care costs associated with preterm delivery. It has been estimated that universal use of 17-OHPC in at risk women would reduce the societal cost of prematurity by $2 billion annually[6,7] and may actually reduce prematurity rates.[8,9]

B.3. Issues with 17-OHPC for PTB Prevention

Currently, 17-OHPC is offered to all women with a prior spontaneous PTB, which accounts for roughly 133,000 women annually.[10] A dose of 250 mg is administered intramuscularly weekly from weeks 16-21 until week 36 of gestation. This dosing regimen does not have a sound pharmacologic basis and is based on a meta-analysis[11] and a single clinical trial[12]

suggesting benefit but no dose-ranging studies or concentration controlled trials on the safety or efficacy of 17-OHPC have been performed to date. Considerable skepticism about the efficacy and safety of 17-OHPC exists despite the drug's approval by the FDA. Skepticism regarding the findings in the Meis trial stem from the unexpectedly high rate of preterm birth in the placebo group (52%) compared with the 17-OHPC treated group (37%).[4, 11] In other studies of women having a prior preterm birth, the rate of recurrent preterm birth in untreated women has been closer to the 37% range. As part of the conditional FDA approval for Makena, the sponsor was asked to complete another randomized placebo-controlled trial. That study was recently completed and the results were reviewed by the FDA-Advisory Committee. There was no benefit of 17-OHPC over placebo in that study. The FDA Advisory Committee on October 29, 2019 voted 9 to 7 to withdraw approval of Makena (17-hydroxyprogesterone caproate, 17-OHPC). The FDA will decide on whether or not to accept the Advisory Committee's recommendation; the close vote suggests that the FDA choice is far from clear. Furthermore, there are several issues related to the trial that confound the issue of 17-OHPC efficacy. The Prolong study had difficulty recruiting in the United States since 17-OHPC had been considered 'standard of care' for all women with a singleton pregnancy and a prior PTB. Consequently, most subjects were recruited overseas primarily in Russia and the Ukraine. The sample size was large (1708 subjects) but the primary outcome (delivery<35 weeks) was uncommon occurring in only 12% of the subjects and rates were similar in both treatment groups. The populations studied were very different in the MFMU study which showed a benefit and the Prolong study which did not. In the MFMU study 59% of enrollees were African American vs 7% in the Prolong trial. Also risk factors for PTB such as smoking, >1 prior preterm birth and other PTB risk factors were greater in the MFMU trial , 20 vs 8%, 32 vs 12%, 91 vs 48%, respectively. Importantly, no significant harms from 17-OHPC were identified.

The challenge faced by the FDA as well as providers of care is how to integrate the Prolong data into clinical care for women with a prior preterm birth. Currently, both the Society for Maternal-Fetal Medicine (SMFM) and the American College of Obstetricians and Gynecologist (ACOG) support continuing use of 17-OHPC for women with a prior PTB. The perspective is that the Prolong trial does not negate the findings of the MFMU (Meis) trial. Those skeptical of the effectiveness of 17-OHPC have suggested using vaginal progesterone in women with a prior spontaneous PTB despite the fact that the largest placebo controlled randomized

control trial demonstrated that vaginal progesterone was ineffective in women with a prior PTB.[13] This suggestion, however, is appealing to some and limits enthusiasm for a large clinical trial of 17-OHPC.

The findings from our current trial may provide important information on the effectiveness of 17-OHPC. In particular, if there is a relationship between 17-OHPC concentration and the rate of PTB, a higher dose may be needed. The failure of the Prolong trial and the relatively low risk reduction (33%) in the Meis trial may be due to an inadequate dose. Our recent data, which are summarized below, suggest that the 250 mg dose may be inadequate in a substantial portion of women receiving this therapy.[14] From a safety point of view, doses as high as 1000 mg weekly have been used in pregnant women without any apparent adverse effects; this provides substantial latitude in dosing.[15-17]

B.4. Pharmacology of 17-OHPC

Until recently, the only pharmacological data published on 17-OHPC were obtained in non-pregnant women who were receiving the medication for treatment of uterine cancer.[18] The doses used varied widely and as much as 1000 mg was administered daily for 5 days. The dosing regimen used in non-pregnant women with uterine cancer is not applicable to pregnant women and cannot be used as a justification for the use of 250 mg weekly for prevention of preterm delivery. In the MFMU study reported by Meis et al, blood was not collected.[4] Therefore, there was no way to evaluate the relationship between plasma concentrations of 17-OHPC and the outcome of interest, the rate of PTB. We have utilized one small and two large clinical cohorts to evaluate the pharmacokinetics and pharmacodynamics of 17-OHPC.[14,19-21] The terminal half-life of 17-OHPC administered intramuscularly was estimated to be around 16 days in women with a singleton gestation. There was a good correlation ($r = 0.83$) between the trough plasma concentrations of 17-OHPC and the area under the plasma concentration vs time curve (AUC), a measure of patient exposure to the drug at steady state. In all cohorts, a fixed dose of 250 mg resulted in a large variation in the trough plasma concentrations of 17-OHPC suggesting that there was a large variation in drug exposure in these patients. At least part of the observed variability in the trough plasma concentrations of 17-OHPC in these patients may be attributed to maternal BMI with heavier women having lower plasma concentrations. Variation in the expression and activity of the CYP3A4/5 enzyme that is primarily responsible for the metabolism of 17-OHPC

does not appear to impact plasma concentrations (unpublished data). We have analyzed data from two large multicenter trials from the Maternal-Fetal Medicine Units Network (STTARS and Omega-3 trials) and one multicenter study from the Obstetrical-Fetal Pharmacology Research Units Network (Caproate PK study).[14,19-21] Two of these were performed in women with singleton gestation and one study was performed in women with twin gestation. The twin study performed in the MFMU Network (STTARS Trial) was the first study of 17-OHPC in pregnant women. Both pharmacokinetic and a pharmacodynamic studies were performed in women with twin gestation.[19,20] More relevant to the present proposal are the two studies performed in women with singleton gestation.[14,21] In the OPRU Caproate PK study[21], we defined the pharmacokinetic characteristics of 17-OHPC in a small (n=61) cohort of women with singleton gestation and a prior preterm birth. These women received weekly injections of 17-OHPC and underwent 2 pharmacokinetic studies at 20 0/7 to 24 6/7 weeks' gestation (PK1) and again at 31 0/7 to 34 6/7 weeks' gestation (PK 2). Figure 1 depicts the mean and SD of 17-OHPC concentrations in these women. Concentrations of 17-OHPC were higher during PK 2 than during PK 1. In 18 women, blood samples were obtained over a 28-day period beyond the last injection (extended study, Figure 2).[21] Maternal and/or cord blood were obtained at delivery. The half-life (median ±SD) of 17-OHPC was 16.2 ± 6 days. Body mass index affected maternal 17-OHPC concentrations. The trough plasma concentration of 17-OHPC was a good surrogate marker of drug exposure AUC (r=0.83). The cord to maternal 17-OHPC concentration ratios averaged 0.2; 17-OHPC was detectible in cord plasma 44 days after the last maternal injection. The trough plasma concentration of 17-OHPC was a good surrogate marker of drug exposure AUC (r=0.83). This study also showed

Figure 1. Plasma 17-OHPC Trough Concentrations After Weekly IM Injections of 250 mg

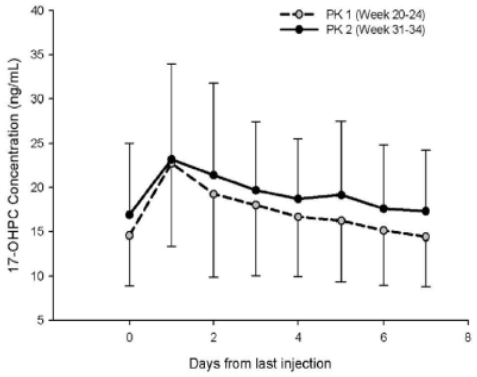
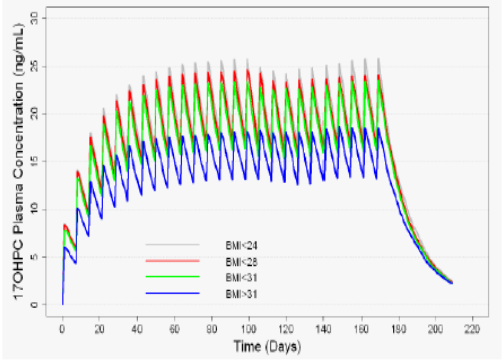


Figure 2. Impact of BMI on Simulated Plasma 17-OHPC Concentrations After Weekly Injections of 250 mg



a large variation in the trough plasma concentrations of 17-OHPC in subjects receiving a fixed 250 mg weekly dose. Some of that variation was attributable to maternal BMI (Figure 2) which seems to account for < 30% of the variability in plasma concentrations when POPPK analysis is performed.

We have also studied the pharmacology of 17-OHPC in the laboratory. Using human liver *microsomes*, hepatocytes and expressed enzymes we have demonstrated that 17-OHPC is primarily metabolized by the CYP3A enzymes.[22] Using adult and fetal *hepatocytes* we have shown that CYP3A4, CYP3A5 and CYP3A7 are capable of metabolizing 17-OHPC.[23] Using human liver *microsomes* we have recently shown that the metabolism of 17-OHPC is inhibited by progesterone[24] and several other drugs commonly used in pregnancy. Fourteen co-medications listed below were identified as inhibitors (>30% inhibition) of the metabolism of 17-OHPC: ketoconazole, fluconazole, itraconazole, voriconazole, nelfinavir, ritonavir, esomeprazole, montelukast, bergamottin, sertraline, trazodone, haloperidol, tacrolimus, and fluticasone.[25] We have also demonstrated that transporters are involved in the disposition of 17-OHPC which is transported into and out of hepatocytes by an active efflux process which is significantly inhibited by cold temperature, cyclosporine, verapamil and rifampin.[23] The active efflux mechanism was observed in both adult and fetal hepatocytes.

B.5. Preliminary Data Relevant to the Primary Outcome

The overall efficacy of 17-OHPC (33% reduction in preterm birth rates), might be improved if a pharmacologically-based dosing regimen was utilized rather than a single dose "one fits all"

Figure 3. Histogram of 17-OHPC concentrations after weekly injections of 250mg

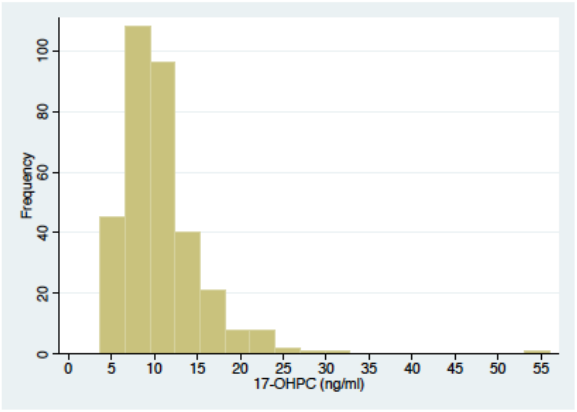
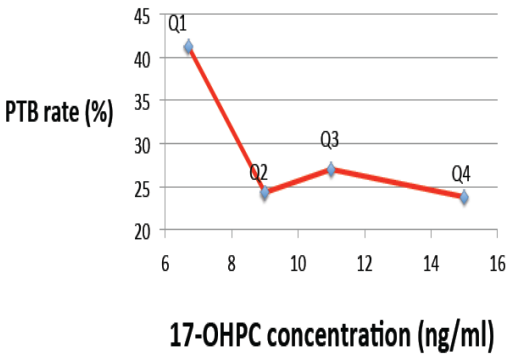


Figure 4. Relationship Between Median 17-OHPC Concentration within each quartile and PTB Rate



strategy. To better understand the relationship between drug exposure and outcome, we have recently performed a secondary analysis of a cohort of women with a singleton gestation and a prior PTB using data obtained in the Omega-3 study from the MFMU Network.[14]

In this study, a single blood sample was obtained at 24-28 weeks' gestation in 331 subjects who received weekly injections of 250 mg 17-OHPC. The trough plasma concentration of 17-OHPC in this cohort of women varied widely, ranging from 4-56 ng/ml (Figure 3) with a median value of 9.9 ng/ml. The rate of preterm birth was significantly associated with the plasma 17-OHPC concentration with the highest rates seen in women with the lowest quartile of plasma concentrations (Figure 4). The significant relationship between quartiles of the plasma concentrations and the outcome of preterm birth persisted after adjustment for potential confounders such as body mass index (BMI), gestational age at study entrance, gestational age at blood sampling, race and ethnicity (Figure 5). The apparent plateauing effect of efficacy with concentrations above 10 ng/ml (Figure 4) could be due to a true plateau effect on preterm birth rates or alternatively it may be due to the small sample size at the higher concentrations. To address this issue, we examined the relationship between the rate of preterm birth and plasma concentrations of 17-OHPC expressed as a continuous variable rather than as quartiles.

Figure 5. Proportion of Subjects remaining Pregnant According to Quartile of Plasma 17-OHPC Concentration

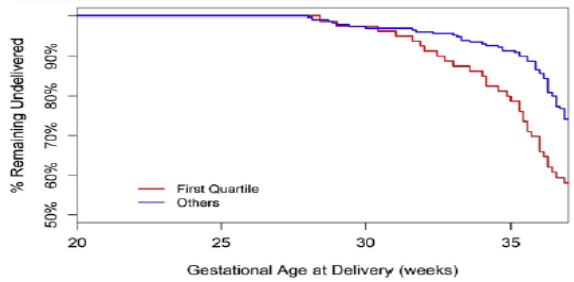
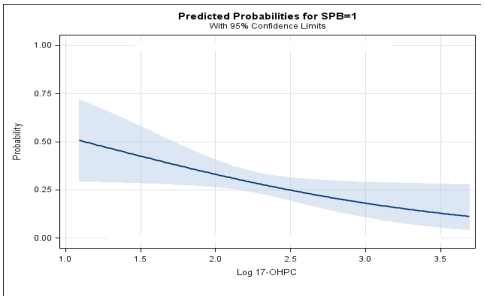


Figure 6. Relationship between Plasma 17-OHPC and PTB

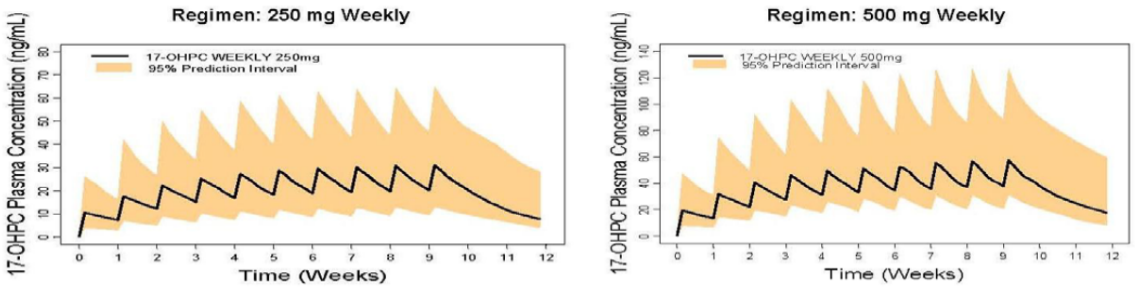


After log transformation of the concentration data we demonstrated a significant relationship ($p<0.04$) between the outcome of preterm birth and plasma concentrations of 17-OHPC; the hazard ratio for preterm birth decreased by 0.49 for every unit increase in the log of the 17-OHPC concentration (Figure 6). This relationship is linear and demonstrates that the rate of PTB does not appear to plateau as suggested in Figure 4. In the secondary analysis of the Omega-3 study, women with plasma concentrations of 17-OHPC >10 ng/ml had a rate of preterm birth of 25% while women with concentrations < 10ng/ml had a preterm birth rate

of 37% (Figure 4). Importantly, only 50% of the women in this study achieved plasma concentrations > 10 ng/ml and only 5% of subjects achieved concentrations greater than 20 ng/ml (Figure 3). The median concentration of 17-OHPC at the highest quartile was only 15 ng/ml (Figure 4). These data suggest that that the rate of preterm birth could be reduced further with higher plasma concentrations of 17-OHPC. The higher concentrations could impact two aspects of 17-OHPC efficacy. First, the percentage of women below what appears to be the lowest level of efficacy, i.e., 10 ng/ml would be increased with a higher dose. Secondly, the apparent plateauing effect may disappear with more concentrations in excess of 20 ng/ml. If indeed there is no plateauing of efficacy at higher concentrations, then the overall success rate could be increased beyond the reported 33% overall efficacy of 17-OHPC. This study provided proof of the efficacy of 17-OHPC because the pharmacodynamic end point, PTB was dependent on plasma 17-OHPC concentrations. This evidence addresses those skeptics of the utility of 17-OHPC in preventing recurrent preterm birth.

Using data from the Omega-3 study, we are able to model 17-OHPC pharmacokinetics. Figure 7 depicts plasma concentrations expected from both a 250 and 500 mg weekly dose of 17-OHPC. With the higher doses of OHPC in the proposed study, we anticipate that plasma concentrations will range from ~ 4 -110 ng/ml with a median concentration of 10 ng/ml with the 250 mg dose and 20 ng/ml with the 500 mg dose based on these simulated data.

Figure 7. Simulated concentrations of 17-OHPC (with 95% CI) following weekly injections of either 250 or 500 mg. **B.6.**



Rationale for Proposed Study

Clearly, research that evaluates the dose/ concentration relationship between 17-OHPC and PTB rates is urgently needed. Such a study should correlate a measure of drug exposure such as steady state trough plasma concentrations with the outcome of preterm birth. The potential benefit of identifying a therapeutic concentration range and of optimizing the dosage of 17-

OHPC are substantial but several challenges exist for such investigations. The *first challenge* relates to the fact that animal models cannot be used to identify a therapeutic concentration range of 17-OHPC for several reasons. In all animals, other than monkeys and guinea pigs, progesterone decreases dramatically prior to birth.[26] Only in humans, monkeys and guinea pigs is plasma progesterone withdrawal not an antecedent to parturition. However, neither the monkey nor guinea pig is a good model for human preterm labor since in the human, preterm birth is multifactorial and in many cases related to infection/inflammation, circumstances not typical in these animals. This challenge will be met by performing the study in humans.

A *second challenge* to establishing a therapeutic concentration and optimizing the dosage of 17-OHPC is the lack of availability of 17-OHPC at a higher concentration than the standard 250 mg/ml concentration that is currently used. The FDA-approved product, Makena, is available but only at a concentration of 250 mg/ml and this product costs anywhere from $900-$1600 per dose. We considered several options for administering a 500 mg weekly dose of 17-OHPC using the 250 mg Makena product including: twice weekly injection of 1 ml of the 250 mg product, administering 2 injections of 250 mg Makena at one time or administering a single injection of 2ml of Makena for a total of 500 mg. All of these options would be very costly as insurance would not necessarily cover the 250 mg dose at all centers and none would cover the 500 mg dose. We explored the option of utilizing a compounding pharmacy to prepare both a 250 mg/ml and a more concentrated 500 mg/1.4 ml product. We also considered utilizing the compounding pharmacy to supply only the more concentrated solution for those subjects assigned to the 500 mg weekly dose while those assigned to the 250 mg dose would use whatever product is used at their respective institutions. Use of a compounding pharmacy requires one that is appropriately qualified based on FDA requirements. We did identify such a pharmacy in Tennessee that prepared 17-OHPC for one of the MFMU studies. We have been unable however, to obtain the 17-OHPC powder from a qualified distributor in the United States making the compounding option moot. We initiated discussions with AMAG Pharmaceuticals the distributor of Makena and they have agreed to provide us the Makena product free of charge for the entire study. Women assigned to the 250 mg/week group will receive a 1 ml injection (250 mg) weekly while women assigned to the 500 mg/week group will receive either a single 2 ml weekly injection or two 1 ml weekly injections administered at the same time. The option of using the Makena product is by far the best for this RCT as all participants will get the same FDA-approved product, the study leadership will not need to negotiate with a 17-OHPC supplier

or a compounding pharmacy. The primary disadvantage is that women randomized to the 500mg injection rather than the standard 250 mg injection may experience more discomfort. We are somewhat encouraged by **our reproductive endocrinology colleagues who typically administer 2 ml progesterone in oil on a daily basis** to women undergoing Assisted Reproductive Technologies. Thus, the second challenge, the availability of a 250 and 500 mg product, has been met.

A *third challenge* to further explorations regarding optimization of the dose of 17-OHPC relates to the challenges in conducting a large clinical trial in pregnant women. A large sample size is needed to demonstrate significant improvement in preterm birth rates with a different dose of 17-OHPC since the current dose of 250 mg of 17-OHPC appears to reduce preterm birth rates by 33%. A large sample size is also required because several maternal characteristics may affect plasma concentrations of 17-OHPC[14,19-21] and several single nucleotide polymorphisms of the progesterone receptor gene appear to impact the drug's efficacy both positively and negatively.[27] This challenge is met by the participation of 5 centers with large populations of women receiving 17-OHPC. All three centers have participated in multiple trials of 17-OHPC by the MFMU.

In order to enhance sample size for this trial, we will enroll a separate cohort of subjects (ancillary cohort) who are not in the parent trial described above. Women already receiving 250 mg 17-OHPC weekly based on their clinical history will be approached prior to 26 weeks gestation. Eligible subjects either refused the Randomized Controlled Trial (RCT) or they were never approached for the trial. These subjects will be asked to provide two blood samples: one at 26-30 weeks and one 6-9 weeks later to assure steady state. This ancillary study will be implemented initially at the UPITT site only but may be expanded to other sites, as required.
Thus, the current design appears to address the challenges described above. Given the observation reported here from the Omega-3 study, it appears that many women receiving 17-OHPC are inadequately treated. It is therefore imperative that this study be performed to establish the relationship between plasma 17-OHPC concentration and the rate of PTB and to determine if higher plasma concentrations will reduce the risk of PTB to a greater degree than reported in the Meis trial. An understanding of the relationship between plasma concentrations of 17-OHPC and PTB rates will lead to an optimized dosing regimen that will benefit a higher

percentage of patients who would otherwise deliver babies prematurely. Preterm birth rate is the only acceptable outcome of interest in assessing the optimal dosing regimen of 17-OHPC as no surrogate biomarker for PTB has been identified at this point. Alternative methods for addressing this problem do not exist.

The findings of the proposed study will have a dramatic impact on clinical practice and will energize the research community to further explore the potential mechanism of action and target organ(s) of this therapy. Demonstration that the rate of PTB is related to plasma 17-OHPC concentration will lead to a different dosing regimen for women with a prior preterm birth. This would impact not only practice in the United States but throughout the world as treatment with 17-OHPC is internationally embraced. Positive findings from this project will have a substantial public health impact. Approximately 133,000 pregnant women annually with a history of a prior preterm birth would be eligible for this treatment.10 If the preterm birth rate is reduced even 10% from its current rate with the optimized dosing regimen the impact would be substantial. In dollar terms each preterm birth cost nearly $52,000. Preventing 10% of these 13,000 preterm births will save $663 million annually. Incremental reductions in preterm birth would lead to comparable changes in financial savings.

Some may suggest that the striking relationship between plasma 17-OHPC concentration and the rate of preterm birth we have reported from the MFMU Omega-3 trial provides sufficient proof to adopt a strategy of merely doubling the standard dose of 17-OHPC. ***We strongly disagree*** with such a strategy because all women in our study received Omega-3 supplementation and the impact of this supplement on the metabolism and plasma concentration and efficacy of 17-OHPC is not completely clear. Furthermore, as a secondary analysis the Omega-3 study was not designed to address the relationship between plasma concentrations of 17-OHPC and the outcome of preterm birth. The Omega 3 trial utilized only weekly injections of 250 mg of 17-OHPC and therefore data from this trial cannot address the issues of what impact higher plasma concentrations would have on the PTB rate. Recently, another study evaluated the relationship between 17-OHPC concentrations and preterm birth and a concentration relationship with the rate of preterm birth could not be demonstrated [28]. These findings emphasize the need for a prospective study that relates plasma 17-OHPC concentrations and PTB rates.

The proposed research will shift both fundamental research and clinical practice paradigms. Paradigm is defined by Webster as "a model for something that may be copied or a group of ideas about how something should be done, or thought about". The proposed study will clearly challenge two existing paradigms; one related to the regulation of parturition and the second related to the study of medications in pregnancy. The demonstration of a relationship between dose or concentration of 17-OHPC and the outcome of preterm birth will not only impact the clinical care of women at risk for preterm birth but will also impact the scientific community and the long held concepts of what regulates human parturition. Progesterone withdrawal preceding parturition is a characteristic of most mammalian species except humans, monkeys and guinea pigs.[26] In humans, progesterone concentrations increase as pregnancy progresses and do not fall prior to labor onset. Plasma progesterone concentrations are high and in excess of concentrations required to half saturate (EC50) the progesterone receptor. The suggestion that progesterone supplementation might delay human parturition, therefore, seems biologically improbable. However, several clinical trials have demonstrated a benefit with progesterone supplementation in certain conditions. A benefit is seen with 17-OHPC in women with prior preterm birth but not in women with twin or triplet gestation or a shortened cervix.[4,29-31] Vaginal progesterone appears to confer benefit to women with a shortened cervix but not to women with prior preterm birth.[13,32.33] Each of these clinical trials has been subject to criticism and skepticism abounds. For 17-OHPC, only a single large trial has shown benefit and in that trial the rate of preterm birth in the control group was much higher than expected leading some to suggest that the medication did not provide a benefit as the preterm birth rate in the 17-OHPC group was identical to the rate reported in other studies in women with a prior preterm birth and no treatment.[4,34] Vaginal progesterone appears to reduce preterm birth in women with a shortened cervix but this treatment failed to gain FDA approval because, in the opinion of the FDA, the study failed to demonstrate benefit in women residing in the United States. Thus, although clinical trials suggest that progesterone supplementation may impact the parturitional process, proof of concept is clouded. If the proposed study demonstrates a concentration relationship between 17-OHPC and preterm birth rates, then the role of progesterone as a regulator of human parturition will be established

beyond doubt. This will be innovative because it will dramatically "change ideas about how something should be done, made, or thought about".

Likewise, demonstration that the pharmacologic characteristics of a medication can impact drug concentration and efficacy should set a new standard in Obstetric Pharmacology as to what evidence is required before medications are prescribed for pregnant women. The proposed study brings concepts to Obstetric Pharmacology that are not innovative by scientific standards in other disciplines but in dealing with pregnant women and their fetuses, Obstetrical Pharmacology is far less advanced. The FDA does not allow medications to be administered to non-pregnant individuals without knowledge of the proper dose or the risks and benefits of treatment. Efficacy studies are not undertaken without first establishing an optimal dosing regimen. These rules do not apply to medications used by pregnant women. Drug administration regimens for pregnant women are based on dosage regimens derived from men and non-pregnant women. Few of the medications taken by pregnant women have been studied adequately. At a time when the most precise information is needed to protect the pregnant woman and her baby, data are sparse and the patient and provider are uninformed about proper dosing regimens and the risks or benefits of any treatment. Too many pharmaceutical interventions in obstetrics have been tested and failed because of inadequate pharmacological assessment of the medication. Ritodrine was approved as a labor inhibition treatment but inadequate pharmacological data led to maternal deaths due to an excessive intravenous dosing regimen while an inadequate oral maintenance dose led to treatment failures.[35,36] The approach taken in the proposed study that seeks to establish a relationship between drug concentrations and the outcome of preterm birth while exploring the impact of maternal covariates as modifiers of maternal:fetal drug concentrations and efficacy will be innovative and, in fact, transformational in the field of Obstetric Pharmacology. Knowledge of the relationship between maternal:fetal drug concentration ratios and metabolic activity should be fundamental to any drug administered to pregnant women.

C. Study Design

C.1. Infrastructure

The OPRC program is comprised of representatives from the NICHD (Bethesda, MD) and three Clinical Sites: Northwestern University (Chicago, IL), University of Texas Medical Branch (Galveston, TX) and Magee-Womens Hospital (Pittsburgh, PA). Two other sites, The University of Texas at Houston and the University of Utah, were added as additional sites; however, each site is conducting the study under separate INDs. The research is overseen by an internal Steering Committee, an external Data Monitoring Committee (DMC), the local Institutional Review Boards (IRBs) of each clinical site and the FDA. Details regarding the composition and responsibilities of the Steering Committee and OPRC locations are provided in the Network Policy Manual. Details regarding the composition and responsibilities of the DMC are provided in its charter.

C.2. Primary and Secondary Objectives

We will recruit 300 pregnant women with one or more prior preterm births. **Recruitment will occur at the three OPRC clinical sites and the two additional sites**. Ideally, each center will enroll approximately 100 subjects; however, enrollment may differ substantially between centers. Enrolled subjects will be randomized in a 2:1 ratio to receive a single weekly injection of 500 mg or 250 mg 17-OHPC. The 500 mg dose can be administered either as a single 2 ml weekly injection (500 mg) or two 1 ml (250 mg) weekly injections administered at the same time. Those assigned to the 250 mg weekly dose will receive 1 ml of 250 mg of 17-OHPC.

The Primary Objective of this study is to determine whether the rate of preterm birth is related to the plasma concentration of 17-OHPC.

Secondary Research Questions include the following:

1. What is the impact of race or BMI on plasma 17-OHPC concentrations?
2. Are maternal side effects increased at higher doses or higher plasma concentrations of 17-OHPC?
3. Is there an increase in adverse fetal effects from a higher dose or higher concentrations of 17-OHPC?
4. What is the relationship between dose of 17-OHPC and plasma concentrations?

5. Do single nucleotide polymorphisms in the maternal progesterone receptor, P-glycoprotein affect efficacy or the placental transfer of 17-OHPC?
6. Is efficacy of 17-OHPC affected by the gestational age at therapy initiation, the number of prior PTBs or the gestational age at delivery of prior PTBs?
7. What is the relationship between cord blood concentrations of 17-OHPC and maternal plasma concentrations?
8. Does the 17-OHPC concentration in maternal plasma or whole blood correlate better with certain pharmacological and clinical outcomes?

Subjects in the ancillary cohort will already be receiving 250 mg 17-OHPC weekly as part of their standard of care, based on their clinical history. The *primary objective* in the ancillary cohort is identical to the RCT.

The *secondary research questions* are similar to the RCT except for numbers 2, 3, and 7 above. Data will be collected from subjects in the ancillary cohort, but will not be utilized for secondary research questions 2 and 3 above. These research questions cannot be adequately addressed because subjects in the ancillary cohort are not randomized. Research questions 5 (cord blood only) and 7 cannot be addressed because biological fluids will not collected at delivery in the ancillary cohort.

C.3. Screening Procedures

All patients who present for prenatal care before 20 6/7 weeks are eligible for screening. The latest gestational age to start treatment in the RCT is 21 6/7 weeks. Subjects who either refuse the RCT or are not approached may be eligible for participation in the ancillary cohort. The inclusion/exclusion criteria will be reviewed with the patient's chart. Potentially eligible subjects will be told about the study and asked to sign a medical records release so that the medical records of her previous deliveries may be obtained. Documentation of the previous deliveries will be reviewed to ensure the patient meets the inclusion criteria. If a patient appears to meet the criteria for enrollment and expresses interest in the study, she will be told about the study and asked to sign the informed consent form. A copy of the consent form will be provided to the patient.

C.3.i. Eligibility Criteria

a. RCT Eligibility Criteria

Inclusion Criteria - Subjects in the RCT must meet all of the following:

1. Pregnant female with documented prior birth between 16 0/7- 35 6/7 week gestation from spontaneous preterm labor or preterm premature rupture of membranes
2. Gestational age (GA) < 22 weeks, based on study determined GA (as treatment must start between 16 0/7 and 21 6/7 weeks)
3. Singleton gestation
4. Age between 18 – 45 years
5. Able to give informed consent and undergo study procedures

Exclusion Criteria - Subjects in the RCT must not meet any of the following:

1. Plan for cerclage
2. Plan for progesterone treatment other than study medication
3. Known major fetal anomaly or chromosomal anomalies that might affect gestational age at delivery
4. Malformation of uterus (uterine didelphus, septate uterus or bicornuate uterus) or known cervical length <2.5 cm
5. Participation in another trial that may affect gestational age at delivery
6. Planned delivery at other institution where pregnancy outcome data cannot be obtained
7. Medical or obstetrical complication that might affect gestational age at delivery, such as active ulcerative colitis, liver tumors, liver disease/failure, renal disease/failure, undiagnosed vaginal bleeding unrelated to pregnancy, or hypertension requiring 2 or more agents
8. Current or history of thrombosis or thromboembolic disorders
9. Known or suspected breast cancer, other hormone-sensitive cancer, or a history of these conditions
10. Moderately severe depression (PHQ-9 score ≥15, EPDS score of >13, or suicidal ideation)

b. Ancillary Cohort Eligibility Criteria

Inclusion Criteria - Subjects in the ancillary cohort must meet all of the following:

1. Pregnant female with documented prior birth between 16 0/7- 35 6/7 week gestation from spontaneous preterm labor or preterm premature rupture of membranes
2. Receiving 250 mg 17-OHPC weekly- must be compliant with that treatment based on interview and reviewing the medical record
3. Gestational age (GA) <26 weeks, based on study determined GA

4. Singleton gestation
5. Age between 18 – 45 years
6. Able to give informed consent and undergo study procedures

Exclusion Criteria - Subjects in the ancillary cohort must not meet any of the following:

1. Inclusion in the RCT of 250 vs 500 mg OPRC study
2. Cerclage in place
3. Plan for progesterone treatment other than study medication
4. Known major fetal anomaly or chromosomal anomalies that might affect gestational age at delivery
5. Malformation of uterus (uterine didelphus, septate uterus or bicornuate uterus) or known cervical length <2.5 cm
6. Participation in another trial that may affect gestational age at delivery
7. Planned delivery at other institution where pregnancy outcome data cannot be obtained
8. Medical or obstetrical complication that might affect gestational age at delivery, such as active ulcerative colitis, liver tumors, liver disease/failure, renal disease/failure, undiagnosed vaginal bleeding unrelated to pregnancy, or hypertension requiring 2 or more agents
9. Current or history of thrombosis or thromboembolic disorder.
10. Known or suspected breast cancer, other hormone-sensitive cancer, or a history of these conditions.
11. Moderately severe depression (PHQ-9 score ≥15, EPDS score of >13, or suicidal ideation)- based on criteria in the RCT

C.3.ii. Gestational Age Determination

We will use the criteria recommended by the American Congress of Obstetricians and Gynecologists (ACOG) [37].

C.4. Informed Consent Procedures and Documentation

Written informed consent will be obtained before entry into the trial. Full disclosure of the nature and potential risks of participating in the trial will be made. Each center will develop its own consent form according to the requirements of its IRB. Women who are not fluent in English will be enrolled by a person fluent in their language and both verbal and written

informed consent obtained in that language; if such are not available, they will not be included.

C.5. Randomization Procedures

Eligible and consenting women will be enrolled and randomized by clinical site and prior preterm birth history (based on earliest SPTB, dichotomized to 30 weeks or less or >30 weeks) to the standard 17-OHPC dose of 250 mg or to a dose of 500 mg intramuscularly weekly starting at 16 0/7 – 21 6/7 weeks and continuing till
36 6/7 weeks gestation. We will stratify at randomization by site and pregnancy history due to the dramatic ethnic, racial and socioeconomic differences in the population at the 3 centers and the demonstrated impact of gestational age at a prior preterm birth on subsequent pregnancy duration. This will be an open label study. Blinding is not required since the primary outcome of interest is the rate of preterm birth according to plasma concentration achieved. Knowledge of the dose by the provider or subject does not impact the primary outcome, i.e., the rate of preterm birth or the plasma concentration. We will not stratify subjects at randomization by any maternal variables since an objective of the study is to define which maternal characteristics might impact 17-OHPC concentrations or efficacy. Our prior work demonstrates a clear impact of maternal BMI but this effect is modest i.e., ~17%.[38]
Subjects enrolled in the ancillary cohort will not be randomized as they are already on the 250 mg dose.

C.6. Study Procedures

C.6.i. Study Drug Dispensation

- **Randomized Clinical Trial:** For subjects in the RCT, the weekly injections will be administered into the buttocks/hip by either research staff, physician's office staff or other qualified medical personnel, or a person selected by the subject who is trained to administer the medication in the subject's home. That person will attend a study visit with the subject, conducted prior to the start of home injections, where research staff will demonstrate the technique of injection and withdrawal of medication from the vial in a fashion that maintains sterility. The subject will be given 6-9 weeks' worth of medication to take home with her until the next visit with research staff. Every injection will be recorded as the research staff will be in contact with the subject weekly. The

medication will be the standard 250 mg of 17-OHPC in 1 ml castor oil, or 500mg 17-OHPC in 2 ml castor oil. In order to minimize the discomfort associated with the 500mg dose, we will offer women the option of either a single 2 ml injection (500 mg) or two 1 ml injections (250 mg) for a total dose of 500 mg. All of the 17-OHPC will be dispensed in a 5 ml multi-dose vial. Information regarding drug dispensation will be recorded at each visit/encounter with the participant from randomization up until the last encounter at 36 6/7 weeks, or delivery, whichever occurs first.

- **Ancillary Cohort:** Subjects in the ancillary cohort will have 17-OHPC prescribed by their healthcare provider as part of their standard of care. Subjects will be offered the GMP produced Makena drug for consistency with the RCT and also to potentially enhance recruitment if the prescribed 17-OHPC medication is not covered by the subject's insurance. Although it is preferred that subjects use the Makena medication, it is not a requirement for the ancillary cohort. We will document the source of the medication. Study drug will be distributed to subjects during routine OB visits or during study visits.

  Research staff will not be responsible for administering injections to subjects enrolled in the ancillary cohort; however, they may administer the injections on an as needed basis. Study drug will be distributed to subjects during routine OB visits or during study visits.

C.6.ii. Randomization and Baseline

At randomization, we will record height and weight from the medical record and collect information about demographics (e.g., age and race), social history (e.g., marital status, years of education, alcohol use, tobacco use and illicit drug use), medical and obstetric history, prenatal labs and ultrasound findings from the index pregnancy, and concomitant medication and supplement use. Self-reported information will be confirmed with the medical record. This visit is anticipated to last approximately 30 minutes.

C.6.iii. Study Visits

- **Randomized Clinical Trial:**

Each center will see subjects enrolled in the RCT every 6-9 weeks to provide additional medication and to perform other aspects of the study including examination of the injection sites, as needed. All subjects in the RCT will have venous blood draws performed at the times described in the protocol. Research staff will contact subjects weekly to record injection information, ask about possible side effects of the medication experienced since the last injection, and about the use of other medications. They will also ask about any hospitalizations and any pregnancy complications that have been diagnosed since the last contact. If complications of the pregnancy arise, such as need for a cervical cerclage, or need for vaginal progesterone, they will be recorded. If the patient is hospitalized for any reason including preterm labor, treatment should continue. The local principal investigator will evaluate the event's expectedness, relatedness and severity. Events will be reported to the local IRBs per institutional procedures and the participating investigators and FDA, as required. Details of monitoring, documenting and reporting serious adverse events (SAEs) will be documented in the study's safety monitoring plan. Weekly communication will occur until the patient delivers.

Subjects in the RCT will be assessed for depression using either the Patient Health Questionnaires (PHQ-2 and PHQ-9, if required) or the Edinburgh Postnatal Depression Scale (EPSDS)[40] at the following study visits when maternal blood samples are collected: Screening Visit, 26-30 week visit, and 6-9 weeks after the 26-30 week visit.

The Patient Health Questionnaire-2 (PHQ-2) will be administered as a screen for depression. If the PHQ-2 score is 3 or greater, major depressive disorder is possible and subjects will be further evaluated with the Patient Health Questionnaire-9 (PHQ-9). The PHQ-9 is a multipurpose instrument for screening, diagnosing, monitoring and measuring the severity of depression. Alternatively, the EPDS consists of 10 questions and is a valuable and efficient way of identifying patients at risk for perinatal depression.

- A PHQ-9 score of ≥ 15 or an EPDS score >13 represents moderately severe depression. Subjects with a PHQ-9 score of ≥ 15 or an EPDS score >13 at the Screening Visit will be excluded from enrolling in the study and will be referred to a mental health provider. Currently enrolled subjects who develop depression and have a PHQ-9 score

≥ 15 or an EPDS score >13 will not automatically be withdrawn from the study, but a risk-benefit discussion for 17-OHPC treatment is appropriate. Subjects will be offered a referral to a mental health provider, if not already in treatment. If the patient is in treatment, we will continue to monitor her depression and notify her treatment professional, as appropriate.

Note: Question 9 on the PHQ-9 and question 10 on the EPDS are single screening questions on suicide risk. If a subject answers yes to question 9 on the PHQ-9 or has a score ≥1 for question 10 on the EPDS, they are ineligible to continue with the study and will be immediately referred to a mental health provider for further assessment.

- A PHQ-9 score of ≥ 10 and < 15 or an EPDS score >10 and ≤13 indicates moderate depression. Subjects with moderate depression will not be excluded or withdrawn from the study, but a risk-benefit discussion for 17-OHPC treatment is appropriate. Patients will be offered a referral to a mental health provider, if not already in treatment. If the patient is in treatment, we will continue to monitor her depression and notify her treatment professional, as appropriate.

- **Ancillary Cohort:**

All subjects in the ancillary cohort will have venous blood draws performed at the times described in the protocol. Study drug will be dispensed to subjects during routine OB visits or during study visits. Subjects will be given enough medication to last 6-9 weeks. The administration of the injections will be managed by the prescribing physician.

Research staff will contact subjects weekly to record injection information, ask about possible side effects of the medication experienced since the last injection, and about the use of other medications. They will also ask about any hospitalizations and any pregnancy complications that have been diagnosed since the last contact. If complications of the pregnancy arise, such as need for a cervical cerclage, or need for vaginal progesterone, they will be recorded.

C.6.iv. Delivery

At delivery, pregnancy and neonatal outcome information will be obtained from the medical record. Biological samples will not be collected at delivery for subjects in the ancillary cohort.

C.6.v. Specimen Collection

a. RCT Specimen Collection

Maternal blood will be collected on four occasions by a health care provider or research staff trained in phlebotomy:

1. Prior to the 2nd injection (1 tube of 7 ml), ideally on day 7 from the first injection
2. Between 26-30 weeks - but only after a minimum of 7 injections have been administered (1 tube of 7 ml will be drawn just prior to the next injection; this will be a trough level)
3. Between 6-9 weeks after the 26-30 week specimen but not later than 37 weeks (1 tube of 7 ml will be drawn just prior to the next injection; this will be a trough level)
4. At delivery (1 tube of 7 ml), when possible

A second tube of blood (7 mL) will be drawn with any of the first 3 blood draws for genotyping of various genes related to preterm birth if mother consents to this optional test.

Umbilical cord blood (7 mL) will be collected at delivery. A second cord blood specimen (7 ml) will be drawn for genetics studies in the baby if mother consents. These blood specimens will be collected, when possible, and used to determine the concentrations of various biochemicals, 17-OHPC and its metabolites, and for genetic studies.

A 3 x 3 cm piece of placenta will be collected, when possible, for p-glycoprotein and other transporters.

Labor and Delivery biological specimens will be collected when possible. All biological specimens will be frozen at -70 to -80° C and stored locally until assayed at the University of Pittsburgh.

b. Ancillary Cohort Specimen Collection

Maternal blood will be collected on two occasions by a health care provider or research staff trained in phlebotomy:

1. Between 26-30 weeks - but only after a minimum of 7 injections have been administered (1 tube of 7 ml will be drawn just prior to the next injection; this will be a trough level)
2. Between 6-9 weeks after the 26-30 week specimen but not later than 37 weeks (1 tube of 7 ml will be drawn just prior to the next injection; this will be a trough level)

A second tube of blood (7 mL) will be drawn with any of the first 2 blood draws for genotyping of various genes related to preterm birth if mother consents to this optional test.

C.7. Patient Management

No attempt will be made to alter or mandate clinical management of the subjects.

C.7.i Criteria for Stopping Treatment

In the event that either of the two scenarios below occur, treatment with 17-OHPC will stop and the subject will be retained in the study but their data related to 17-OHPC concentration will not be utilized since they will have stopped the medication. Additional subjects will be recruited to maintain the sample size.

a. Systemic drug allergy, thrombosis during pregnancy, cholestatic jaundice, suicidal ideation, or other concern regarding the patient's safety
b. Pregnancy termination (not planned at the time of eligibility determination)

The ancillary cohort is an observational cohort. This section is not applicable to the ancillary cohort, as the 17-OHPC medication is prescribed and managed by the subject's healthcare provider.

C.7.ii Non-Compliant Subjects

Compliance with drug administration will be documented by discussion with the subject during the weekly contact with the subject and by counting the used vials. Non-compliant subjects will be retained in the study and treatment with the randomized dose of 17-OHPC will continue; however, their data related to 17-OHPC concentration will not be utilized for the primary outcome. Additional subjects will be recruited to maintain the sample size. Non-compliance will be defined as:

a. Subjects who miss any injections in the 4 weeks preceding the 26-30 week blood draw.
b. Subjects who, after enrollment, admit to missing 2 or more injections after the first injection.

C.7.iii Subjects that Withdraw from the Study

If a subject in the RCT withdraws their consent, we will continue to provide the standard dose of 250 mg until treatment can be transitioned to the patient's healthcare provider, if desired, to ensure there is no lapse in patient care. Additional subjects will be recruited to maintain the sample size.

Patients who are greater than 21 6/7 weeks GA when they elect to withdraw from the study may not be eligible for insurance coverage of the standard 250 mg weekly dose administered by their healthcare provider. In this case, the study will continue to provide the standard 250 If mg weekly dose until 36 6/7 weeks gestation or until delivery.

If a subject in the ancillary cohort withdraws from the study, her treatment will continue to be managed by her healthcare provider. Any data collected will be retained and used as appropriate.

C.8. Study Halting Criteria

We have established study halting criteria since the 500 mg dose is not FDA approved. We will use subjects in the 250 mg RCT group as a comparator for adverse effects and to establish criteria for stopping the study. We will not use subjects in the ancillary study who are receiving the 250 mg dose as a comparator since they will not have been randomized.

The DMC will review enrollment and AEs on a regular basis (every 4 months). Enrollment will not stop unless the Network is directed to stop by the DMC or the FDA. Criteria that warrant discussion of halting the study by the DMC will include more than expected occurrence of the SAEs previously known to be associated with 17-OHPC use, or SAEs unexplained by the patient's medical condition. Preterm birth is an expected pregnancy complication in this group of subjects and is associated with significant and serious fetal and neonatal morbidities and mortality mostly related to severe prematurity. Therefore, when the DMC evaluates SAEs, a distinction will be made as to whether the SAE is thought to be related to the patient's medical

or pregnancy condition (e.g. severe preeclampsia, extreme prematurity, etc) or whether it is thought to be related to study medication. Study halting criteria include:

1. Fetal and neonatal deaths.

A safety signal for an excessive number of early pregnancy losses and stillbirths was identified in the Meis study by the FDA in their review of the Makena NDA. The rate of loss was 3.6 in the 17-OHPC group and 1.3 % in the control group. If this signal is related to plasma concentrations of 17-OHPC, then a greater number of these adverse outcomes might be seen. Fetal and neonatal deaths will be evaluated on an ongoing basis. Study coordinators will report serious adverse events to the site PI, the LCC, and the study Lead PI within 24 hours of notification. Information not available at the time of the initial report should be submitted as a follow-up report within 48 hours of notification. It would appear reasonable to set some criteria to alert the Medical Monitor and the DMC that a review is necessary. We have set this alert at a rate of pregnancy loss (20 0/7 weeks till delivery) or stillbirth of 1.5 times higher in the 500 mg weekly group than in the 250 mg group. The DMC should review all deaths to determine the relationship to study drug and to determine what if any action is needed. Enrollment in the study will continue during this review. After the review the DMC, in conjunction with NICHD and the Medical Monitor, will determine whether the study should be stopped or continued.

2. Thromboembolic Events (deep vein thrombosis or pulmonary embolus)

There is a potential risk of thrombosis from progestin treatment but this risk is low. Genetic predisposition to clotting is seen in over 5% of the population and pregnancy increases the risk of thrombosis so clotting during pregnancy is uncommon but NOT an unexpected event. Doses of 17-OHPC of 250 mg weekly have not been associated with an increased risk of clotting but the potential exists that higher doses of 17-OHPC may increase the risk of thrombosis. We would expect no more than 3 cases of thrombosis (3%) in the 150 subjects recruited to the 250 mg weekly group. If the number of cases of deep vein thrombosis or pulmonary embolus in the 500 mg weekly group exceeds the number of cases in the 250 mg group by more 3% (3 cases) then these cases will be identified by the Medical Monitor and reviewed by the DMC for 17-OHPC causality. All SAEs are reported promptly to the site PIs, Medical Monitor and study lead and are reviewed by the DMC. The DMC should review all the thrombotic events to determine the relationship to study drug and to determine what if any action is needed.

C.9. Medical monitor and Logistic Coordinating Core

A Medical Monitor has been selected for this trial. The Medical Monitor will receive reports from each clinical center which will be compiled by the NICHD funded **Logistic Coordinating Core (LCC)**. The LCC facilitates and coordinates the OPRC clinical study activities and ensures that all activities are implemented.

The role of the Medical Monitor will include:

- Ongoing monitoring of SAE reports submitted by the clinical centers to identify safety concerns and suggest protocol modifications, if necessary
- Advise IND sponsor, Principal Investigator(s), and DMC regarding SAEs and safety concerns
- Provide reports when necessary to the Principal Investigator and DMC

D. Statistical Analysis and Sample Size Justification

D.1. Primary and Secondary Outcomes

The primary outcome is the rate of preterm birth (delivery prior to 37 0/7 weeks) according to 17-OHPC concentration.

Secondary outcomes include:

- The rate of spontaneous PTB prior to 32 0/7 and 35 0/7 weeks according to the concentration of 17-OHPC.
- The impact of dose, race, BMI and co-medications on plasma 17-OHPC concentrations
- Plasma concentrations of 17-OHPC, mono-hydroxylated metabolite of 17-OHPC
- Concentrations of progesterone and 17-hydroxprogesterone in maternal and cord blood according to the weekly dose of 17-OHPC
- The frequency of maternal side effects according to dose and plasma concentration
- The frequency of neonatal complications according to dose and plasma concentration
- Impact of dose on rates of spontaneous PTB
- The correlation of progesterone and 17-hydroxyprogesterone in maternal plasma and whole blood to pharmacological and clinical outcomes

The primary outcome will be the rate of preterm birth (<37 weeks) according to the plasma concentration of 17-OHPC (as a continuous variable) from the 26-30 week sample. The distribution of plasma concentration is expected to be highly positively skewed; thus, a natural logarithmic transformation will be applied to plasma concentrations to produce normally distributed values. A Cox proportional hazard model will be employed to analyze the effect of continuous log-transformed plasma concentration on event-free survival (in this case, "event-free survival" meaning "not birthed until term"). Specifically, the hazard of delivering preterm will be modeled as a function of log-transformed plasma concentration and additional covariates that may influence treatment effectiveness (e.g., BMI, race, gestational age at study entry). Adjusted survival curves will be calculated using the relationship between the hazard function and the survival function. A Wald test will be performed to determine whether the hazard ratio corresponding to a one-unit increase in log-transformed plasma concentration is significantly different from zero. Wald and likelihood ratio tests will also be employed to test for the significance of additional covariates, and stratified analyses may be considered if appropriate (i.e., race-specific analyses). This semi-parametric modeling approach will allow us to analyze the effect of, and not just the presence of, covariates on event-free survival in a robust manner.

Secondary outcomes that are also survival outcomes (such as spontaneous PTB, spontaneous PTB before 32 weeks and before 35 weeks, and indicated PTB) will be analyzed in a similar fashion to the primary outcome analysis described above. For other secondary outcome measures that are continuous variables (maternal and infant hospital days, maternal and fetal plasma concentrations of progesterone, hydroxyprogesterone, 17-OHPC and its metabolite, etc.), linear regression models with appropriate covariates will be utilized.

We will also compare preterm birth rates between treatment groups (250 or 500 mg). Descriptive statistics will be generated for key characteristics of the treatment groups at baseline. For continuous variables, we will report means and standard deviations or medians and interquartile ranges (the latter in the case of skewed distributions or large outliers). Counts and percentages will be reported for categorical variables. We will then construct a Cox model and produce adjusted survival curves to assess the magnitude of differences in PTB rate

between groups while adjusting for covariates that may influence treatment effectiveness (e.g., BMI, race, gestational age at study entry).

We have previously performed population pharmacokinetic analysis of 17-OHPC in twin and singleton pregnancies. In this study, we will also use population pharmacokinetic analysis to evaluate the contribution of various covariates to the observed variability in the trough plasma concentrations of 17-OHPC, a surrogate measure of drug exposure.

D.2. Sample Size and Power

A Cox proportional hazards regression model will be used to assess the effect of log-transformed 17-OHPC concentration on event-free survival, while adjusting for covariates such as maternal BMI.

The recruited cohort of women will be sufficiently large to provide a wide range of drug exposures and enable evaluation of several potential covariates on plasma concentration of 17-OHPC and to describe the association between plasma concentration of 17-OHPC and its efficacy. Based on the large variation in 17-OHPC concentrations we have reported in clinical trials with the standard dose, we are confident that the range of concentrations of 17- OHPC that will be achieved with the two doses selected will provide a sufficiently wide range (between 4 and 112 ng/ml) to enable the performance of a robust concentration response assessment (Figure 7). Based on these simulated data, we estimate that 99% of subjects will achieve a concentration > 10 ng/ml with the 500 mg/week dose.

Based on the simulated distribution of 17-OHPC plasma concentration at higher doses in this study, we expect the variance of log-transformed concentration to be 0.226. Given the results of the prior study, we have estimated the hazard ratio corresponding to a one-unit increase in log-transformed 17-OHPC concentration to be 0.49. We also anticipate that 28% of study participants will deliver preterm. Thus, a Cox regression based on a sample of 208 women will have 80% power at the α=0.05 significance level to detect a hazard ratio of 0.49. This sample size will be adjusted for the presence of other covariates in the Cox model; assuming that additional covariates such as BMI account for 30% of the variation in log-transformed plasma concentration, we require a sample size of **296 women** to achieve 80% power (Table 1). Our

published data indicate that only maternal BMI affects plasma concentrations of 17-OHPC and accounts for no more than 30% of the variation in plasma concentrations (Figure 7). We will recruit 300 subjects overall to account for non-compliance or non-collection of plasma for analysis of 17-OHPC. Data from non-compliant subjects will not be used for any analysis related to plasma 17-OHPC concentration. In addition, data analysis related to plasma 17-OHPC concentration in those subjects who stop 17-OHPC treatment because of a drug allergy, thrombosis or cholestatic jaundice will not be utilized, but their outcomes will be kept and included in the secondary outcomes that are not related to plasma 17-OHPC concentrations. The sample size will be sufficient to address secondary outcomes since the variation in the variables studied will be far smaller than the variation in plasma 17-OHPC concentration.

All three centers have recruited very well in other studies of 17-OHPC from the MFMU. The pool of subjects annually available for recruitment will exceed 300. Even at a study acceptance rate as low as 30%, there are more than enough subjects to enable us to reach our recruitment goal. We anticipate an acceptance rate closer to 50%. All power analyses were performed using PASS 13. [CITE: PASS 13 Power Analysis and Sample Size Software (2014). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass.]

Table 1. Total Sample Size Required for 80% Power

		% variation accounted for by additional covariates			
		0%	10%	20%	30%
% participants delivering preterm	26%	223	248	279	319
	27%	215	239	269	307
	28%	208	231	259	**296**
	29%	200	223	250	286

E. Data Management and Quality Assurance

E.1. Investigational Product Supply

All the 17-OHPC will be provided by AMAG Pharmaceuticals Inc. the supplier of the FDA approved Makena product. The product will contain 17-hydroxyprogesterone caproate 250 mg/ml with concentrations of benzyl benzoate and castor oil of 46% and 44-54%, respectively. To assure the concentration of 17-OHPC is as stated in the product label, we will

save a 1 ml aliquot from one vial of each product lot used in this trial for future analysis of 17-OHPC content.

E.2. Clinical Data Management

Clinical data will be acquired throughout the subject's participation in the clinical trial and will include information related to screening, randomization, enrollment, pregnancy outcome, adherence, and adverse outcomes. These data will be collected on case report forms (CRFs) specific to this study.

All key data from case report forms will be entered into the University of Pittsburgh (UPITT) REDCap database program, which will be used as a secure web application for research data storage and archiving. The password protected database provides system validation, metadata, and is supported by UPITT Information Technology with back-up via UPITT servers. The database provides features including auto-validation, calculated fields, branching/skip logic, and data quality tools such as Data Resolution Workflow which tracks query generation, data alterations, and resolution.

E.3. Laboratory Test Results

Analyses of samples (e.g. blood, DNA, placenta and cord blood) will be performed at the University of Pittsburgh (Dr. Raman Venkataramanan's lab) or at another NICHD supported research center. Analysis of 17-OHPC, its primary monohydroxylated metabolite, progesterone and hydroxyprogesterone will be simultaneously measured in human plasma by a specific and sensitive validated HPLC-MS-MS assay developed in his lab.[39] This assay is sensitive down to a plasma concentration of 1 ng/ml for 17-OHPC.[39] The assay may be further validated to detect lower concentrations of 17-OHPC.

Analysis of 17-OHPC will be performed using plasma and whole blood samples at the Magee-Womens Hospital site. Northwestern University and the University of Texas Medical Branch will collect plasma samples only.

These laboratory results will be sent to the Lead Site PI and merged with the data for this study. All data from CRFs and biological fluid analysis will be linked using a participant identification number assigned at study entry.

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