# A Randomized Controlled Trial of Pravastatin to Prevent Preeclampsia in High Risk Women

## Protocol

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# 1 Introduction

## 1.1 Study Abstract

Preeclampsia complicates approximately 3% to 5% of pregnancies and remains a major cause of maternal and neonatal morbidities and mortality. Women who experience preeclampsia in one pregnancy are at higher risk of developing preeclampsia in a subsequent pregnancy than those who have never experienced the condition. There is evidence from laboratory studies and clinical trials, as well as biological plausibility, to suggest that HMG-CoA reductase inhibitors (statins) may prevent the development of preeclampsia by reversing various pathophysiological pathways associated with preeclampsia. Pravastatin has a favorable safety profile and pharmacokinetic properties. The beneficial effects of pravastatin are likely to contribute substantially to preventing preeclampsia, and provide biological plausibility for its use in this setting.

This protocol describes a double-blind randomized placebo-controlled trial of 1,550 high-risk women to assess whether daily treatment with pravastatin administered early in pregnancy reduces the rate of preeclampsia in high-risk women.

## 1.2 Primary Hypothesis

In women who have a prior history of preeclampsia and were delivered by 34 weeks 0 days of gestation, prophylactic treatment of pravastatin prevents a composite outcome of preeclampsia, fetal loss, and maternal death.

## 1.3 Purpose of the Study Protocol

This protocol describes the background, design and organization of the randomized clinical trial and may be viewed as a written agreement among the study investigators. The Data and Safety Monitoring Committee (DSMC) and the External Advisory Committee review the protocol. Before recruitment begins, the protocol is approved by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network Steering Committee, and the single Institutional Review Board (IRB) of record. Other study materials must also be approved by the IRB before study start. Any changes to the protocol during the study period require the approval of the Steering Committee and the single IRB of record. The protocol and any subsequent changes will also be submitted as IND amendments to the FDA. A manual of operations supplements the protocol with detailed specifications of the study procedures.

# 2 Background

## 2.1 Introduction

Preeclampsia is a pregnancy-specific syndrome characterized by widespread endothelial damage in multiple organ systems in the second half of pregnancy. Clinical presentation includes hypertension, proteinuria, and end organ dysfunction.[1] For the mother, preeclampsia may lead to seizure, stroke, intracranial bleeding, uncontrolled hypertension, renal failure, pulmonary edema, and death. For the fetus, it may lead to intrauterine growth restriction, death, and complications of prematurity.[1] Apart from the use of low dose aspirin, which remains controversial, there is no effective prophylactic therapy, and delivery remains the main approach to preventing maternal morbidity and mortality. However, this is frequently achieved at the expense of premature delivery and the associated neonatal morbidities and mortality. Women with underlying hypertension, pregestational diabetes, multifetal gestation, renal or autoimmune disease are at increased risk. Women with a previous history of preeclampsia, especially if early and severe, are at a substantially high risk (up to 65%) for the disease.[1,2]

## 2.2 Etiology of Preeclampsia

The pathogenesis of preeclampsia is not completely understood, but the origin of the disease is thought to be related to impaired early placental development mainly through defective trophoblast invasion and remodeling of the spiral arterioles.[3] Many mechanisms have been proposed for the pathogenesis, but abnormalities in the following processes have generally been well accepted: angiogenesis, endothelial injury, oxidative stress, and inflammation.[4-7]

### 2.2.1 Angiogenic Imbalance

Imbalances in pro- and anti-angiogenic factors are thought to play a role in preeclampsia.[5-7] Two anti-angiogenic factors, soluble Fms-like tyrosine kinase (sFlt-1) and soluble endoglin (sEng), have been shown to bind vascular endothelium growth factor (VEGF) and placental growth factor (PlGF) in the circulation and suppress their effects. Over expression of these anti-angiogenic factors results in a preeclampsia-like condition in animal models.[8,9] The concentration of sFlt-1 in the maternal circulation correlates with the severity of the preeclampsia-like condition.[8] In the case of over expression of sFlt-1, lowering the circulating levels of free sFlt-1 below a critical threshold reverses some of the pathological findings of preeclampsia and lowers the blood pressure.[10] In humans, both sFlt-1 and sEng are known to increase dramatically weeks prior to the onset of clinical manifestations of preeclampsia.[5,6]

Findings in the animal models strongly support a role for sFlt-1 in the temporal relation between elevated sFlt-1 levels and later development of preeclampsia.[5,6,8,9,11] This angiogenic imbalance may represent a "common pathway" responsible for the expression of the clinical features of preeclampsia. The trigger for the cascade of events leading to preeclampsia remains unknown, but may include immunologic, inflammatory, and/or genetic susceptibilities, and may be related to a mismatch between placental metabolic demands versus supply.[3,4] The end result is excessive release of vasoactive factors, cytokines, and maternal endothelial dysfunction, which then triggers the clinically apparent maternal syndrome characterized by hypertension, proteinuria, and other systemic manifestations of end-stage organ damage.[3]

### 2.2.2 Endothelial Dysfunction, Oxidative Injury, and Inflammation

Preeclampsia is accompanied by endothelial dysfunction. This dysfunction results in abnormal vascular relaxation and platelet activation and is associated with inflammation and oxidative imbalance.[4,12] The activation of the inflammatory cascade that occurs in normal pregnancy is further exaggerated in preeclampsia.[13] Markers of inflammation, such as the C-reactive protein (CRP) or its high-sensitivity

form (hs-CRP), are elevated in patients who later develop preeclampsia; this association no longer persists when body mass index is added to the multivariable model implying that inflammation might be part of a causal pathway between obesity and preeclampsia.[14] The risk of developing preeclampsia in patients with elevated hs-CRP increases by 10% for every 5 unit increases in hs-CRP level.[15] In addition, preeclampsia is associated with elevated cytokines such as tumor necrosis factor-α (TNF-α), TNF receptor, interleukin-6 (IL-6), and IL-12. Some of these cytokines are known to activate the inflammatory cascade and increase free radical generation and oxidative stress, thus contributing to generalized endothelial injury.[16-18]

In addition to the dyslipidemia associated with preeclampsia, studies have shown increased antibodies for the oxidized form of LDL (ox-LDL)[19] in patients with preeclampsia, a finding that is consistent with oxidative stress and similar to changes noted in atherosclerotic disease.[20] Preeclampsia may also be associated with suppression of the heme oxygenase-1/carbon monoxide pathway. Heme oxygenase-1 (HO-1) is an inducible anti-oxidant enzyme essential for the degradation of heme into biliverdin in a three-step process that liberates carbon monoxide (CO). HO-1 has anti-inflammatory properties and is known to have a protective effect against oxidative stress in the vascular system.[21] HO-1 messenger ribonucleic acid (mRNA) is down-regulated in placental samples obtained at chorionic villous sampling (CVS) from women who subsequently developed preeclampsia.[22] In addition, patients with preeclampsia have significantly decreased CO concentrations in their exhaled breath, signifying either decreased levels or activity of HO-1.[23]

## *2.3 Preeclampsia and Cardiovascular Disease*

Although preeclampsia is unique to human pregnancy, it shares biological and pathological similarities as well as many risk factors (e.g., obesity, diabetes, dyslipidemia, hypertension, metabolic syndrome, prothrombotic state, and family history) with adult cardiovascular diseases.[24-26] Endothelial dysfunction and inflammation are fundamental mechanisms for the initiation and progression of both atherosclerosis[27,28] and preeclampsia.[3,26] Preeclampsia is considered by many as either an early manifestation of cardiovascular disorders unmasked by the challenges and characteristics of pregnancy or a risk factor for future cardiovascular disease. This association is demonstrated in studies that showed that a diagnosis of preeclampsia increases the patient's risk of hypertension, ischemic heart disease, and ischemic stroke later in life by two- to three- fold.[29-31] Moreover, the severity and gestational age at diagnosis of preeclampsia are important determinants of the risk of cardiovascular disease later in life. When compared with patients who did not develop preeclampsia, the relative risk (RR) of developing cardiovascular disease later in life was 2.0 (95% confidence interval [CI] 1.8-2.2) for patients who had mild preeclampsia and 5.4 (95% CI 4-7.3) for patients who had severe preeclampsia.[32] Similarly, the RR of death from cardiovascular disease later in life is 2.1 (95% CI 1.3-3.6) for patients who had preeclampsia at term and 9.5 (95% CI 4.5–20.3) if the preeclampsia resulted in delivery at less than 34 weeks.[33]

## *2.4 Prevention of Preeclampsia*

Numerous attempts at primary and secondary prevention of preeclampsia, using various supplements and medications, have failed. Use of antihypertensive medications in women with chronic hypertension, who are at higher risk of preeclampsia, was found to control severe hypertension, without decreasing the risk of preeclampsia.[2,34] Supplementation with fish oil, calcium, or antioxidant vitamins C and E did not show any benefit in reducing the rate of recurrence or severity of preeclampsia.[2,35-40]

### 2.4.1 Low-dose Aspirin to Prevent Preeclampsia

Low-dose aspirin, by selectively inhibiting the production of the vasocontractile thromboxane A2 without affecting the production of the vasorelaxant prostacyclin, was thought to protect the vasculature and thus

theoretically prevent preeclampsia. However, the benefits of low-dose aspirin in preeclampsia prevention were not supported by large randomized studies that included high- and low-risk women in the USA.[41,42] The Perinatal Antiplatelet Review of International Studies (PARIS) Collaborative Group performed an individual patient meta-analysis of the effectiveness of antiplatelet agents (predominantly aspirin) for the prevention of preeclampsia. This meta-analysis, which included 31 randomized trials involving 32,217 women, found a small benefit in reducing the occurrence of preeclampsia: 0.90 (95% CI 0.84-0.96).[43] In the pre-specified subgroup analyses, including early versus late gestation, no interaction term was positive. Therefore the analysis did not identify a particular group of women that would benefit more or less.

The authors of another meta-analysis reported that low-dose aspirin reduces the risk of preeclampsia when started before 16 weeks gestational age (RR = 0.47; 95% CI 0.36-0.62), with no benefit if aspirin was started after that point.[44] This meta-analysis, which included data from 34 studies, has been heavily criticized. Unlike the PARIS collaboration, the meta-analysis was not comprehensive (for example, it did not include the MFMU Network Low Risk Trial, which was large and well-conducted in comparison with many of those included). The authors did not conduct an interaction test to determine whether there was heterogeneity before concluding that the aspirin effect was different by gestational age category. The analysis was also subject to publication bias, wherein smaller, negative trials are not published and therefore unavailable for inclusion in meta-analyses.[45] This meta-analysis was particularly susceptible to bias. The trials designated as recruiting women at ≤ 16 weeks were all small with sample size between 33 and 255 women. Because it was not based on individual patient data, the authors were unable to separate the results by less than 16 weeks versus greater in many trials where the gestational age range spanned 16 weeks. This included two of the largest trials, the High Risk trial conducted by the MFMU Network and the Collaborative Low-dose Aspirin Study in Pregnancy (CLASP)[46] where the lower limit of eligibility was 12-13 weeks. These trials were placed in the greater than 16 weeks category although they would have contributed a substantial number of women randomized by 16 weeks. The limitations and biases of misclassification, inclusion of small studies and inappropriate subgroup analyses were confirmed in a recent IPD meta-analysis by the same authors, which included 3,293 women, from larger clinical trials, who were started on aspirin before 17 weeks. In this meta-analysis, low dose aspirin started before 17 weeks did not reduce risk of preeclampsia (RR 0.93; 95% CI 0.75-1.15), or small for gestational age (RR 0.84, 95% CI 0.56-1.26).[47]

Despite the limitations of the aspirin data, and the fact that the majority of the large aspirin trials regarding prevention of preeclampsia have been negative or contradictory, the United States Preventive Services Task Force (USPSTF) recommended the use of low dose aspirin in high-risk women including those with previous history of preeclampsia. The American College of Obstetricians and Gynecologists (ACOG) initially recommended that low dose aspirin be considered for women with preeclampsia in a prior pregnancy that resulted in delivery before 34 weeks gestation, but updated their guidelines in 2016 and now currently recommends the use of low dose aspirin in all women with prior preeclampsia, irrespective of gestational age at delivery in the index pregnancy, and this practice is currently part of the standard of care in the U.S.[1,48] This practice is also supported by patient advocacy groups such as the Preeclampsia Foundation.[49]

### 2.4.2 Biologic Plausibility of Statins to Prevent Preeclampsia

The properties and mechanisms of action of statins make them highly promising candidates for the prevention and/or treatment of preeclampsia due to their ability to improve vascular health broadly. Statins up regulate endothelial nitric oxide synthase (NOS3), which in turn increases nitric oxide (NO) production in the vasculature.[50] They also promote VEGF and PlGF release, which may moderate the effects of sFlt-1, and also upregulate the transcription and expression of HO-1 which in turn suppresses the production of sFlt-1 and s-Eng in the placenta and endothelium.[51] Furthermore, using various rodent models of preeclampsia, daily pravastatin administration was found to prevent the hypertensive vascular phenotype, attenuate kidney injury, restore trophoblast invasiveness and placental blood flow, upregulate

NOS3 in the vasculature, reverse the angiogenic imbalance (reduce circulating sFlt-1 and sEng levels, and upregulate VEGF and PlGF), and prevent growth restriction without any deleterious effects on the pups, and without affecting maternal cholesterol levels.[52-56]

Statins are also known to have anti-inflammatory properties and have been shown to decrease hs-CRP (37%) in parallel with the decrease in cardiovascular mortality and morbidities even in patients with normal cholesterol levels.[57] Preeclampsia is associated with reversal of the Th1 and Th2 responses (increase in Th1 proinflammatory cytokines, such as TNF-α, IL-1, IL-2, IFN-γ, and decrease in Th2 anti-inflammatory cytokines such as IL-4, IL 10).[58] Statins are known to up regulate Th2 cell cytokine production and down regulate Th1 cell differentiation.[59] These and other pleiotropic actions on free oxygen radical formation and smooth muscle cell proliferation, as well as immunomodulatory and anti-inflammatory effects,[60] make statins highly promising candidates for the prevention and/or treatment of preeclampsia.

In conclusion, it appears that statins are capable of 1) reversing the pregnancy-specific angiogenic imbalance associated with preeclampsia, 2) restoring global endothelial health, and 3) preventing oxidative and inflammatory injury, actions that provide biological plausibility for the use of statins in the prevention of preeclampsia.

## *2.5 Safety of Pravastatin in Pregnancy*

Pravastatin was previously labeled as a U.S. Food and Drug Administration (FDA) category X drug, signifying that "studies in animals or humans have demonstrated fetal abnormalities and/or there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits". The FDA recently changed the designation of drugs in pregnancy and discontinued categorization of medications. The new rule requires that the pregnancy subsection in the package insert provides information relevant to the use of the drug in pregnant women, such as dosing and potential risks to the developing fetus, and requires information about whether there is a registry that collects and maintains data on how pregnant women are affected when they use the drug or biological product. The package insert for Pravachol (pravastatin) was updated in 2016 and still reports pravastatin as contraindicated for use in pregnant women. This is predominantly due to the limited published safety data and no known benefit to therapy with pravastatin during pregnancy.[61] Potential concerns are addressed in the subsections below.

### 2.5.1 Congenital Anomalies

Pravastatin was neither embryolethal nor teratogenic in rats at doses up to 1000 mg/kg daily or in rabbits at doses of up to 50 mg/kg daily. These doses resulted in 10 times (rabbit) or 120 times (rat) the human exposure at 80 mg/day maximum recommended human dose based on surface area (mg/m2).[62]

In humans (Table 1), an increased risk of congenital malformations has not been seen with pravastatin. The Medical Genetics branch of the National Institutes of Health reviewed 214 ascertained pregnancy exposures to statins that were reported to the FDA from 1987 to 2001. Of the 70 evaluable cases reviewed in the final report, 20 cases of pravastatin exposure were included. No congenital malformations or adverse pregnancy outcomes occurred in the pravastatin exposed group compared with 22 cases of structural defects, 4 cases of growth restriction, and 5 cases of fetal demise with exposure to lipophilic statins (cerivastatin, lovastatin, atorvastatin, or simvastatin).[63]

Recent epidemiologic data from two large case-control studies of birth defects, the National Birth Defects Prevention Study and the Slone Epidemiology Center Birth Defects Study failed to show any link between pravastatin exposure and a pattern of birth defects. In this database analysis, the authors identified preconception statin exposure in 22 cases of birth defects. Three of these defects were reported

with exposure to pravastatin; however, two patients were diabetic and the third infant had Down syndrome.[64]

A Canadian population based pregnancy registry that collected data on women exposed to statins and other cholesterol-lowering agents prior to and in the first trimester of pregnancy showed no pattern or increased rate of congenital abnormalities in 288 women with live births.[65] The rate of congenital anomalies was 3/64 (4.69%; 95% CI 1.00, 13.69) in women exposed to statins prior to and in the first trimester, and 7/67 (10.45%; 95% CI 4.19, 21.53) in women exposed to statins between 1 year and 1 month before conception, but not during pregnancy. The adjusted odds ratio for congenital anomalies in women exposed to statin prior to and in the first trimester of pregnancy compared with those exposed to statin prior to conception was 0.36 (95% CI 0.06, 2.18). Of note, no cases of congenital anomalies were found in women exposed to pravastatin. In addition, a prospective observational cohort study by the Motherisk program in Toronto did not find any malformation patterns or increased malformations in infants of 64 women with first trimester exposure to statins (atorvastatin [n = 46], simvastatin [n = 9], pravastatin [n = 6], and rosuvastatin [n = 3]) compared with women without exposure to known teratogens.[66] The rate of major congenital malformations was not significantly different between the statin-exposed and control groups (2.2% vs. 1.9%; p=0.93). In addition, the rates of live birth (71.9% vs. 81.2%), spontaneous abortions (21.9% vs. 17.2%), therapeutic abortions (4.7% vs. 0%), and stillbirths (1.5% vs. 1.6%) were similar between the two study groups.

A multicenter observational prospective controlled study from the European Network of Teratology Information Services followed 249 pregnant women who were exposed to statins in the first trimester (32 were exposed to pravastatin), and another 249 pregnant women who were not exposed to any teratogenic drugs as a matched control group. There was not a significant association between exposure and congenital malformations (4.1% vs. 2.7%; OR 1.5 95% CI 0.5 – 4.5) or miscarriage (hazard ratio 1.36, 95% CI 0.63–2.93, P = 0.43) compared with the control group.[67]

Lastly, using data from more than 800,000 pregnant women enrolled in the Medicaid program, Bateman et al. compared the outcomes of 1,152 women exposed to statins during the first trimester with those not exposed. Using propensity score analysis and after controlling for confounders, particularly pre-existing diabetes, there was no increased risk of congenital malformation (aOR 1.07; 95% CI 0.85 - 1.37) or organ specific malformations.[68]

The findings from these studies, and various systematic reviews and meta-analyses,[69-71] support the lack of teratogenicity of pravastatin. One limitation is that most of the statin-exposed patients discontinued statin use as soon as pregnancy was confirmed.

The reassuring findings from these studies are to be expected because of pravastatin's unique pharmacokinetic properties.[72] Pravastatin is one of the most hydrophilic (polar) statins and a substrate of the efflux pump P-glycoprotein and thus is expected to have limited transplacental transfer. This was confirmed in recent placental transfer studies, which demonstrated, using an ex-vivo placental perfusion model, that pravastatin transfer across the placenta appears to be limited and slow,[73] and its clearance is higher from the fetal-to-maternal direction than the maternal-to fetal direction.[74] This is secondary to pravastatin being a substrate of placental efflux transporters such as P-glycoprotein and multidrug resistance-associated protein 2.[74] Moreover, pravastatin is one of the least potent inhibitors of HMG-CoA reductase compared with other statins and its inhibitory activity is specific to hepatocytes.[75] This hepatoselectivity is also a result of pravastatin being actively and preferentially taken up into hepatocytes via a Na+-independent multispecific anion transporter. In addition, pravastatin is cleared through both hepatic and renal routes, which reduces the need for dose reduction in case of liver/renal impairment; and CYP3A-dependent metabolism represents only a minor pathway in pravastatin elimination with no clinically important pharmacokinetic interaction between pravastatin and CYP3A inhibitors.[72]

**Table 1. Summary of studies assessing the use of statins in pregnancy**

| Study | Population | Statin (n) | Control (n) | Congenital anomalies |
|---|---|---|---|---|
| Edison et al [63] 2004 case series | Reports of maternal statin exposure spontaneously reported to the FDA from 1987 to 2001 | Atorvastatin, ceriva-statin, lovastatin, pravastatin, simva-statin (n=70; prava-statin n=20) | None | 22 reports of congenital anomalies, **none with pravastatin** |
| Ofori et al [65] 2007 cohort | Women (15-45 years) prescribed statins in the year before and/or during pregnancy from 1997-2003 | Atorvastatin, fluva-statin, lovastatin, pravastatin, simva-statin (n=64; prava-statin n=12) | Use of statins only before conception (1 year – 1 month) (n=67) | Exposed vs non-exposed: 4.7% vs.10.5% aOR 0.36 (95% CI 0.06-2.18) **No anomalies with pravastatin** |
| Taguchi et al [66] 2008 cohort | Hypercholesterolemic women using statins during pregnancy and contacting the teratogen information service from 1998 to 2005 | Atorvastatin, prava-statin, simvastatin, rosuvastatin (n=64; pravastatin n=6) | No exposure to known teratogens (n=64) matched with women who contacted the Motherisk program with ordinary therapeutic uses of nonteratogens | Exposed vs non-exposed: 2.2% vs 1.9%; p = 0.93 |
| McGrogan et al[76] 2017 cohort | Women (aged 10–49 years) using statins 3 months before or during the 1st trimester 1992 - 2009 | (n = 281, pravastatin n=8) | No statin use (n = 2,643) matched on maternal age, year of pregnancy, and hypertension & diabetes diagnosis | Exposed vs non-exposed: 3.2% vs. 2.8%, OR* 1.6 (95% CI 0.72-3.64) |
| Winterfeld et al[67] 2013 cohort | Women with statin exposure in first trimester, who contacted European teratology information services between 1990-2009. | (n=249; pravastatin n=32) | Exposure to agents known to be non-teratogenic (n=249) | Exposed vs non-exposed.: 4.1% vs. 2.7%, OR 1.5 (95% CI 0.5 – 4.5) |
| Bateman et al[68] 2015 cohort | Women (12-52 years) with live birth, from US Medicaid data 2000-07; used statin in the 1st trimester | Simvastatin, lovastatin, pravastatin, uvastatin, atorvastatin, ceriva-statin, and rosuvastatin (n=1152) (pravastatin n=75) | No statin use in the first trimester (n=3327 in propensity score matched cohort; 885,844 in overall cohort) | Exposed vs non-exposed: 6.3% vs. 3.6%, aOR 1.04 (95% CI 0.85-1.37) |

*Unadjusted OR calculated from data in report.

### 2.5.2 Cholesterol Synthesis in the Fetus

Cholesterol is an essential component of fetal development because it is an integral part of the cell membrane and is also the precursor of other steroid hormones such as progesterone.[77] The majority of fetal cholesterol originates from de novo synthesis (fetus or placenta) rather than from maternal sources. Defects in cholesterol synthesis pathways in the fetus lead to congenital malformations. Six are extremely rare and often lethal. A seventh (Δ7 reductase) is more common and leads to Smith-Lemli-Optiz syndrome. Fetuses with null mutations in Δ7 reductase (i.e., they do not synthesize cholesterol) have only

very low amounts of cholesterol in their tissues and blood, confirming the minor role of maternal exogenous sources.[77] In the presence of normal fetal sterol synthesis, maternal cholesterol supply is of minimal importance.[77] Additionally, women with abetalipoproteinemia or hypobetalipoproteinemia and those consuming low-cholesterol diets have low plasma cholesterol concentrations during gestation and yet there are no adverse effects on pregnancy or the fetus.[77]

In a group of women with diets different in cholesterol content (from cholesterol-free to high cholesterol) during pregnancy, maternal serum total cholesterol at term ranged from 188 to 291 mg/dL. Switching to a cholesterol-free diet decreased total serum cholesterol from 234 to 187 mg/dL, a 20% drop without any adverse fetal or pregnancy effects.[78] Similarly, the Tarahumara Indians of Mexico are known to consume little cholesterol/fat and have a low plasma lipid profile. The average total cholesterol and LDL cholesterol are 141 and 89 mg/dL in non-pregnant females and 193 and 122 mg/dL during pregnancy. Although the degree of increase in cholesterol during pregnancy is similar to that observed in women consuming normal diets in other populations, cholesterol levels during pregnancy are still lower in the Tarahumara Indians compared with American women (193 vs. 250–300 mg/dL during pregnancy). Yet no increased rates of congenital malformations or pregnancy complications are observed in this Indian group.[79] The lower cholesterol levels during pregnancy in these populations are in accordance with the reduction in total cholesterol levels observed with a 40 mg dose of pravastatin.[80,81] Lower doses of pravastatin would be expected to have a smaller reduction in cholesterol levels. In summary, concerns regarding the effect of maternal intake of pravastatin during pregnancy on fetal cholesterol are not supported.

### 2.5.3 Fetal Brain Development

Pravastatin is unlikely to cross to the fetal brain as it has limited ability to cross the blood-brain barrier. No traces of pravastatin have been found in the cerebrospinal fluid of adult patients taking pravastatin. Pravastatin is a substrate of P-glycoprotein, an efflux pump in the endothelial cells of the blood-brain barrier.[72] This supports the previously reported cohort studies that did not find any association between pravastatin exposure during early pregnancy and risk of congenital (especially central nervous system) malformations.

#### 2.5.3.1 Preclinical Studies

In pregnant rats given oral gavage doses of 10, 100, and 1000 mg/kg/day from gestation day 17 through lactation day 21 (weaning), increased mortality of offspring and developmental delays were observed at 100 mg/kg/day systemic exposure, twelve times the human exposure at 80 mg/day (maximum recommended human dose, MRHD) based on body surface area ($mg/m^2$).[82]

The approval package for Pravachol[83] reports that when administered to juvenile rats on postnatal days 4 through 80 at 5-45 mg/kg/day, no drug related changes were observed at 5 mg/kg/day. At 15 and 45 mg/kg/day, slight thinning of the corpus callosum at the end of the recovery period was observed. This finding was not evident in rats at the completion of the dosing period, and was not associated with any inflammatory or degenerative changes in the brain. The biologic relevance of the corpus callosum finding was noted to be uncertain. Neurobehavioral changes (enhanced acoustic startle responses and increased errors in water-maze learning) combined with evidence of generalized toxicity were noted at 45 mg/kg/day during the later part of the recovery period.

It was also reported that studies performed since the marketing approval in 1991 consisted of direct dosing neonatal rats at postnatal days 4 to 80 to address neurodevelopmental events specifically (such as myelination) which occur prenatally in humans but postnatally in the rat. Decreased thickness of the corpus callosum was observed at 20X systemic exposure following an 80 mg clinical dose. At higher exposures (40X) this finding in conjunction with decreased relative brain weights and functional alterations in reflex response (acoustical startle) and learning (water-maze) were observed.

Together these studies were deemed to indicate a perturbation in myelination. The No Observed Adverse Effect Level (NOAEL) of 5 mg/kg/day or 5X systemic exposure following an 80 mg human dose was established in the rat neonatal neurodevelopmental study."

Data from a preeclampsia-like rodent model in which dams were treated with pravastatin at a dose of 5 mg/kg/day, demonstrated that 6-month old mice offspring born to preeclamptic dams, and who were exposed in-utero to pravastatin from embryo day 9 till birth performed similarly on assays testing vestibular function, balance, and coordination (righting reflex, negative geotaxis, balance beam, and climbing) compared with control offspring born to non-preeclamptic dams. In fact, pravastatin corrected some of the findings observed in offspring from preeclamptic dams in the placebo group.[84] Additionally, whole brains from these offspring underwent magnetic resonance imaging (MRI) to evaluate the volumes of 28 regions of interest, including areas involved in adaptation and motor, spatial and sensory function. Offspring born to preeclamptic dams and who were exposed to pravastatin in utero had similar brain volumes compared with those born to non-preeclamptic dams. Similar to the functional data, pravastatin prevented the changes seen in mice offspring born to preeclamptic dams in the placebo group (changes seen in the fimbria, periaquaductal gray, stria medullaris, ventricles, lateral globus pallidus, neocortex, fasciculus retroflexus, inferior colliculus, thalamus, and lateral globus pallidus).[85] Neuronal quantification exhibited decreased cell counts in the neocortex of offspring born to preeclamptic dams, and prenatal pravastatin treatment prevented these changes.

Data from another murine model of preeclampsia (C1q KO model) in which mice destined to develop preeclampsia were treated with pravastatin 10 microgram i.p., a human equivalent dose of 20 mg, showed that pravastatin treatment in pregnancy prevented the microglial activation seen in the brains of 30 day old (P30) offspring born to preeclamptic dams assigned to placebo.[86]

Other studies using other statins showed that simvastatin attenuates the hypomyelination induced by hypoxia-ischemia injury in PND 7 rats, through its anti-inflammatory properties via suppression of microglial activation.[87] Simvastatin also improves functional outcomes in neonatal rat stroke models through reduced cytokine induction and dampening of the inflammatory response.[88]

2.5.3.2 Clinical Studies

Data from the NICHD Obstetrics Fetal Pharmacology Research Centers (OPRC) Network pilot studies in which pravastatin 10 mg and 20 mg were used (described in section 2.6.2), showed the following: 1) the only central nervous system anomaly observed was ventriculomegaly and was seen in a patient who received placebo, 2) there were no cases of holoprosencephaly or agenesis (or hypogenesis) of the corpus callosum on prenatal ultrasound examination, 3) neurologic injury markers in the cord blood (NSE and S100-B) were not different between the pravastatin or placebo arms of either cohort, 4) all newborns in both cohorts who were exposed to pravastatin passed either the Auditory Brain Stem Response (ABR) or Otoacoustic Emissions (OAE) tests before discharge from the hospital after birth, 5) neither the 10 mg dose nor the 20 mg dose of pravastatin resulted in a difference in neonatal head circumference compared with placebo ($34 \pm 1.8$ cm vs. $34.2 \pm 0.6$ cm, $p=0.6$ and $31.1 \pm 4.7$ vs. $32.4 \pm 3.1$, $p=0.5$), and 6) pravastatin drug concentrations in the cord blood were undetectable for the majority of newborns born to mothers on active treatment for both the 10 mg cohort (5 out of 7 available cord blood concentrations were less than the lower limits of quantification (LLOQ) and the 20 mg cohort (6 out of 7 available cord blood concentrations were less than LLOQ).

### 2.5.4 Maternal Safety

A thorough evaluation of the safety profile for a therapeutic intervention for preeclampsia prevention must also focus on potential maternal risks. Pooled data from the large-scale placebo-controlled randomized clinical trials in non-pregnant patients have provided significant information on the risks and side effects associated with use of pravastatin and have reassured clinicians that serious side effects (e.g.,

liver injury and rhabdomyolysis) are extremely rare. In general, pravastatin is considered to be safe and tolerable. Three long-term (4.8- to 5.9-year) placebo-controlled trials of pravastatin (the West of Scotland Coronary Prevention Study, the Cholesterol and Recurrent Events Study, and the Long-term Intervention with Pravastatin in Ischemic Disease Study) collectively included 19,592 patients randomized to pravastatin (40 mg daily) or placebo and accumulated more than 112,000 person-years of exposure.[80,81,89] During five years of exposure, the rates of marked elevations of aminotransferases were low and similar between the two groups (<1.2%). In addition, experience with pravastatin in these trials suggests that liver function test abnormalities observed during pravastatin therapy were usually asymptomatic, not associated with cholestasis, and did not appear to be related to treatment duration. On the other hand, the rate of myopathy, defined as muscle aching or muscle weakness with elevated creatine phosphokinase (CPK) serum levels greater than 10 times the upper limit of normal, was rare (<0.1%) in pravastatin clinical trials.[61,90]

Adverse events were assessed in an indirect comparison meta-analysis of all statins including pravastatin (higher doses than proposed in this trial). In pravastatin exposed patients, rhabdomyolysis occurred in 120 (0.6%; data available from 10 randomized trials; 40,394 individuals), increased AST (3xULN) occurred in 244 (1.4%; data available from seven randomized trials; 35,350 individuals), increased ALT (3xULN) occurred in 134 (1.8%; data available from four randomized trials; 15,200 individuals), and 10 fold increase in creatine kinase (CK) without muscle symptoms occurred in 156 (1.2%; data available from seven randomized trials; 26,407 individuals).[91]

Regarding side effects, pravastatin is generally considered safe, and side effects associated with its use are usually mild and transient. In short-term (4-month) placebo-controlled trials, the rate of study drug discontinuation was similar between pravastatin- and placebo-treated patients (1.7% vs. 1.2%).[61] Of patients on pravastatin (any dose) or placebo, 10.1% and 10.2% reported localized muscle pain; however, only 1.4% and 1.5% of these events were attributed to study medication. The rate of myalgia, regardless of causality, was 2.3%; however, that attributed to pravastatin use was 0.6%.[61] On the other hand, data from 21,483 patients from seven long-term (1.9- to 5.1-year) placebo-controlled pravastatin (40 mg) trials showed similar safety and tolerability profiles for pravastatin compared with placebo (47,613 patient-years of exposure to pravastatin). In these long-term trials, the most common reasons for discontinuation were mild, nonspecific gastrointestinal side effects.[61] Finally in the PRIMO observational study, the rate of muscle related symptoms with pravastatin 40 mg was 10.9%.[92] Findings from the OPRC pilot trials are similar to data from non-pregnant subjects and are summarized in section 2.6.2.

### 2.5.5 Interactions with Other Medications

Studies regarding the interaction of pravastatin and other medications did not include pregnant women. The effects of pregnancy on such interactions are unknown.

The concurrent use of erythromycin, niacin, cyclosporine, or fibrates with HMG-CoA reductase inhibitors other than pravastatin has been thought to be associated with increased risk of myopathy. However, except for fibrates, the risk has not been proven with concurrent use of these medications and pravastatin. Therefore, it is recommended not to co-administer pravastatin with fibrates and colchicines; and to reduce the pravastatin dose when given together with niacin.[61]

On the other hand, inhibitors of cytochrome P450 3A4- such as diltiazem, itraconazole, ketoconazole, mibefradil, and erythromycin- were not found to significantly affect the pharmacokinetic properties of pravastatin when used concurrently. That may be explained by the fact that pravastatin is a weak substrate of cytochrome P450 3A4.[72]

The concurrent use of bile acid resins (cholestyramine, colestipol) and pravastatin resulted in approximately 40-50% decrease in the mean area under the curve (AUC) of pravastatin. Therefore, it is recommended to administer pravastatin at least 1 hour before or 4 hours after the administration of the resin. For pravastatin and cimetidine (H2-receptor antagonist), there was 30% increase in the AUC of

pravastatin compared with when pravastatin was taken alone. On the other hand, when it was taken concomitantly with digoxin, there were no differences in digoxin bioavailability, but the AUC of pravastatin increased by 23%. Concomitant use with clarithromycin resulted in increase in pravastatin AUC by 110%, therefore it is recommended to limit pravastatin to 40 mg daily when used concomitantly with clarithromycin. Regarding cyclosporin, studies in transplant patients taking both medications concurrently did not show any significant effect on cyclosporine levels, however, pravastatin AUC was increased by 282% and Cmax by 327%; and it is recommended to limit pravastatin dosage to 20 mg once daily for concomitant use with cyclosporine.[61,72]

## *2.6 Preliminary Studies of Pravastatin as a Modifier of Outcomes Associated with Preeclampsia*

### 2.6.1 Animal Models

The ability of pravastatin to reverse pathophysiological pathways associated with preeclampsia and to ameliorate the preeclampsia phenotype was evaluated in several studies using different rodent models of preeclampsia (adenoviral overexpression of sFlt-1;[52,54,93] CBA/J x DBA/2 model of immunologically-mediated preeclampsia[55]; C1q-deficient (C1q-/-) mice[56]; and lentiviral vector-mediated placenta-specific sFlt-1 overexpression[53]). These models are based on various pathophysiological pathways thought to be associated with preeclampsia. Findings from these studies support the benefit of pravastatin in preventing preeclampsia and did not raise any safety concerns regarding pravastatin use in pregnant dams. Pravastatin was demonstrated to 1) improve angiogenic balance by reducing sFlt-1 and sEng serum concentrations, increasing PlGF serum concentration, and increasing the expression of PlGF and VEGF in the placenta; 2) lower blood pressure, likely through improvement in vasculature reactivity and up-regulation of endothelial nitric oxide synthase in the vasculature; 3) prevent kidney damage (decrease albuminuria, improve glomerular blood flow, prevent glomerular endotheliosis, and reduce fibrin deposition); 4) improve placental blood flow, restore trophoblast invasiveness, up-regulate a pro-survival/anti-apoptotic MAPK pathway in the placenta, restored placental weight; and 5) prevent pup growth restriction.[51-55,93] These findings were observed without any increase in pup resorption or deformations.

Long-term follow-up of offspring was reassuring and showed prevention of metabolic, cardiovascular, and neuromotor dysfunction, and normalization of postnatal growth in offspring of dams treated with pravastatin, as compared with the altered fetal programming in the offspring of the preeclampsia-like syndrome dams that were not treated with pravastatin.[52-54,56,86]

### 2.6.2 NICHD OPRC Pilot Study

The NICHD Obstetrics Fetal Pharmacology Research Centers (OPRC) network conducted a phase I/II pilot study (Clinicaltrials.gov Identifier NCT01717586, IND # 114205) to assess the safety and pharmacokinetic properties of pravastatin in women at high-risk for preeclampsia (due to history of severe preeclampsia that required delivery prior to 34 completed weeks). The initial study consisted of two double-blind placebo-controlled trials conducted in sequence, the first with a pravastatin dose of 10 mg and the second with a dose of 20 mg. The FDA requested the dosing schedule and sample size. Each trial consisted of 20 patients (10 randomized to pravastatin and 10 to placebo) with singleton non-anomalous pregnancies and a history of prior severe preeclampsia that required delivery before 34 completed weeks. A third cohort was added later using 40 mg (in order to complete the pharmacokinetic profile of pravastatin in pregnancy).

Results of the first two trials are summarized in Table 2.[94] Combining data from the two cohorts, there were no differences in rates of maternal demographic characteristics. Low dose aspirin was also used by 25% of patients in the 10 mg cohort and 70% in the 20 mg cohort with no differences in aspirin use between the pravastatin and placebo groups. The rates of adverse and serious adverse events were similar

between the two groups with the most common side effect among patients who received pravastatin being heartburn and musculoskeletal pain. There were no reports of rhabdomyolysis or liver injury, but one patient in the 20 mg cohort assigned to pravastatin developed muscle weakness and pain without increase in creatine kinase, and study medication was discontinued followed by complete reversal of symptoms. In addition, there was no maternal, fetal or infant death in either group. The rate of any congenital anomaly was not different between the two groups (20% in the placebo group compared with 10% in the pravastatin group). Congenital anomalies in the pravastatin group included hypospadias (infant's father also had hypospadias) and coarctation of aorta. Congenital anomalies in the placebo group included polydactyly, ventriculomegaly, hypospadias and atrial septal defect.

Overall maternal and neonatal outcomes were more favorable in women randomized to pravastatin compared with placebo, with reduced rates of preeclampsia, preeclampsia with severe features and indicated preterm delivery before 37 weeks. Infants of women assigned to pravastatin were born at a later gestational age (mean difference 1 week, 95% CI -0.5 – 2.5 weeks for the 10 mg cohort and 1.9 weeks, 95% CI -1.6 – 5.4 weeks for the 20 mg cohort) and were heavier at birth (mean difference 141 grams, 95% CI -309.3 – 591.3, for the 10 mg cohort and 456.4 grams, 95% CI -502.1 – 1414.8 grams, for the 20 mg cohort compared with those assigned to placebo, but these differences were not statistically significant. The concentrations of PLGF were higher and those of sFlt-1 and sEng were lower among women assigned to pravastatin compared with placebo, but the differences were not statistically significant. Maternal cholesterol and LDL concentrations were marginally decreased in patients receiving pravastatin (consistent with pravastatin's weak cholesterol lowering properties). Cord blood cholesterol concentrations were not significantly different.

**Table 2. Results of the OPRC pilot trial using pravastatin versus placebo to prevent preeclampsia**

| | 10 mg cohort | | 20 mg cohort | | Overall | |
|---|---|---|---|---|---|---|
| **Variable** | Placebo (N= 10) | Pravastatin (N= 10) | Placebo (N=10) | Pravastatin (N= 10) | Placebo (N=20) | Pravastatin (N=20) |
| Use of low-dose aspirin | 3 (30) | 2 (20) | 6 (60) | 8 (80) | 9 (45) | 10 (50) |
| Heartburn | 3 (30) | 4 (40) | 1 (10) | 4 (40) | 4 (20) | 8 (40) |
| Musculoskeletal pain | 1 (10) | 4 (40) | 4 (40) | 4 (40) | 5 (25) | 8 (40) |
| Maternal, fetal, or infant death | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhabdomyolysis | 0 | 0 | 0 | 0 | 0 | 0 |
| Liver injury | 0 | 0 | 0 | 0 | 0 | 0 |
| Myopathy (weakness without increase in CK) | 0 | 0 | 0 | 1 | 0 | 1 |
| Congenital anomalies | 2 | 2 | 2 | 0 | 4 (20) | 2 (10) |
| Preeclampsia (any) | 4 (40) | 0 | 5 (50) | 2 (20) | 9 (45) | 2 (10) |
| | | | | | **RR 0.22 (0.05-0.90) p=0.03** | |
| Preeclampsia with severe features | 3 (30) | 0 | 5 (50) | 0 | 8 (40) | 0 (0) |
| | | | | | **p=0.003** | |

(continued next page)

**Table 2. Results of the OPRC pilot trial using pravastatin versus placebo to prevent preeclampsia (continued)**

| | 10 mg cohort | | 20 mg cohort | | Overall | |
|---|---|---|---|---|---|---|
| **Variable** | Placebo (N= 10) | Pravastatin (N= 10) | Placebo (N=10) | Pravastatin (N= 10) | Placebo (N=20) | Pravastatin (N=20) |
| Gestational age at delivery (wks) | 36.7± 2.1 | 37.7± 0.9 | 34.5 ± 4.1 | 36.4 ± 3.2 | Mean difference 1.5 (-0.4, 3.3), p=0.11 | |
| Indicated preterm delivery < 37 weeks | 5 (50) | 1 (10) | 6 (60) | 3 (30) | 11 (55) | 4 (20) |
| | | | | | **RR 0.36 (0.14-0.95) p=0.048** | |
| Indicated preterm delivery < 34 weeks | 1(10) | 0 (0) | 3 (30) | 1 (10) | 4 (20) | 1 (5) |
| | | | | | RR 0.25 (0.03-2.0) p=0.34 | |
| Total cholesterol at 34- 37 weeks (mg/dl) | 252 ± 27 | 207 ± 31 | 209 ± 40 | 197±44 | Mean diff -26.7 (-55.3, 1.9), p=0.07 | |
| LDL cholesterol at 34-37 weeks (mg/dl) | 130.8 ± 46.7 | 90.4 ± 21.9 | 97.5 ± 29.3 | 91.2 ± 40.8 | Mean diff -22.1 (-48.6, 4.4), p=0.10 | |
| Birth weight (g) | 2,877±630 | 3,018± 260 | 2356 ± 1024 | 2812 ± 1016 | Mean diff 299 (-215, 812), p=0.2 | |
| Highest level of care routine nursery | 5 (50) | 8 (80) | 4 (40) | 6 (60) | 9 (45) | 14 (70) |
| NICU length of stay ≥ 48 hours | 3 (30) | 0 | 5 (50) | 4 (40) | 8 (40) | 4 (20) |

Data presented as mean±SD, median [IQR], n (%), statistics as RR (95% CI) or mean difference (95% CI)

### 2.6.3 Other Studies of Pravastatin in Pregnancy

The use of pravastatin to prevent adverse pregnancy outcomes in women with antiphospholipid syndrome and prior poor obstetrical history was recently investigated in a prospective cohort study of 21 patients. All patients received low dose aspirin and low molecular weight heparin, which was continued when they all developed preeclampsia and/or intrauterine growth restriction. In 11 patients, 20 mg pravastatin daily was added. Compared with patients in the control cohort, those who received pravastatin had improved uterine artery Doppler velocimetry, lower blood pressure, and delivered infants with higher birth weight (2390 grams, IQR [2065-2770] vs. 900 grams, IQR [580-1100]), at a more advanced gestational age (36 weeks, IQR [35-36] weeks vs. 26.5 weeks, IQR [26-32]).[95]

Another proof of concept randomized trial conducted in the United Kingdom (Statins to Ameliorate early onset Preeclampsia (StAmP) trial) and designed to examine the utility of pravastatin 40 mg to improve angiogenic imbalance in women with early-onset preeclampsia before 32 weeks (www.controlled-trials.com; ISRCTN23410175) has not yet reported results.

## *2.7 Rationale for a Randomized Trial*

Multiple clinical trials, using various supplements and medications, have failed to find a satisfactory preventative approach to preeclampsia or its complications. Preeclampsia and adult cardiovascular diseases have biological and pathological similarities and share many risk factors. Endothelial dysfunction and inflammation are fundamental for the initiation and progression of both atherosclerosis and preeclampsia. Primary and secondary prevention of cardiovascular mortality and other cardiovascular events in non-pregnant patients using 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or statins, through their pleiotropic and lipid-lowering actions is widely accepted.[96,97]

Pravastatin has been shown in various preclinical and clinical studies to reverse various pathophysiological pathways associated with preeclampsia. The data on degree of intrauterine exposure and fetal cholesterol synthesis presented above, combined with pravastatin's low potency for inhibition of HMG-CoA reductase, and limited ability to cross the placenta are reassuring and add support to the use of pravastatin in pregnancy.[72,75,98] In animal studies, a possible signal of disrupted myelination based on thinning of the corpus callosum is reported in the pravastatin sodium labeling. While the potential for myelination disruption or risk of neurodevelopmental insults from any medication is of concern, it is worth noting that children born to preeclamptic mothers especially if born prematurely are also at increased risk of neurodevelopmental adverse outcomes.[99-101] Pravastatin has not been shown to be teratogenic in animal or human cohort studies, and data from the cardiovascular trials in non-pregnant women and men suggest a favorable maternal safety and tolerability profiles.

Data from the *Eunice Kennedy Shriver* NICHD-OPRC Network pilot study also support the favorable maternal and fetal safety profiles of pravastatin and its effectiveness to prevent preeclampsia. All of the above provide the rationale for the use of pravastatin in the prevention of preeclampsia in high-risk women.

# 3 Study Design

## 3.1 Primary Research Question

The randomized trial will address the primary research question:

In women with a prior history of preeclampsia with preterm delivery less than 34 weeks, does early treatment with pravastatin in a subsequent pregnancy reduce the rate of a composite outcome of preeclampsia, fetal loss and maternal death, when compared with placebo?

## 3.2 Secondary Research Questions

Secondary research questions this study will address are:

- Does treatment with pravastatin alter the risk of:
    - pregnancy associated hypertensive disorders,
    - indicated and spontaneous preterm delivery,
    - fetal/neonatal mortality or neonatal morbidity including small for gestational age
    - severe maternal and perinatal morbidity?
- Does pravastatin increase neonatal birth weight?
- Does pravastatin prolong the gestational age at delivery?
- Does pravastatin improve the maternal angiogenic profile by decreasing sFlt-1 and sEng, and increasing PlGF?
- Are there specific subgroups in which treatment with pravastatin is more efficacious (see Section 6.3.1 for a discussion of subgroup analyses)?
- Does treatment with pravastatin affect child neurodevelopment, behavior, hearing or vision at 2 and 5 years of age?

## 3.3 Design Summary

The study is a randomized controlled multi-center clinical trial of 1,550 women with a prior history of preeclampsia that required delivery at less than 34 weeks gestation, randomized to one of two arms at participating MFMU Network clinical centers.

- 20 mg pravastatin daily
- Identical appearing daily placebo

20 mg was chosen as a dose, since it showed a significant effect in the pilot study, is the same as the pediatric dose and assumed to be safer than 40 mg. The dose proposed is significantly lower than the reported no-observed-adverse-effect-level in rats. The Data and Safety Monitoring Committee will evaluate maternal and neonatal safety data after the first 50 and 310 patients have delivered and will make the recommendation to continue the 20mg dose or lower the dose to 10mg.

An initial cohort of 50 women will be randomized to evaluate the safety profile through delivery and to assess long-term follow-up of the children through 5 years. Recruitment beyond the first 50 will continue after obtaining approval from the FDA.

## *3.4 Eligibility Criteria*

### 3.4.1 Inclusion Criteria

1. 16 years or older at time of consent with ability to give informed consent
2. Single or twin gestation with cardiac activity in one or both fetuses. Higher order multifetal gestations reduced to twins, either spontaneously or therapeutically, are not eligible unless the reduction occurred by 13 weeks 6 days project gestational age (see below).
3. Gestational age at randomization between 12 weeks 0 days and 16 weeks 6 days based on clinical information and evaluation of the earliest ultrasound as described in Gestational Age Determination in Section 3.4.2 below.
4. Documented history (by chart or delivery/operative note review) of prior preeclampsia with delivery less than or equal to 34 weeks 0 days gestation in any previous pregnancy. If in the index pregnancy, the woman was induced by 34 weeks 0 days gestation and delivered within 48 hours in the same hospitalization, that woman would be eligible. Severe gestational hypertension managed as preeclampsia and reported as an indication for delivery also qualifies.
5. Normal serum transaminase (AST/ALT) concentrations documented in the last 6 months.

### 3.4.2 Gestational Age Determination

Gestational age is determined using criteria proposed by the American Congress of Obstetricians and Gynecologists, the American Institute of Ultrasound in Medicine and the Society for Maternal-Fetal Medicine and is denoted "project gestational age".[102] The project EDD (estimated date of delivery), which is based on the project gestational age, cannot be revised once a determination has been made. If the pregnancy is conceived by in-vitro fertilization, project gestational age is calculated from the date of embryo transfer and the embryo age at transfer. If the pregnancy is conceived spontaneously (including ovulation induction and artificial insemination) information from the earliest dating ultrasound and the last menstrual period are used to determine project gestational age. If no dating ultrasound has been performed previously, one must be performed before the patient can be randomized.

The following algorithm is used:

- The first day of the last menstrual period (LMP) is determined, and a judgment made as to whether or not the patient has a "sure" LMP date.
- If the LMP date is unsure, ultrasound measurement(s) of the single fetus or larger twin obtained at the patient's first dating ultrasound examination is used to determine the project gestational age. If the first dating ultrasound was conducted before 14 weeks 0 days, the measurement must be based on crown rump length (CRL).
- If the LMP date is sure, project gestational age is determined by a comparison between the gestational age by LMP and by ultrasound measurement of the single fetus or larger twin based on the earliest dating ultrasound. If the ultrasound confirms the gestational age by LMP as in the table below, the LMP-derived gestational age is used to determine the project gestational age. Otherwise, project gestational age will be determined based upon the ultrasound measurement of the larger twin (if applicable).

**Table 3. Cutoffs for Using LMP to Determine Gestational Age for Sure LMP**

| Gestational age at first ultrasound by LMP | Ultrasound method of measurement | Ultrasound agreement with LMP |
|---|---|---|
| Up to 8 weeks 6 days | CRL | ± 5 days |
| 9 weeks 0 days to 13 weeks 6 days | CRL | ± 7 days |
| 14 weeks 0 days to 15 weeks 6 days | Per institution | ± 7 days |
| 16 weeks 0 days to 21 weeks 6 days | Per institution | ± 10 days |

### 3.4.3 Exclusion Criteria

1. Monoamniotic gestation because of the risk of fetal demise
2. Known chromosomal, genetic or major malformations
3. Fetal demise or planned termination of pregnancy. Selective reduction by 13 weeks 6 days gestation, from triplets to twins or twins to singleton is not an exclusion.
4. Contraindications for statin therapy:
    a. Hypersensitivity to pravastatin or any component of the product
    b. Active liver disease: acute hepatitis or chronic active hepatitis
5. Statin use in current pregnancy
6. Patients with any of the following medical conditions:
    a. Uncontrolled hypothyroidism with a TSH level above 10 mIU/L, because of increased risk of myopathy
    b. HIV positive, because of increased risk of myopathy with use of protease inhibitors
    c. Chronic renal disease with baseline serum creatinine ≥1.5 mg/dL, because of association with adverse pregnancy outcomes
7. Current use of concomitant medication with potential for drug interaction with statins (i.e.,, cyclosporine, fibrates, niacin, erythromycin). Patients will not be excluded if the drug is discontinued (at least one week) prior to randomization.
8. Participating in another intervention study that influences the primary outcome in this study
9. Plan to deliver in a non-network site
10. Participation in this trial in a previous pregnancy. Patients who were screened in a previous pregnancy, but not randomized, do not have to be excluded.

## *3.5 Informed Consent Criteria*

Written informed consent must be obtained from patients before they can be randomized into the study. Full disclosure of the nature and potential risks of participating in the trial is to be made. Patients will be given a document summarizing the safety and side effects of pravastatin. This will also be discussed with the patient in detail. A copy of the signed consent form will be provided to the patient.

Women who are not fluent in English will be enrolled by a person fluent in their language, if possible. Both verbal and written informed consent and authorization will be obtained in that language; if this is not possible the patient will be excluded.

## *3.6 Randomization Method and Masking*

Consenting women will be assigned to pravastatin or placebo in a 1:1 ratio according to a randomization sequence prepared and maintained centrally by the Biostatistical Coordinating Center (BCC). The two study medication arms of the study (pravastatin or placebo) are double masked; neither the patient nor the clinical staff will be aware of the treatment assignment.

The simple urn method will be used to generate the randomization sequences because it provides a high probability of balance in treatment assignments, it is unpredictable, and it allows an explicit randomization analysis to be conducted with relative ease.[103] Randomization will be stratified by clinical site to assure balance between the two treatment groups with respect to anticipated differences among the clinic population and possible differences in patient management.

# 4 Study Procedures

## *4.1 Screening for Eligibility and Consent*

Women who present for prenatal care before 17 weeks 0 days are potentially eligible for screening. Women may also be contacted using an approved phone script. The inclusion/exclusion criteria will be reviewed in the patient's medical records and documented on the screening log. If an ultrasound examination has not been performed, one must be arranged prior to randomization to confirm gestational age and check for exclusion criteria.

If a patient meets all the inclusion criteria for enrollment, does not have any of the exclusion criteria, and expresses interest in the study, she will be approached by research personnel and fully informed in detail about the study, the medication, and its potential effects on her and her fetus, and she will be asked to sign the informed consent form if she is willing to participate. Eligibility will be confirmed by reviewing the delivery note/ record, admission note, discharge summary or other parts of the medical record of the qualifying delivery. Patients will be asked for permission to maintain/update their contact information for future contacts, and to sign a release of medical records form in the event that they deliver at different hospital. A copy of the signed consent form will be provided to the patient and another one kept in the medical record.

After consent is obtained and before randomization, the patient's liver enzymes (AST, ALT) will be assayed, if these results are not available in the past 6 months in the patient medical record. If the results are normal, the patient will be scheduled as soon as possible for the randomization visit.

## *4.2 Randomization*

If the patient is eligible and willing to participate, study-certified research staff will make an entry on the next line of the randomization log. This assigns a study drug code to the patient and defines the point of randomization. The study drug code corresponds to a blinded study medication kit packaged according to the randomization sequence that consists of a sufficient number of bottles to last the remainder of her pregnancy.

## *4.3 Baseline Procedures*

In addition to information collected for eligibility, project gestational age, and project EDD determination, the following information will be obtained at randomization from a patient interview followed by a review of her chart:

- Demographic information: age, race, ethnicity, insurance status
- Medical history: pre-pregnancy weight, height, medication use (e.g., aspirin, calcium, vitamins, anti-platelet agents, prior statins, antihypertensives), blood pressure, and thyroid and chronic disease history.
- Obstetrical history including outcomes of all prior pregnancies and which pregnancy(ies) was complicated by preeclampsia.
- Social history including marital status, years of education, alcohol and tobacco use.

During the randomization visit, blood pressure, height and weight will be recorded. Blood (27 ml) will be collected for Creatine Kinase (CK) and run in real-time at the local laboratory to serve as a reference in the event the patient develops a condition that suggests the possibility of myopathy and a repeat CK is warranted (Section 4.5). The remainder of the sample will be stored for analysis of DNA, lipids and

angiogenic markers. Proteinuria status at baseline is required for assessment of the primary outcome. Proteinuria will be evaluated by dipstick if not available from a recent prenatal visit. Dipstick values of 1+ or higher will require a protein:creatinine ratio or 24-hour urine collection for confirmation if not done clinically.

Treatment will be initiated at the randomization visit between 12 weeks 0 days and 16 weeks 6 days, in order to 1) avoid the period of organogenesis, and 2) start as early in pregnancy as possible. Treatment will start the same day as randomization, and the patient will be asked to take one capsule daily orally, with or without food. Treatment will continue until delivery or until a condition develops requiring discontinuation of the study drug.

## *4.4 Study Procedures*

All patients will have monthly assessments (in-person or virtual) with a research nurse to assess drug side effects and the occurrence of adverse events, and compliance by pill count. Patients will receive a 35 day supply of study drug to last until their next visit (in case it is slightly more than 4 weeks). Specifically, at each study visit, the following procedures will be performed:

- Assess drug side effects and adverse events
- Record weight
- Record blood pressure
- Perform pill count and dispense new supply
- Record current medication list

Treatment will continue until delivery or until a condition develops requiring discontinuation of the study drug (see Section 4.5). Blood will be collected and stored for retrospective analysis at two time periods, 1) 23-$28^{6/7}$ weeks gestation and 2) at 33-$37^{6/7}$ weeks gestation to measure lipids, angiogenic markers and pravastatin concentration (20 ml). Cord blood will be collected at delivery for the first 50 women randomized to measure pravastatin concentration (6 ml).

**Table 4. Study visit procedures**

| | **Timing** | **Procedures** |
|---|---|---|
| **Screening** | < $16^{6/7}$ weeks | Consent, AST/ALT (if not available), verify eligibility criteria |
| **Randomization** | $12^{0/7}$–$16^{6/7}$ weeks | Baseline data, record blood pressure and weight |
| | | Blood collected for:<br>• Creatine Kinase (to be run immediately)<br>• lipid profile(to be run at the end of study)<br>• angiogenic markers (to be run during study)<br>• maternal DNA (may be run in future) |
| **Study visits** | Monthly | Assessment of side effects, pill count, record BP and weight |
| | $23^{0}$–$28^{6/7}$ weeks | Blood collected for:<br>• lipid profile (to be run at the end of study)<br>• angiogenic markers (to be run during study)<br>• maternal pravastatin concentration (may be run in future) |
| | $33^{0}$-$37^{6/7}$ weeks | Blood collected for:<br>• lipid profile (to be run at the end of study)<br>• angiogenic markers (to be run during study)<br>• maternal pravastatin concentration (may be run in future) |

| | | |
|---|---|---|
| **Delivery** | | Clinical outcomes (maternal and neonatal)<br>Cord blood collected for pravastatin concentration (first 50 only) |
| **Postpartum** | 6 weeks postpartum | Assessment of adverse events, postpartum complications, record blood pressure and weight |
| **Child Two-year Follow-up** | 2 years of age | Medical history, height and weight<br>Cognitive, behavior, motor, visual and hearing assessment |
| **Maternal Two-year Follow-up** | 2 years of child's age | Medical history<br>Blood pressure & anthropometric (height, weight, waist, hip) assessments<br>Blood collection for lipid profile and additional analytes |
| **Child Five-year Follow-up** | 5 years of age | Medical history, height and weight<br>Cognitive, behavior, and vision test |

Information on interventions during the pregnancy such as receipt of antenatal corticosteroids, tocolytics, or antihypertensive medications, pregnancy complications, and delivery outcomes will be collected by trained research personnel from chart review. Blood pressure measurements made during labor will be abstracted from the patient's chart. For the neonate, birth weight, length, head circumference, and ABR/OAE testing, will be obtained from the infant's chart. Neonatal data on all infants will be collected through discharge or 120 days after birth, whichever occurs first.

All patients will be followed up at 6 weeks postpartum, and information on any postpartum complications, including readmissions for blood pressure control, postpartum preeclampsia, infection or other morbidities, will be recorded.

The children will be followed for five years with study visits performed at 2 years and 5 years of age, including neonates who fail both the ABR and OAE testing at birth. To keep in touch with the family, follow-up coordinators will use a variety of methods, including contacting the family by telephone/e-mail/text every six months. Comprehensive pediatric neurodevelopmental testing will be performed at these two time points by study examiners that are trained and certified for the study. At the time of the 2-year child visit, maternal assessments will also be performed.

### 4.4.1 Child 2-year Follow-up

The following assessments will be performed at the child 2-year visit:

- Medical history including hearing loss and need of hearing aids
- Height and weight
- Cognitive, Motor and Language Scores from the Bayley Scales of Infant and Toddler Development 4
- The Gross Motor Function Classification System (GMFCS) and the Manual Abilities Classification System (MACS)
- Visual and hearing assessment
- Child Behavior Checklist (CBCL)

### 4.4.2 Maternal 2-year Follow-up

The following assessments will be performed at the same time as the child 2-year visit:

- Medical history
- Blood pressure

- Anthropometrics including height, weight, waist and hip circumferences
- Blood collection

Maternal blood will be processed for lipids, HbA1c, fasting glucose and insulin, hs-CRP, Lipoprotein A and other analytes.

### 4.4.3 Child 5-year Follow-up

The following assessments will be performed at the child 5-year visit:

- Medical history including hearing loss and need of hearing aids
- Height and weight
- Differential Ability Scales-II (DAS-II) cognitive assessment. The DAS II general conceptual ability score correlates well with full scale IQ as measured by the Wechsler Preschool and Primary Scale of Intelligence (0.89).
- Behavior Rating Inventory of Executive Function (BRIEF 2nd Edition)
- Vineland Adaptive Behavior Scales, 3rd Edition
- The Gross Motor Function Classification System (GMFCS) and the Manual Abilities Classification System (MACS)
- Child Behavior Checklist (CBCL)
- Opthalmologic exam including visual acuity/refraction and cover test (evaluate strabismus)

## *4.5 Patient Management*

Use of low dose aspirin is recommended, as it is currently standard of care in the U.S. for women with prior preeclampsia according to the ACOG guidelines.

Patients' pregnancy management (including antenatal testing, ultrasounds, control of hypertension, inpatient vs. outpatient management, and mode and timing of delivery) will be performed as recommended by standard prenatal care as defined by the respective participating institution. Inpatient vs. outpatient management of patients with preeclampsia (without severe features), single vs. double courses of antenatal corticosteroids in the setting of preterm labor, and other obstetric interventions will be left to the discretion of the treating physician.

Although chronic use of the drugs that may have potential significant interactions with pravastatin are listed as an exclusion criteria, the occasion may arise that a pregnant woman in the study may require one or more of these medications. Therefore, and to avoid any potential (or unknown) interactions; patients and providers in this study will be advised of the following:

- Study drug should be taken at least 1 hour before or 4 hours after the administration of cholestyramine.
- Cimetidine (H2-receptor antagonists) should be taken at least 1 hour before study drug.
- There is no need to change the timing or stop study drug if also taking aspirin, digoxin, or diltiazem.
- In the event that a patient requires erythromycin (e.g., rupture of membranes), an alternative antibiotic will be recommended if available. If not, the study drug will be stopped for the duration of erythromycin intake (usually 7 days). The study drug will be resumed at the end of that period.

In this study, the following events that could be related to pravastatin use will be defined and monitored as Adverse Events of Special Interest (AESI).

- Myalgia: diffuse muscle pain, cramps, or soreness without elevation in CK
- Muscle weakness: muscle weakness documented on physical exam without elevation in CK
- Myositis: muscle symptoms (myalgia or weakness) with CK elevations between 3 and 10 times upper limit of normal (ULN) in pregnancy or between 3 and 10 times the baseline CK value, whichever is higher
- Myopathy: muscle symptoms (myalgia or weakness) plus CK ≥ 10x ULN or 10x baseline CK
- Rhabdomyolysis: muscle symptoms (myalgia or weakness) plus CK ≥ 10x ULN or 10x baseline CK plus an elevation in serum creatinine (usually with brown urine and myoglobinuria) or need for medical interventions such as hospitalization and intravenous hydration.[104-109]
- Serious liver injury defined as persistent AST or ALT of 3 x ULN or increased AST or ALT levels with clinical symptoms and hyperbilirubinemia or jaundice, in the absence of severe preeclampsia or other hepatic disease

Severe Adverse Events of Special Interest will be defined as maternal myositis, myopathy, rhabdomyolysis, or serious liver injury. Patients will be advised throughout the study to report promptly unexplained muscle pain, tenderness or weakness, particularly if accompanied by malaise or fever. If myositis, myopathy, rhabdomyolysis, or serious liver injury occurs, the study drug (regardless of actual treatment assignment) will be discontinued permanently. If a patient develops myalgia or muscle weakness (documented on physical exam) with CK elevation <3 x ULN (or baseline CK), then the following will take place, 1) study drug will be stopped temporarily, 2) a workup will be done to ensure the patient does not have any of the other more serious muscle related serious adverse events, and 3) risk and precipitating factors will be evaluated. The patient will be monitored until complete resolution of symptoms and the CK values are back to normal (if applicable). The CK values will be checked weekly if above the ULN or baseline CK. Upon resolution, the patient will be re-challenged with pravastatin to be used every day or every other day.

Any woman who withdraws from taking study medication for any reason, including discontinuation for side effects, will be encouraged to continue study visits as planned, including the postpartum contact and follow-up of the child through 5 years of age.

## *4.6 Study Outcome Measures and Ascertainment*

### 4.6.1 Primary Outcome

The primary outcome of the study is a composite of fetal loss, maternal death, and preeclampsia diagnosed prior to 48 hours postpartum as follows:

1. In previously normotensive women, preeclampsia is diagnosed based on
    a) Severe hypertension (systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg) at ≥ 20 weeks in gestation on two occasions with measurements taken at least 4 hours apart, or 1 occasion with subsequent anti-hypertensive therapy, or
    b) Mild hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) at ≥ 20 weeks in gestation on two occasions with measurements taken at least 4 hours apart, with any of the following:
        i. New-onset proteinuria ≥300 mg in a 24-hour period, protein to creatinine ratio ≥ 0.3, or 1+ protein on maternal urine dipstick (only if 24-hour urine collection or protein to

creatinine ratio is not possible) or a doubling in protein in women with baseline proteinuria

ii. Thrombocytopenia (platelet count <100,000 per microliter)

iii. Progressive renal insufficiency (serum creatinine >1.1 mg/dl or doubling of the serum creatinine in women with abnormal baseline values (>1.1 mg/dl) and in the absence of other renal disease)

iv. Impaired liver function (elevated transaminases ≥ 70 U/L , or severe persistent right upper quadrant or epigastric pain unresponsive to medication)

v. Pulmonary edema diagnosed clinically by exam or chest x-ray

vi. New-onset and persistent (non-responsive to supportive treatment) cerebral or visual symptoms.

2. In women with baseline chronic hypertension, preeclampsia is diagnosed based on any of the following (not accounted for by alternative diagnoses):

   a) Severe hypertension (systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg) ≥ 20 weeks in gestation on two occasions with measurements taken at least 4 hours apart, or 1 occasion with subsequent anti-hypertensive therapy, or an escalation of antihypertensive medications to control blood pressure

   b) New onset of proteinuria or a doubling in protein in women with baseline proteinuria

   a) Thrombocytopenia (platelet count <100,000 per microliter)

   b) Progressive renal insufficiency (serum creatinine >1.1 mg/dl or doubling of the serum creatinine in the absence of other renal disease)

   c) Impaired liver function (elevated transaminases ≥ 70 U/L , or severe persistent right upper quadrant or epigastric pain unresponsive to medication)

   d) Pulmonary edema diagnosed clinically by exam or chest x-ray

   e) New-onset and persistent (non-responsive to supportive treatment) cerebral or visual symptoms.

3. HELLP [hemolysis, elevated liver enzymes and low platelet count] syndrome defined as the occurrence of all of the following (not accounted for by alternative diagnoses):

   a) Hemolysis: evidenced by (1) serum total bilirubin ≥ 1.2 mg/dL (20 µmol/L), (2) serum lactate dehydrogenase (LDH) ≥ 600 IU/L, or (3) hemolysis on peripheral smear

   b) Thrombocytopenia (platelet count < 100,000 / microliter)

   c) Serum aspartate aminotransferase (AST) or alanine transaminase (ALT) ≥ 70 IU/L

4. Atypical HELLP defined as the occurrence of 2 of the 3 following (not accounted for by alternative diagnoses)

   a) Hemolysis: evidenced by (1) serum total bilirubin ≥ 1.2 mg/dL (20 µmol/L), (2) serum lactate dehydrogenase (LDH) ≥ 600 IU/L, or (3) hemolysis on peripheral smear

   b) Thrombocytopenia (platelet count < 100,000 / microliter)

   c) Serum aspartate aminotransferase (AST) or alanine transaminase (ALT) ≥ 70 IU/L

5. Eclampsia defined as an occurrence of a seizure without any known cause

6. Competing outcomes also qualify as the primary outcome

a) Maternal death before delivery

b) Fetal loss less than 20 weeks, 0 days

All charts of women who have any elevated BP ≥ 140/90 mmHg or a diagnosis of proteinuria, will be centrally reviewed by teams of three Protocol Subcommittee members (three investigators/nurse coordinators) blinded to assignment to ensure integrity of the primary and key secondary outcomes.

### 4.6.2 Maternal Secondary Outcomes

1. Preterm birth < 37 weeks (major secondary outcome)
2. Indicated preterm birth < 37 weeks
3. Preterm birth < 34 weeks
4. Preeclampsia with severe features as defined by the ACOG diagnostic criteria (i.e., severe hypertension, thrombocytopenia, impaired liver function, progressive renal insufficiency, pulmonary edema, new-onset and persistent cerebral or visual symptoms)
5. Postpartum preeclampsia diagnosed after 48 hours
6. Gestational hypertension defined as new onset hypertension in the absence of accompanying proteinuria or other features of preeclampsia
7. Pregnancy associated hypertension (gestational hypertension or preeclampsia)
8. Gestational diabetes
9. Adherence to study medication
10. Adverse Events of Special Interest (AESI) defined as myalgia and muscle weakness, and serious AESI defined as maternal myositis, myopathy, rhabdomyolysis, and serious liver injury
11. Gestational age at delivery
12. Length of hospital stay for the delivery admission and number and length of maternal hypertension related and overall hospitalizations during the pregnancy
13. Concentrations of angiogenic factors (sFlt-1, sEng, and PlGF)
14. Concentrations of cholesterol (total, low density lipoprotein, high density lipoprotein) and triglycerides
15. A composite of severe maternal morbidity of either maternal death, eclampsia, HELLP syndrome, cerebral vascular accident, heart failure, myocardial infarction, acute respiratory distress syndrome requiring mechanical ventilation, disseminated intravascular coagulopathy, pulmonary edema, renal failure, liver rupture, or placental abruption

### 4.6.3 Fetal and Neonatal secondary outcomes

1. Fetal and neonatal death
2. Birth weight and rate of "small for gestational age" as measured by birth weight: a) < 5th percentile and b) < 10th percentile for gestational age, assessed specifically by sex and race of the infant based on United States birth certificate data [110,111]
3. Admission to neonatal intensive care unit/intermediate nursery and total length of stay in that unit
4. Complications of prematurity including:
    a. Mechanical ventilation in the first 72 hours of life and duration
    b. Oxygen support and duration

c. Respiratory distress syndrome (RDS), defined as the presence of clinical signs of respiratory distress (tachypnea, retractions, flaring, grunting, or cyanosis), with an oxygen requirement and confirmed by a chest x-ray

d. Bronchopulmonary dysplasia (BPD), defined as oxygen requirement at 28 days of life and at 36 weeks corrected gestational age

e. Necrotizing enterocolitis (NEC), defined as modified Bell Stage 2 (clinical signs and symptoms with pneumatosis intestinalis on radiographs) or Stage 3 (advanced clinical signs and symptoms, pneumatosis, impending or proven intestinal perforation)

f. Intraventricular hemorrhage (IVH) grade III-IV

g. Periventricular leukomalacia (PVL), diagnosed by neuroimaging

h. Retinopathy of prematurity (ROP) stage III or higher

i. Neonatal sepsis (within 72 hours and > 72 hours after birth). The diagnosis of sepsis will require the presence of a clinically ill infant in whom systemic infection is suspected with a positive blood, CSF, or catheterized/suprapubic urine culture; or, in the absence of positive cultures, clinical evidence of cardiovascular collapse or an unequivocal radiograph confirming infection.

j. Composite outcome of: fetal or neonatal death, RDS, Grade III-IV IVH, PVL, Stage 2 or 3 NEC, BPD, Stage III or higher ROP, or early onset sepsis

k. Seizures

5. Congenital anomaly / birth defect (excluding any conditions that must have been present before randomization)
6. Neonatal auditory brain stem response (ABR)/ Otoacoustic Emissions (OAE)

### 4.6.4 Child Secondary Outcomes

1. Body mass index for age at 24 corrected months and 5 years of age using CDC pediatric growth charts
2. Cognitive, Language and Motor Scale Scores from the Bayley Certified Scales of Infant Development 4 at 24 months age
3. Level from the Gross Motor Function Classification System at 24 months of age
4. Hearing loss or vision problems (severe nearsightedness or farsightedness, and eye movement problems) at 24 months of age
5. Total problems score and syndrome scale (emotionally reactive, anxious/depressed, somatic complaints, withdrawn, attention problems, aggressive behavior) scores from the Child Behavior Checklist at 24 months and 5 years of age
6. General Conceptual Ability score and subscale (verbal ability, non-verbal reasoning ability, and spatial ability) scores from the Differential Ability Scales at 5 years of age
7. Global Executive Composite score and index (behavioral, emotion, and cognitive regulation) scores from the Behavior Rating Inventory of Executive Function at 5 years of age
8. Adaptive Behavior Composite score and domain (communication, daily living skills, socialization and motor skills) scores from the Vineland Adaptive Behavior Scale at 5 years of age
9. Visual acuity and strabismus from visual assessment at 5 years of age

## *4.7 Adverse Event Reporting*

Detailed information concerning adverse events will be collected and evaluated throughout the conduct of the protocol.

Any maternal death, neonatal death, life threatening maternal event or Serious Adverse Event of Special Interest as defined in Section 4.5 must be entered into the adverse events database within twenty-four hours of being notified. If a death is reported, a copy of the patient's de-identified medical record will be uploaded to the adverse events database.

Adverse events which do not qualify under the above definition must be entered into the database within 7 days of being notified.

## *4.8 Emergency Unblinding*

The only indication for breaking the randomization code is when it is medically necessary to unmask the study drug assignment to be able to treat the patient, i.e. when treatment options would differ based on the knowledge of the medication. In such a situation, the center should try to contact the BCC before unmasking but in an emergency situation, the PI of the center, the Nurse Coordinator, the alternate PI or the site PI may authorize the unmasking.

# 5 Data and Safety Monitoring Plan

## *5.1 Data and Safety Monitoring Committee*

The Data and Safety and Monitoring Committee (DSMC) for the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network is an independent group appointed by the NICHD to oversee MFMU Network trials. The primary objective of the DSMC is to ensure the safety of study subjects and to provide NICHD, and for this study, NHLBI with advice on the ethical and safe progression of the trial. The DSMC also advises the Institutes on research design issues, data quality and analysis, as well as ethical and human subject aspects of studies. Recommendations will be made to the NICHD and NHLBI and disseminated to the Steering Committee.

### 5.1.1 DSMC Membership Qualifications

The DSMC members are appointed by the Director of NICHD in accordance with established NIH policies governing the use of advisors. DSMC members are chosen for their expertise in one or more of the following fields:

- Maternal-fetal medicine or obstetrics
- Neonatology
- Ethics
- Biostatistics/epidemiology
- Clinical trial methods

One of the members with expertise in obstetrics or maternal-fetal medicine is appointed as Chairperson. In addition, a layperson or member of the public is appointed as a patient advocate.

In addition to the members of the DSMC, an NICHD representative who is not part of the MFMU Network is appointed as a liaison between the Institute and the committee. NHLBI may also appoint a representative. The NIH representatives participate in the deliberations, but do not vote.

### 5.1.2 Conflict of Interest and Confidentiality

Members of the DSMC may not be affiliated with any of the clinical centers or the BCC and must be financially and intellectually independent of the trial investigators. As part of their service on the Committee, members must sign an annual conflict of interest and confidentiality declaration. If a new conflict arises for a member, it must be disclosed in a timely manner (within 30 days) to the NICHD. The NICHD will determine whether the conflict limits the ability of the DSMC member to participate in the committee.

All data and information remain confidential. Interim data analysis reports and any other reports provided to DSMC by the BCC must be returned or destroyed. Investigators from the MFMU Network will not communicate directly with DSMC members about the study except when making presentations or responding to questions regarding review of a protocol during a DSMC meeting or scheduled conference call.

### 5.1.3 Responsibilities of the DSMC

Specifically, the responsibilities of the DSMC members are as follows:

Before the start of the trial:

- To review the research protocol and informed consent document(s) with respect to ethical and safety standards. The study design should be evaluated to determine whether it is adequate to answer the research question(s).

While the trial is ongoing:

- To evaluate whether the study design assumptions are valid and the impact of the assumptions on how well the research question(s) will be answered.
- To monitor the safety of the participants including review of serious adverse events as they arise.
- To monitor recruitment, losses to follow-up, compliance with the protocol by investigators and participants, and data quality.
- To monitor the evidence for treatment harm or benefit and to evaluate evidence for treatment differences in the main efficacy outcome measures.
- To assess the impact and relevance of external evidence on the advisability of trial continuation as well as on the need for design modification including the necessity for modification of the informed consent material.
- To make recommendations for changes to the study design, if necessary, to ensure that the research question(s) will be answered.
- To recommend continuation or termination of a trial for all participants, certain treatment groups, or certain participant subgroups.

## *5.2 Protocol Review*

Before the start of the trial, the DSMC reviews the final protocol and provides comments. The Chair of the Protocol Subcommittee or designee may present the protocol, and be available to answer questions. The subcommittee Chair will report the DSMC's comments to the Steering Committee for consideration and possible modification of the protocol. The trial cannot start until the DSMC approves the protocol. Approval is by consensus.

If the Steering Committee proposes major changes to the protocol during the course of the trial (e.g. eligibility criteria), the DSMC must review and approve the changes before they can go into effect, provided that the DSMC has not already been unblinded to study treatment in an interim analysis.

## *5.3 Ongoing Safety Monitoring*

As described in Section 4.7 any maternal death, neonatal death, life threatening maternal event or serious Adverse Event of Special Interest (myositis, myopathy, rhabdomyolysis or serious liver injury as defined in Section 4.5) is reported electronically within twenty-four hours of notification. Other adverse events are reported within seven days of notification. The BCC is automatically notified by the adverse event system of the addition of a new adverse event. BCC staff review each report for completeness before passing it on to the NICHD – approved medical monitor. The medical monitor reviews the adverse event reports including the accuracy of the classification of seriousness, unexpectedness and relatedness to the study interventions. The medical monitor is masked to treatment assignment.

The adverse events reports are then forwarded electronically to the DSMC Chair, the NIH representative(s) to the DSMC, and any other DSMC member who requests notification. Deaths, life threatening events, serious Adverse Events of Special Interest and SUSARS (see below) are reported immediately. Other adverse events are forwarded on a monthly basis. The DSMC Chair decides whether the adverse event reports should be disseminated to the rest of the committee, whether a follow-up call or meeting is required, and whether the treatment assignment should be unmasked. If NIH or the Steering

Committee is concerned about a pattern of events, they may request that the DSMC consider their concern.

An FDA Investigational New Drug (IND) safety report will be completed and forwarded to the FDA for any serious unexpected suspected adverse reaction (SUSAR) to the study medication that is fatal or life threatening (whether active or placebo) within 7 days after the event was entered into the database. For other adverse events classified as SUSAR, notification will be reported to the FDA within 15 days. All cumulative adverse events will be reported in the annual IND report.

Finally, cumulative adverse events are reported to the DSMC at each meeting and will be considered along with other interim safety data in the DSMC deliberations.

## *5.4 Data and Safety Monitoring Committee Meetings*

For this study the DSMC will meet at least every six months, once in person and once most likely by conference call. The committee may meet more often as needed. DSMC meetings and conference calls are organized into open and closed sessions, and if requested, an executive session. Definitions for each of the session types are included below.

- Open session – Open sessions are attended by the NICHD Project Scientist, the NHLBI Project Scientist if desired and may be attended by Steering Committee member(s), or the Steering Committee Chairperson as requested by NICHD Project Scientist.
- Closed session – Closed sessions are attended only by the NICHD (and NHLBI representative if desired), the BCC PI, and BCC personnel involved in the trials being monitored. The Project Scientist(s) do not attend the closed session. For closed session conference calls, the BCC will provide participants with the dial-in information shortly before the call.
- Executive session – An executive session may be requested at any time by the committee. Only the full voting members, appropriate ad hoc members of the DSMC, and NICHD/other Institute DSMC representatives attend. BCC staff do not attend the executive session.

Meetings will always be scheduled to include the DSMC Chair and a biostatistician. A quorum of this DSMC is considered to be two-thirds of the full members (i.e. not including the NIH representatives). Agreement is reached by consensus.

### 5.4.1 Study Report to the DSMC

For the bi-annual meeting, and any other meeting called by the DSMC to review data, a report will be generated by the BCC in advance. Data are presented by treatment group and by center. For security purposes, the treatment groups will be coded in the report (e.g. A or B). The decision to unmask the treatment group at the meeting is at the discretion of the DSMC. A full report on a study that has accrued sufficient patients to evaluate outcome will include the following:

- Enrollment data
- Baseline data
- Compliance and side effects
- Adverse events
- Protocol adherence, including data quality, data completeness, and timeliness
- Primary outcome
- Secondary outcomes

- Additional study specific information as requested by the DSMC

However, the main emphasis is on the primary outcome as well as maternal safety outcomes (including maternal myositis, myopathy, rhabdomyolysis and serious liver injury).

### 5.4.2 Interim Analysis

Two special interim analyses will be performed to review the maternal and neonatal safety outcomes by treatment group. The first after the first 50 women randomized have delivered and the second after the first 310 women randomized (20%) have delivered. After each of these special interim analyses, the DSMC will make the recommendation to continue the 20mg dose or lower the dose to 10mg.

Given that the sample size estimates involve a number of assumptions, it is planned that the assumptions and the resulting primary outcome rate in the placebo group be examined after the first 310 women randomized have delivered. The DSMC would be charged with making a recommendation regarding potential revision of the sample size.

Once the first 50% of the patients have been accrued into the trial and delivered, the report also will include a formal interim analysis evaluating the primary outcome by treatment group on this cohort of patients. A second interim analysis will be performed after 75% of the patients have been accrued and delivered. Each cohort consists of all patients randomized before a certain date so that the analysis cohort does not depend on gestational age at delivery. Additional interim analyses of the primary outcome will be at the discretion of the DSMC.

### 5.4.3 Trial Stopping Rules

For the primary outcome, the group sequential method of Lan and DeMets will be used to characterize the rate at which the type I error is spent.[112] This method is flexible with regard to the timing of the interim analyses. Asymmetric stopping boundaries will be used. The upper boundary which describes the stopping rule for benefit will be based on 1-sided type I error of .025 and the Lan-DeMets generalization of the O'Brien-Fleming boundary. The lower boundary will be based on a less stringent stopping rule: 1-sided type I error of .05 and the Lan-DeMets generalization of the Pocock type boundary.

It also planned to calculate conditional power given the observed data to date, and conditional on the future data showing the originally assumed design effect. If this conditional power is low (under 10 percent) the DSMC may consider termination for futility if the accrual rate is slow, with confidence that the Type II error is not greatly inflated.[113]

Specific stopping rules for the trial will be established for monitoring maternal, neonatal and child events relevant to pravastatin risk. For the maternal stopping rule, an occurrence of one of the following events will qualify:

- Serious Adverse Event of Special Interest (serious AESI; myositis, myopathy, rhabdomyolysis or serious liver injury as defined in Section 4.5).
- Non-serious AESI (myalgia or muscle weakness as defined in Section 4.5) that required repeat discontinuation of study medication (initial discontinuation, rechallenge, discontinuation again)

For the neonatal stopping rule the following event will qualify:

- Failed newborn ABR/OAE test

The BCC will continuously monitor these maternal and neonatal events and if there is an excess of women in the pravastatin group who have experienced one or more of the events such that the one sided nominal p-value of a Fisher's exact test is less than 0.05 or such that the lower 90% confidence bound of the relative risk exceeds 1.0 for larger sample sizes of 50 or more, will request that the DSMC review the

data immediately and make a determination on stopping or pausing the trial, or modifying the dose. Likewise if there is an excess of neonates who have failed their hearing tests, the same stopping criteria will apply.

For the child stopping rule, the following events will qualify:

- Cognitive or Motor Scale Score < 70 (2.5$^{th}$ percentile) from the Bayley Certified Scales of Infant Development 4 at 24 months age
- Total problems or syndrome scale score > 97$^{th}$ percentile (clinical range) from the Child Behavior Checklist at 24 months
- Hearing loss at 24 months of age

These childhood events will be monitored by the DSMC at their regular meetings or more frequently upon request once any children reach two years of age. The 1-sided Lan-DeMets generalization of the Pocock type with type I error of .05 will be used as a stopping rule.

It is recognized that any decision to terminate the study would not be reached solely on statistical grounds but on a number of complex clinical and statistical considerations.

## *5.5 Reporting from the DSMC*

At the conclusion of each call or meeting, an open session will be held to report the recommendations of the DSMC to the NICHD MFMU Network Project Scientist (and the NHLBI Project Scientist if desired). If the recommendations are complex and/or if requested by the Project Scientist, the BCC will collaborate with the DSMC Chair and the NICHD representative to write a brief summary of the discussion and recommendations for presentation to the NICHD Director.

In addition the BCC will prepare

1) Within 2 weeks, a brief summary of trial status and DSMC recommendations for NIH and the clinical centers including whether the trial should proceed or not, and whether modifications are required.

2) Within 4 weeks, detailed formal minutes of the DSMC's discussion and recommendations to the NICHD within 30 days of the meeting. The minutes are initially reviewed by the DSMC Chair, and then distributed to the members for final review and approval.

3) Within 4 weeks, a summary report for the IRB which will include a brief accounting of all adverse event reports since the previous meeting and cumulatively. If there are no safety or other protocol-related concerns, the report will state that the DSMC recommended that the study continue without modification. If concerns are identified, the report will outline the concerns and recommendations, as well as any decisions of the NICHD Director.

# 6 Statistical Considerations

## 6.1 Data Relevant to the Primary Outcome

To determine the recurrence rate of preeclampsia based on the gestational age at delivery of the previous preeclampsia, several sources of information were considered.

First, the NICHD MFMU Network conducted a randomized controlled trial to evaluate the efficacy of low dose aspirin compared with placebo in preventing preeclampsia in high-risk women. The trial, which was negative, included four independently powered arms, with one being in women who had prior preeclampsia, without other risk factors.[42] One hundred sixty nine women who had a prior pregnancy complicated by preeclampsia that required preterm delivery were assigned to low dose aspirin, and had a rate of preeclampsia recurrence of 16% (27/169 women, 95% confidence interval 10%-22%).

The second source was a large cohort study in the US that included 6,157 women who had preeclampsia at the time of their first birth. In this study, the rates of recurrence of preeclampsia at their second delivery were 38.6%, 29.1% and 21.9% if the delivery for preeclampsia in the prior pregnancy occurred at 20-28 weeks, 29-32 weeks and 33-36 weeks, respectively.[114] Assuming the same distribution of gestational ages at the index pregnancy as in the MFMU Network High Risk Aspirin trial the rate of recurrent preeclampsia before 34 weeks would be close to 31%.

An individual patient meta-analysis showed that the rate of recurrence of preeclampsia was 16.0% for women with prior preeclampsia (at any gestational age).[115]

In the VIP trial, women with prior history of preeclampsia with delivery < 37 weeks had a 23% recurrence rate of preeclampsia.[116] In another study 25% of primiparous women who experienced preeclampsia and were delivered before 34 weeks, were diagnosed with preeclampsia in their next pregnancy.[117]

In a prospective cohort study using data from the Swedish medical birth register (763,795 women), Hernandez-Diaz and colleagues reported that for patients who had severe preeclampsia in their first pregnancy that required delivery before 34 weeks' gestation, the rate of preeclampsia recurrence was 29% in the subsequent pregnancy.[118] Moreover, in the NICHD-OPRC pilot trials, which included women with history of preeclampsia that required delivery < 34 weeks, the rate of recurrence of preeclampsia in the placebo groups was 45%.[94]

## 6.2 Sample Size and Power

In all of the older studies, except the High Risk Aspirin study, aspirin was not being used routinely. In the High Risk Aspirin trial, the dose was lower than is generally used today and was started later in gestation. Therefore, for the purposes of sample size estimation, it is conservatively assumed that all women will be receiving low dose aspirin, and that the rate of preeclampsia (primary outcome) in the placebo group is 20%. It is estimated that a sample size of 1,550 women is required to demonstrate at least a 30% reduction in preeclampsia with the use of pravastatin (from 20% to 14%), with a power of 85%, and type I error of 5% 2-sided. The sample size is adjusted for a drop-out rate of 5% (i.e. that 5% of the women on pravastatin discontinued their study drug and had the same primary outcome rate as the placebo group) and for the interim 'looks' at 50% and 75% information.

**Table 5. Sample Sizes for Different, Levels of Power and Effect Sizes**

| Reduction in Primary Outcome Rate | Power % | Estimated Sample Size |
|---|---|---|
| **25%** | 80 | 2000 |
| | 85 | 2280 |
| | 90 | 2660 |
| **30%** | 80 | 1360 |
| | 85 | 1550 |
| | 90 | 1810 |
| **35%** | 80 | 980 |
| | 85 | 1120 |
| | 90 | 1290 |

Trials evaluating cardiovascular disease event rates have reported that the combination of pravastatin and aspirin consistently has greater efficacy than pravastatin or aspirin alone indicating at least an additive and possibly a synergistic effect.[119] Therefore, there is minimal concern that concomitant aspirin use would reduce the effect size.

Preterm birth < 37 weeks of gestation is considered to be the major secondary outcome. With a sample size of 1550, there is more than 80% power to detect an effect as small a 20% reduction in preterm birth.

The sample size for this study is also sufficient to detect with at least 80% power a 40% reduction in preterm delivery < 34 weeks and a 30% reduction in NICU/special care admission.

## *6.3 Analysis Plan*

All statistical analyses will be based upon the total cohort of patients randomized into the trial. Although data on some patients may be missing, all relevant data available from each patient will be employed in the analyses. Patients will be included in the treatment group to which they were randomly assigned regardless of compliance.

The primary analysis will consist of a simple comparison of binomial proportions. The relative risks and confidence intervals will be reported. If the treatment groups are found to differ on a pre-treatment factor known to be a risk factor for the outcome, the statistical analysis will adjust for these differences, using a log binomial regression (or log-Poisson when relevant). An evaluation of treatment by center interaction will be included, using the Breslow-Day test. An analysis adjusting by center also will be performed to ensure that center differences do not change the conclusion.

If the primary outcome is significant or the trial is stopped early for benefit, the methods of Maurer and Bretz will be used to test formally the major secondary endpoint of preterm delivery < 37 weeks, while controlling the familywise error rate.[120]

Loss to follow-up will be defined as the inability to ascertain whether a woman has preeclampsia. Those defined as lost to follow-up will not be included in the primary analysis. It is expected that the loss to follow-up rate will be very low. However, a sensitivity analysis will be performed including patients lost to follow-up with different assumptions regarding their outcome, to determine whether the results are

robust. Multiple imputation techniques (or a Bayesian bootstrap technique if the 'missingness' is non-ignorable) will be evaluated if required.[121]

Since many of the secondary endpoints are dichotomous variables like the primary outcome, standard statistical methods for rates and proportions will be appropriate. For normally distributed continuous outcomes, least squares means general linear regression will be used to estimate means and 95% confidence intervals. The Wilcoxon test and the Hodges-Lehmann estimators will be used to compare non-normal continuous variables that cannot be transformed to approximate normality. For time-to-event variables, the log-rank test and Cox proportional hazards model will be used (model assumptions tested by Lin's test).

### 6.3.1 Subgroup Analyses

If the two groups show a difference in the incidence of the primary outcome or major secondary outcome, interactions will be evaluated and subgroup analyses conducted to determine whether the effect prevails throughout particular subgroups of patients. Indeed, NIH guidelines require investigators to evaluate consistency between the genders and across racial subgroups. It should be noted, however, that subgroup analyses have been greatly abused, particularly when there is no overall treatment difference.[122] There is a strong temptation to search for a specific subpopulation in which the therapy is nevertheless effective. Yusuf et al. concluded "*the overall 'average' result of a randomized clinical trial is usually a more reliable estimate of the treatment effect in the various subgroups examined than are the observed effects in individual subgroups*."[123] Thus subgroup analyses will be interpreted with care.

It is generally acknowledged that subgroup analysis that is pre-specified in the protocol has more validity than ad-hoc comparisons. The following factors will be considered for subgroup analysis, if there is a significant interaction between the factor of interest and the treatment effect:

- Race/ethnicity
- Baseline BMI category
- Gestational age at randomization
- Actual or prescribed aspirin use at baseline
- Pregestational diabetes
- Chronic hypertension at baseline
- Singleton pregnancy

For subgroup analyses, there will be more limited power. However, if an interaction test were positive for the primary outcome, indicating the presence of heterogeneity between subgroups, and 1) the subgroups were each 50% of the sample size and 2) the placebo outcome rate in the subgroup were the same as assumed overall (20%), then there would be more than 85% power to detect a 40% difference between treatment groups within one of the subgroups.

The racial/ethnic composition of women recruited into the MFMU Network trials varies. Assuming for this trial that the composition is 31% African-American and 24% Hispanic (estimates based on site specific rates reported among preeclamptics in the RCT of Antioxidants to Prevent Preeclampsia CAPPS trial) there is more than 85% power to detect a 50% reduction in the primary outcome in African American women and 77% power to detect a 50% reduction in the primary outcome in Hispanic women within these separate subgroups.

# 7 Data Collection

## *7.1 Data Collection Forms*

Data will be collected on standardized forms on which nearly all responses have been pre-coded. Each form is briefly described below:

- Screening Log
- Eligibility and Randomization Form is completed for all patients eligible for the study.
- Study Drug Randomization Log: lists patients randomized to pravastatin or placebo, and provides the drug code number.
- Baseline Form is completed for all randomized patients. This form includes detailed demographic and social data, medical and obstetrical history, and current pregnancy complications.
- Previous Pregnancy Outcome Form
- Study Visit Form documents monthly study visits, possible side effects and compliance
- Creatine Kinase Log documents CK values reported for the participant.
- Study Drug Dispensing and Compliance Log documents the dispensing of study medication and the return of any unused study medication.
- Study Drug Alteration Log for Side Effects documents the modification of study medication due to side effects.
- Unscheduled Visit or Hospitalization Form is completed for all patients who had an unscheduled emergency room, Labor & Delivery, clinic visit or hospitalization between the scheduled monthly visits, including the delivery admission.
- Maternal Delivery and Outcome Form documents specific pregnancy complications since randomization, in addition to labor, delivery and postpartum information.
- Neonatal Baseline Form records date and time of birth, delivery data and status at delivery, for each fetus/infant.
- Neonatal Outcome Form records outcome data for all infants admitted to the NICU or special care nursery.
- Patient Status Form documents loss to follow up/withdrawal status, last date of contact for lost to follow-up patients, side effects since the last dose.
- Follow-up Visit Form documents maternal complications since discharge and the 6-week postpartum study visit.
- Postpartum Readmissions Log is completed for all patients who are readmitted to the hospital after discharge following delivery.
- Adverse Event Form records serious and non-serious adverse events.
- Outcome Diagnosis Form records outcome data for hypertension and preeclampsia
- Two Year Study Visit Form for infants and proprietary forms for neurodevelopmental and behavior testing

- Five Year Study Visit Form for infants and proprietary forms for neurodevelopmental and behavior testing

## *7.2 Web Data Entry System*

For this protocol, web data entry screens corresponding to the study forms listed above will be developed and maintained by the staff of the BCC. Clinical center staff will enter data into the MySQL database located at the BCC through a web data management system (MIDAS). The data are edited on-line for missing, out of range and inconsistent values. A Users' Manual documenting this system is provided to the centers by the BCC.

## *7.3 Centralized Data Management System*

Daily data conversions from the MySQL database create up-to-date SAS datasets. Data are reviewed weekly using edit routines similar to those implemented on-line during data entry, as well as additional checks for data consistency within or across forms. A database of resulting potential data problems is generated in MIDAS for initial review by BCC staff who then evaluate the comments keyed in association with edits on missing or unusual values. Valid edits will be flagged in MIDAS for resolution at the clinical centers.

At regular intervals, specialized data reviews comparing data availability and consistency across forms are run by the BCC staff on the entire database or on a specific subset of data. These reports are also submitted to the centers for correction or clarification.

An audit trail, consisting of all prior versions of each data form as entered in the computer for each patient, is maintained so that the succession of corrections can be monitored.

## *7.4 Performance Monitoring*

The BCC will present regular reports to the Protocol Subcommittee, the Steering Committee, and the Data and Safety Monitoring Committee. These include:

- Monthly Recruitment Reports - reports of the number of women screened and enrolled by month and by clinical center are provided monthly to the subcommittee and all other members of the Steering Committee. Weekly or bi-weekly reports are provided electronically as decided by the Protocol Subcommittee.
- Quarterly Steering Committee Reports - reports detailing recruitment, baseline patient characteristics, data quality, incidence of missing data and adherence to study protocol by clinical center, are provided quarterly to the subcommittee and all other members of the Steering Committee.

# 8 Study Administration

## *8.1 Organization and Funding*

The study is funded by the National Heart Lung and Blood Institute (NHLBI) and by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD). The study is conducted by the NICHD Maternal-Fetal Medicine Units (MFMU) Network, consisting of twelve clinical centers, the Biostatistical Coordinating Center (BCC) and the NICHD.

### 8.1.1 MFMU Clinical Centers

Each of the funded institutions is represented by a Principal Investigator. A complete description of the organization of the MFMU Network is provided in the MFMU Network Policy Manual. The participating Principal Investigators of the clinical centers have agreed to abide by the study protocol, to have comparable staff, facilities and equipment and to ensure the proper conduct of the study at each of their centers including: recruitment and treatment of patients as specified in the protocol, accurate data collection and the transmission of information to the Steering Committee.

### 8.1.2 Biostatistical Coordinating Center (BCC)

The BCC is responsible for all aspects of biostatistical design, data management, interim and final statistical analyses, and preparation of publications based on the study results. The Principal Investigator of the BCC reports to the Steering Committee and the Data and Safety Monitoring Committee.

### 8.1.3 Clinical Coordinating Center (CCC)

For this study, the University of Texas Medical Branch and Columbia University together will serve the role of Clinical Coordinating Center.

### 8.1.4 NHLBI/NICHD

In addition to their role as funding agency, the NICHD and NHLBI participate in the activities of the Network, including the development of the protocol, administration and conduct of the study and preparation of publications.

### 8.1.5 Network External Advisory Committee

Appointed by the NICHD, the members of the Network External Advisory Committee (EAC) consist of a group of experts who are not affiliated with research being conducted by the Network and represent the disciplines of maternal-fetal medicine, neonatology and biostatistics/epidemiology. The role of the EAC includes the review and prioritization of proposed studies, in addition to the identification of scientifically and clinically important questions and ideas that might be conducted by the Network. The NICHD Program Scientist convenes and attends the meetings.

## *8.2 Committees*

### 8.2.1 Steering Committee

This committee consists of fifteen members. The Principal Investigator from each of the twelve clinical centers, the BCC, and the NICHD MFMU Network Project Scientist are all voting members. The Chair of the Steering Committee may vote to break a tie. The Chair, a person independent of the participating institutions, is appointed by NICHD. The Steering Committee has the responsibility for identifying topics for Network studies, designing and conducting study protocols and monitoring study implementation, recruitment and protocol adherence. The committee receives recommendations from the Data and Safety Monitoring Committee and the Network External Advisory Committee.

### 8.2.2 Protocol Subcommittee

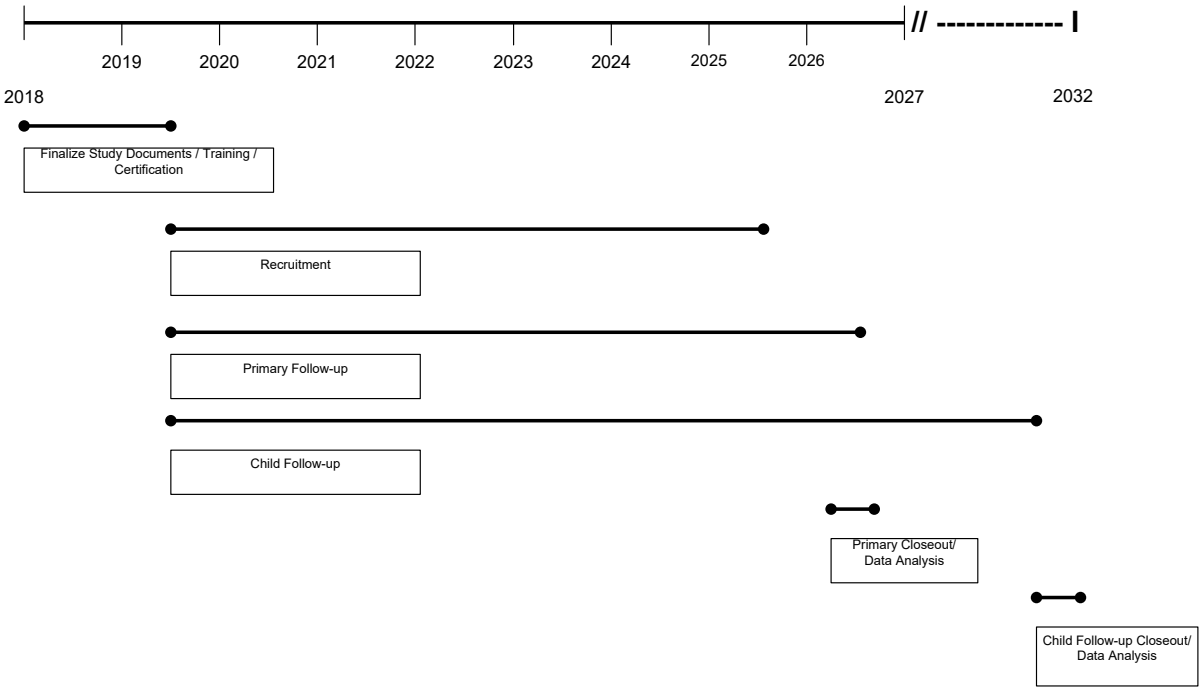
The subcommittee consists of the multiple PIs named in the Clinical Coordinating Center and Data Coordinating Center NHLBI grants, investigators from one or more other clinical centers, , nurse coordinators, outside consultants (if appropriate), the NICHD Network Program Scientist and the NHLBI Project Scientist. The Protocol Subcommittee is responsible for the preparation and conduct of the study, and reporting the progress of the study to the Steering Committee.

### 8.2.3 Publications Committee

The Publications Committee is a standing committee of the Steering Committee. The functions of this committee are to develop publication policies and to review all manuscripts and abstracts prior to submission. The goals of this committee are fair and appropriate authorship credit and high quality publications.

# 9 Study Timetable

**Figure 1. Timetable**




## *9.1 Training and Certification*

Training will be held with the nurse coordinators in July 2018. Each participating center must be certified to start the trial before recruitment at that center can begin. The certification requirements are designed to ensure that personnel involved in the trial are committed to the study and proficient in study procedures, and that the center has satisfied regulatory requirements. The trial is expected to start in June 2019.

## *9.2 Recruitment and Data Collection Period*

### 9.2.1 Estimate of the Prevalence of Previous Preeclampsia

Using the Swedish Medical Birth Registry, Hernandez-Diaz identified a cohort of 763,795 nulliparous women. At the first pregnancy the cohort was subdivided into those who had preeclampsia and those who did not, and women within each subcohort followed forward to their next pregnancy if they had one. The process was reported until the fourth pregnancy.[118] This can be used to calculate the probability of previous preeclampsia in a general population at the second, third and fourth pregnancies as shown, and naturally takes into account that women who have had a bad experience in one pregnancy may avoid another one. For the grouped category of 5 or more pregnancies it was assumed that 5.5% would have had previous preeclampsia in a previous pregnancy. From the MFMU Network APEX study of a random sample of all deliveries in the MFMU Network over a 3-year period, it is possible to estimate the

prevalence of previous preeclampsia in the MFMU Network population by multiplying the probability of previous preeclampsia at each pregnancy number (1-4, 5+).

Overall it is estimated that 2.58% of the MFMU population would have prior preeclampsia (Table 6). This is likely an underestimate since the MFMU Network incorporates tertiary care centers and the rate of preeclampsia is higher in the US than in Sweden.

**Table 6. Probability of Previous Preeclampsia in the MFMU Network Population**

| Gravida | Estimated probability of previous preeclampsia from Hernandez-Diaz et al. | Percent of women in MFMU population | Percent of MFMU population with previous preeclampsia |
|---|---|---|---|
| 1 | 0 | 40.5 | 0 |
| 2 | 0.0387 | 31.4 | 1.22 |
| 3 | 0.0455 | 16.4 | 0.75 |
| 4 | 0.0517 | 6.9 | 0.35 |
| 5 or more | 0.0550 | 4.8 | 0.26 |
| Overall | | 100.0 | 2.58 |

To determine the percentage of women with previous preeclampsia that would have delivered before 34 weeks the High Risk Aspirin trial was examined. In the High Risk Aspirin trial 28.2% of those who enrolled had prior preeclampsia before 36 weeks which yields a prevalence of 0.73% (2.58% x 28.2%). This may be an overestimate of the prevalence in the population since women who had a worse experience previously may have been more likely to enroll.

Hernandez-Diaz et al. also examined severe preeclampsia, defined as preeclampsia associated with delivery < 34 weeks. Using the same calculations for this outcome as for the overall prevalence, the prevalence of previous preeclampsia <34 weeks is 0.25% . This is probably too low, since the MFMU Network incorporates tertiary care centers and the rate of preeclampsia is higher in the US than in Sweden. Also the cohort represented women who had their first deliveries between 1987-2004 and the rate of preeclampsia has risen over time.

The average of these two rates (0.5%) is used as the estimate of the prevalence of previous preeclampsia with delivery before 34 weeks.

### 9.2.2 Estimate of Recruitment Rate

There are more than 160,000 deliveries per year in the MFMU network which would result in 800 potentially eligible women (160,000 x 0.5%). Assuming that 80% of the women delivering at MFMU network centers would be available for screening (i.e. that they present for care early enough in gestation, at clinics that are accessible and do not meet exclusion criteria), and that 50% of these women consent (a conservative consent rate for studies involving high risk patients in the Network) it is estimated that approximately 320 patients per year or 26-27 patients will be enrolled per month. The first 50 women will be enrolled over 6 months. Recruitment of the remaining 1500 women will take approximately 4.8 years.

## *9.3 Final Analysis*

After a three-month period for completion of data entry for the trial and close-out of the delivery and primary outcome, the data set will be locked and available for the primary and other main analysis. After completion of the 5 year infant follow-up, a two-month period will be dedicated to complete data entry, close-out this follow-up, and lock the follow-up dataset.

# Appendix A Design Summary

OBJECTIVE: To determine whether treatment with pravastatin among women with a prior history of preeclampsia with preterm delivery by 34 weeks reduce the rate of preeclampsia.

## ORGANIZATION

- Clinical Centers: Magee, UAB, Ohio State, Utah, Brown, Columbia, Case Western, UT-Houston, UNC, Northwestern, UTMB-Galveston, U Penn
- Subcommittee: Maged Costantine, MD (Chair)

## DESIGN

- Major Eligibility Criteria:
  - Prior preeclampsia with delivery ≤ $34^0$
  - Gestational age $12^0$ to $16^6$ wks at randomization
  - Contraindications for statin therapy, use in current pregnancy
- Groups:
  - Pravastatin 20mg
  - Matching placebo
- Level of Masking:
  - Double masked
- Stratification:
  - Clinical site
- Sample Size:
  - 1550
- Assumptions:
  - Outcome = Incidence of preeclampsia
  - Placebo group event rate = 20%
  - Pravastatin group event rate = 14% (30% reduction)
  - Type 1 error = 5% 2-sided
  - Power =85%
  - Adjustment for 5% loss to follow-up and scheduled interim analysis
- Interim Analysis:
  - Lan-DeMets group sequential method

## TIMETABLE

- Enrollment:
  - Jun 2019 – Jul 2025
- Data collection:
  - Jun 2019 – Mar 2026 (primary)
- Primary analysis:
  - Apr 2026 – Jun 2026 (primary)
- Child data collection:
  - Jun 2019 – Mar 2028 (child 2 yr follow-up)
  - Jun 2019 – Mar 2031 (child 5 yr follow-up)
- Closeout/final analysis:
  - Apr 2031 – Jun 2031

## SCHEDULED EVALUATIONS / DATA COLLECTION

- Pre-Randomization:
  - History
  - Dating ultrasound and AST/ALT if not done
- Randomization:
  - BP, height, weight
  - Blood collection
- Post-randomization (monthly):
  - Blood collection at $23^0$ to $28^6$ and $33^0$ to $37^6$
  - Study visits monthly to assess side effects and AEs, record weight and BP, pill count and dispense study medication, review current medications
- Delivery:
  - Delivery and neonatal data
  - Cord blood (first 50 participants)
- Postpartum (6 wks):
  - Assess AEs and postpartum complications, record weight and BP
- Maternal Follow-up at 2 yrs:
  - Medical history, BP, height, weight, waist and hip circumferences, blood collection
- Child Follow-up at 2 yrs:
  - Medical history, height, weight, vision & hearing assessment, neurodevelopment & behavior testing
- Child Follow-up at 5 yrs:
  - Medical history, height, weight, vision, neurodevelopment & behavior testing

## MANAGEMENT PROTOCOL

- Masked medication:
  - Daily dose of pravastatin 20mg or placebo until delivery

## OUTCOME MEASURES

- Primary:
  - Preeclampsia, mat death, or fetal loss < 20 wks
- Secondary:
  - Preterm birth < 37 weeks (major secondary)
  - Preterm birth < 34 weeks, gest age at delivery
  - Preeclampsia with severe features
  - Gestational hypertension
  - Gestational diabetes
  - Concentrations of angiogenic factors and lipids
  - Fetal and neonatal death
  - Birth weight and small for gestational age
  - Complications of prematurity
  - Child BMI for age at 2 and 5 years
  - Cognitive, Language and Motor Scale Scores from the Bayley 4 at 2 years
  - General Conceptual Ability score and subscale scores from the Differential Ability Scales at 5 years of age

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