Study Protocol and Analysis Plan

Clinicaltrials.gov: Low Doses of Aspirin in the Prevention of Preeclampsia - NCT04070573

Official Title: A Randomized Controlled Trial Comparing Low Doses Of Aspirin In The Prevention Of Preeclampsia (ASAPP)

Primary Investigator: Line Malha, MD, MS
Weill Cornell Medicine

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Confidentiality Statement

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from WCMC.

List of Abbreviations

All abbreviations used throughout the protocol must be defined.

AE	Adverse Event
AUC	Area Under the Curve
cfRNA	Cell free RNA
BMI	Body Mass Index
CFR	Code of Federal Regulations
Cr	Creatinine
CrCl	Creatinine Clearance
CRF	Case Report Form
DBP	Diastolic Blood Pressure
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
FDA	Food and Drug Administration
FE	Fractional excretion
GA	Gestational Age
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
HIPAA	Health Insurance Portability and Accountability Act of 1996
HRBFA	Human Research Billing Analysis Form
ICF	Informed Consent Form

IND	Investigational New Drug
IRB	Institutional Review Board
NIH	National Institutes of Health
NMR	Nuclear Magnetic Resonance
PE	Preeclampsia
PHI	Protected Health Information
PRA	Plasma Renin Activity
PTH	Parathyroid Hormone
PI	Principal Investigator
REDCap	Research Electronic Data Capture
ROC	Receiver Operating Characteristic
SAE	Serious Adverse Event
SAS	Statistical Analysis Software
SBP	Systolic Blood Pressure
SPE	Superimposed preeclampsia
SUSAR	Suspected Unexpected Serious Adverse Reaction
UAP	Unanticipated Problem
WCMC	Weill Cornell Medical College

Protocol Summary

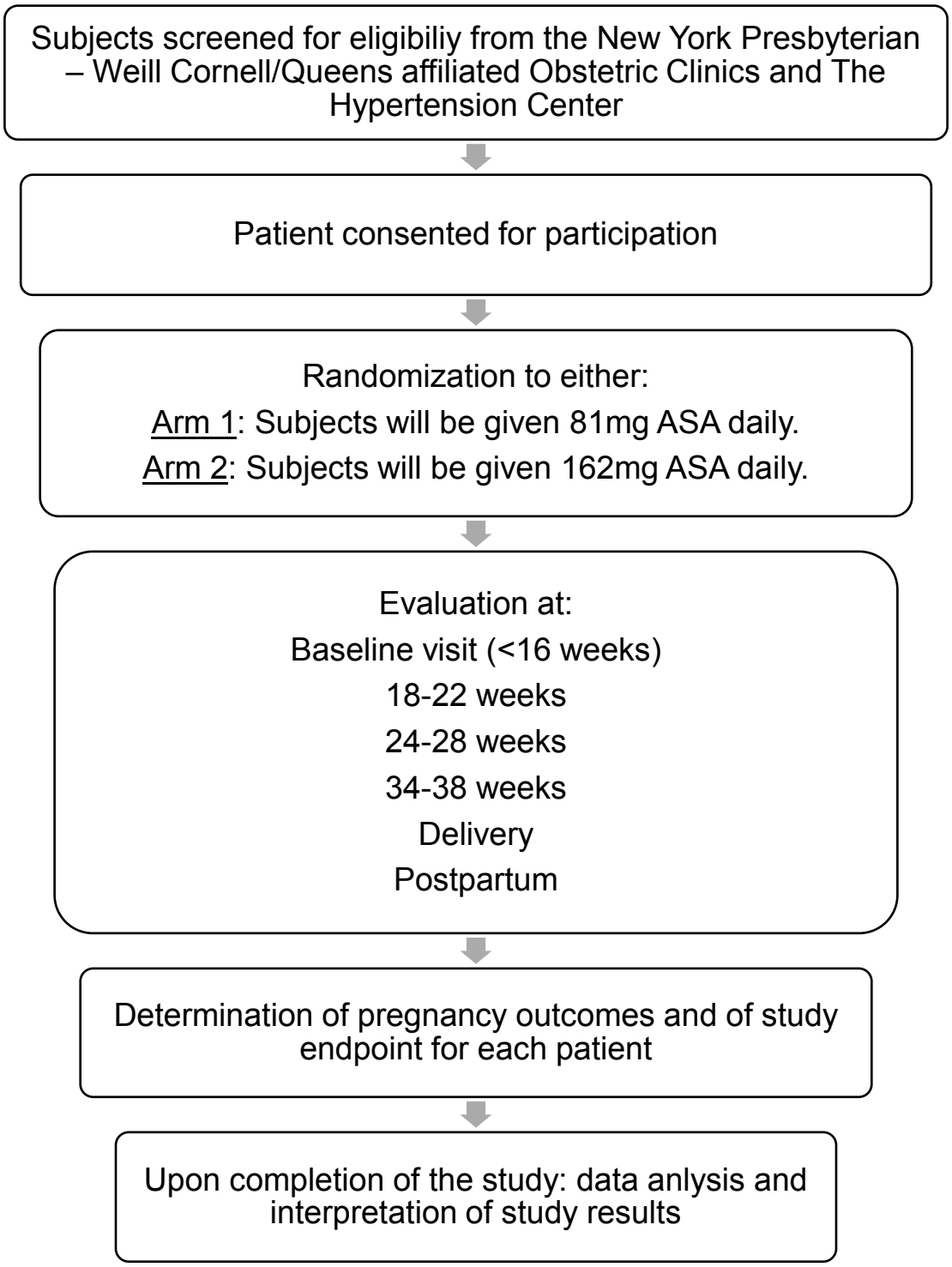
Full Title:	*A Randomized Controlled Trial Comparing Low Doses Of Aspirin In The Prevention Of Preeclampsia (ASAPP)*
Short Title:	*ASAPP*
Clinical Phase:	*III*
Principal Investigator:	*Line Malha, MD*
Sample Size:	*N= 400 which may be modified after the first stage of our study*
Accrual Ceiling:	*400*
Study Population:	*Pregnant women < 16 weeks of gestation at high risk of PE (preeclampsia in a prior pregnancy, multifetal gestation, chronic hypertension, Type 1 or 2 diabetes, renal disease, autoimmune disease).*
Accrual Period:	*5 years*
Study Design:	*Open-label, randomized clinical trial. First stage: pilot to determine feasibility and sample size and second stage: completing the clinical trial*
Study Duration:	*9 months for each patient (from recruitment until 6 weeks postpartum)*
Intervention Description:	*Randomized to 81mg vs. 162mg of aspirin per day*
Primary Objectives:	*To compare the incidence of preterm PE and PE with severe features in women treated with 81mg to those treated with 162mg of aspirin per day.*
Secondary Objectives:	• *Evaluate and compare the adherence of pregnant women to 81 mg and 162 mg of daily low-dose aspirin using a validated, Simplified Medication Adherence Questionnaire (SMAQ).* • *Compare maternal and fetal outcomes in pregnant women at a high risk for preeclampsia who are treated with either 81 mg or 162 mg of daily aspirin during pregnancy, including preeclampsia ≥ 37 weeks, severe maternal hypertension, preterm delivery, fetal growth restriction, placental abruption and maternal/fetal mortality.* • *To compare the time-to-event for developing PE for women treated with 81mg vs. 162mg of aspirin per day.* • *To assess the adherence to low dose aspirin in pregnant women that are a high risk for preeclampsia and compare adherence rates for women on 81mg vs 162mg of aspirin per day.*
Exploratory Objectives:	*To assess the impact of specific co-morbidities (diabetes, chronic hypertension, renal disease and autoimmune disease), blood pressure*

control, age and race on the relationship between treatment group and PE incidence.

To compare cfRNA profiles of the 2 treatment groups (81mg vs. 162mg aspirin per day) and of pre-treatment samples in order to better understand how ASA initiation and dosing affect the transcriptome of pregnancy in women who develop PE and in those who do not

Endpoints: *Incidence of PE, gestational age at onset of PE*

SCHEMA


Subjects screened for eligibiliy from the New York Presbyterian – Weill Cornell/Queens affiliated Obstetric Clinics and The Hypertension Center
Patient consented for participation
Randomization to either:
Arm 1: Subjects will be given 81mg ASA daily.
Arm 2: Subjects will be given 162mg ASA daily.
Evaluation at:
Baseline visit (<16 weeks)
18-22 weeks
24-28 weeks
34-38 weeks
Delivery
Postpartum
Determination of pregnancy outcomes and of study endpoint for each patient
Upon completion of the study: data anlysis and interpretation of study results

1. STUDY OBJECTIVES

1.1. Primary Objective

Compare the incidence of preterm (<37 weeks) preeclampsia and preeclampsia with severe features (American College of Obstetricians and Gynecologists [ACOG] 2019 definition (1)) in high risk pregnant women treated with either 81 mg or 162 mg of daily aspirin during pregnancy

1.2. Secondary Objectives

- Evaluate and compare the adherence of pregnant women to 81 mg and 162 mg of daily low-dose aspirin using a validated, Simplified Medication Adherence Questionnaire (SMAQ).
- Compare maternal and fetal outcomes in pregnant women at a high risk for preeclampsia who are treated with either 81 mg or 162 mg of daily aspirin during pregnancy, including preeclampsia ≥ 37 weeks, severe maternal hypertension, preterm delivery, fetal growth restriction, placental abruption and maternal/fetal mortality.
- To compare the time-to-event for developing PE for women treated with 81mg vs. 162mg of aspirin per day.
- To assess the adherence to low dose aspirin in pregnant women that are a high risk for preeclampsia and compare adherence rates for women on 81mg vs 162mg of aspirin per day.

Exploratory Objective:

- To assess the impact of specific co-morbidities (diabetes, chronic hypertension, renal disease and autoimmune disease), blood pressure control, age and race on the relationship between treatment group and PE incidence.
- To compare cfRNA profiles of the 2 treatment groups (81mg vs. 162mg aspirin per day) and of pre-treatment samples in order to better understand how ASA initiation and dosing affect the transcriptome of pregnancy in women who develop PE and in those who do not

2. BACKGROUND

2.1. Preeclampsia

2.1.1 Preeclampsia, risk reduction with low-dose aspirin

Preeclampsia (PE) is a serious and potentially fatal complication of pregnancy. It is a placental disease characterized by an elevated blood pressure in the 3rd trimester with multisystem involvement (proteinuria, elevated liver enzymes, low platelet count and/or neurologic symptoms). PE can cause pulmonary edema, seizures, or stroke and is a leading cause of maternal mortality. The pregnancy outcomes are further worsened if PE develops before term (2, 3). Women who have a history of PE in a

prior pregnancy, diabetes, preexisting hypertension, kidney disease, multifetal gestation or autoimmune diseases are at an increased risk to develop PE in a subsequent pregnancy (4).

Clinical trials evaluating the benefits of low-dose aspirin (ASA) have used a wide range of doses from 60mg to 150mg orally daily with low-dose being defined as less than 325mg per day. Taking ASA (as opposed to placebo) is thought to reduce the risk of preeclampsia by 17%, without increasing the risk of major obstetric bleeding. The number needed to treat is only 19 women (1). ASA is currently the only prophylactic therapy for PE in high-risk women to be recognized by the US Preventive Task Force and should be initiated early in the second trimester of pregnancy, before 16 weeks of gestation (4).

There has also been more awareness that the efficacy of ASA in preventing preeclampsia is limited by the poor adherence of patients to this therapy. Indeed, a cross-sectional study has estimated that up to 46% of women (n=42) on ASA therapy may not be compliant to it, as determined by a validated Simplified Medication Adherence Questionnaire (SMAQ) (5). Adherence is essential to the efficacy of ASA in preventing preterm preeclampsia (6). It would therefore be of interest to obtain more information about adherence to ASA in women who need this therapy.

2.1.1 Molecular basis of aspirin in preeclampsia risk reduction

The molecular basis for the protective effect of ASA in pregnancy is still a matter of debate (7). Cell free RNAs (cfRNAs) have recently been identified as promising biomarkers for preterm birth in pregnant women. Indeed, a panel of 9 placental cfRNAs (*CGA, CAPN6, CGB, ALPP, CSHL1, PLAC4, PSG7, PAPPA* and *LGSALS14*) were found to accurately predict gestational age at delivery in a cohort of 38 women (8). CfRNA profiling enables the detection of placenta-specific cfRNA in the maternal peripheral blood and can therefore give us a unique, non-invasive, insight into placental health (9). Presently, the molecular effect of ASA on cfRNA profiles in pregnancy has not been described. It is also unknown if ASA dosing or the development of PE affect cfRNA profiles.

In addition to testing our hypothesis that 162mg of ASA daily may be associated with lower incidence of PE than 81mg; our study would serve as a platform to collect and biobank samples (urine and blood). These samples would be invaluable in determining the molecular basis of PE development and the differential effects of ASA doses in PE prevention. Biobanked samples will be used (funding permitting) for cfRNA profiling but may also be used for proteomic, transcriptomic, metabolomic or cell-typing analysis if future promising hypotheses emerge.

2.1.3 What don't we know?

The current literature is lacking in evidence to recommend a specific daily dose of ASA. Recent meta-analyses (10, 11) have suggested that there may be a dose response in the protective effect of ASA for PE. As compared to 60mg per day, an ASA dose of 100mg per day was associated with a lower relative risk of PE (0.44 vs 0.57, p=0.36) (10). A large study of 1776 women has compared a slightly

higher dose of ASA (150mg per day) to placebo and found a decrease in preterm delivery (before 37 weeks) due to PE (OR 0.38, p=0.004) (12). Meta-analyses have shown that any dose of ASA above 60mg per day is protective and should be used to prevent PE in high risk pregnancies (3, 4).

To date, there has not been any studies comparing lower doses of ASA (such as 81mg, the traditional "baby aspirin" dose sold in the US) to higher "low-dose" ASA regimens (such as 162mg) in their ability to prevent preterm or severe PE in women who are at a high risk for this devastating disease.

2.2 Rationale

PE is a morbid and potentially lethal complication of pregnancy and is more common in women with specific risk factors. ASA is currently the only prophylactic therapy for PE in high-risk women to be recognized by the US Preventive Task Force and should be initiated early in the second trimester of pregnancy, before 16 weeks of gestation (4). However, currently there is no literature comparing various low-dose aspirin formulations in the risk reduction of PE. In the United States, the currently available low-dose aspirin is over the counter and is found in 81mg tablets. Therefore, when clinicians initiate therapy with low dose ASA, they may prescribe 1 or 2 tablets of 81mg ASA per day depending on personal preference cannot be assisted by evidence to guide their decision.

The proposed study would determine the incidence of preterm PE or preeclampsia with severe features in women taking either 81mg or 162mg in a randomized setting, from two centers (Weill Cornell/New York Presbyterian and New York Presbyterian Queens). We hypothesize that the information gained from this trial will permit a more accurate sample size calculation for a larger clinical trial powered to accept or reject our testing hypothesis.

If our hypothesis is rejected and 162mg of daily ASA is not associated with a lower incidence of severe or preterm PE in pregnancy compared to 81mg, this may be due to lack of power to detect a smaller effect. We would then evaluate the feasibility and results and determine whether a larger trial is reasonable.

cfRNAs may provide further insight into the pathophysiology of the protective effect of ASA on the development of PE in high risk women. Assessing molecular pathways in the development of PE may allow opportunity for earlier diagnosis, specific triaging of patients to closer monitoring and further development of preventative or curative treatment strategies.

2.3 Risk/Benefit Assessment

There will be no direct benefit to the participants of this study, given the equipoise about which low dose of aspirin would be associate with better outcomes. We hope the information learned from this study will benefit other patients at risk for developing preeclampsia in the future.
Both our study arms are doses that are currently included in the standard of care. We do not therefore anticipate any added risk to routine care by being enrolled in the study. The risks that have been associated with aspirin in routine clinical care include a slight increase in minor bleeding but no

increase in major bleeding (requiring intervention) (3, 4).

Patients may experience discomfort and bruising at the site of the needle stick. Lab work will be drawn at the initial screening visit and at 18-22 weeks, 24-28 weeks and 34-38 weeks gestation.

Another potential risk to participating in the study would include loss of confidentiality and will be avoided as described in following sections (i.e. section 11. Data reporting/regulatory considerations and, section 13. Data and safety monitoring plan).

3. SUBJECT SELECTION

3.1 Study Population

Our study population will include pregnant adult women, less than 16 weeks pregnant that are seen ata New York Presbyterian – Weill Cornell affiliated Obstetric or Hypertension Clinic and are at a high risk for preeclampsia (see section 3.2. for inclusion criteria) who thus meet criteria for low dose aspirin therapy. Subjects will be screened for eligibility from the New York Presbyterian – Weill Cornell and Queens campuses' affiliated Obstetric Clinics and The Hypertension Center by reviewing the list of patients scheduled to be seen at that clinic. Eligible patients will be approached by research staff if deemed acceptable by their clinical provider. Patients who consent to being in the study will be enrolled.

Clinical data in addition to urine and venous peripheral blood will be collected from pregnant women, ≥18 years old, with at least one of the following risk factors for developing PE.

3.2 Inclusion Criteria

Major Inclusion Criteria:
Pregnant patients, ≥18 years old, at less than 16 weeks' gestation (as documented by ultrasound) with at least one of the following risk factors for developing PE:

- PE in a prior pregnancy
- Chronic hypertension (prior to pregnancy or before 20 weeks' gestation)
- Type 1 or 2 diabetes
- Renal disease (proteinuria ≥300mg/day or estimated GFR<90mL/min/1.73 m2)
- Multifetal gestation
- Autoimmune disease (e.g. systemic lupus erythematous, antiphospholipid syndrome)

3.3 Exclusion Criteria

Major Exclusion Criteria:

- Patient with known intention to terminate pregnancy

- Major fetal malformation seen on ultrasound
- Contraindication to ASA therapy (including but not limited to allergy and high bleeding risk)

4. REGISTRATION PROCEDURES

4.1 Patient Registration

Screening will take place at the Women's Health Practice, located at 505 East 70th Street, 5th Floor, New York, NY 10021, New York Presbyterian Queens (56-45 Main St, Flushing, NY 11355) and its affiliated high risk clinics, Weill Cornell Maternal Fetal Medicine, located at 525 East 68th Street, Suite J-130, New York, NY 10065 and The Hypertension Center, located at 424 E 70th St, New York, NY 10021. Patient will be registered with Weill Research Gateway as per IRB/JCTO protocol.

5. STUDY PROCEDURES

Study visits will take place at the Women's Health Practice, located at 505 East 70th Street, 5th Floor, New York, NY 10021, Weill Cornell Maternal Fetal Medicine, located at 525 East 68th Street, Suite J-130, New York, NY 10065, New York Presbyterian Queens (56-45 Main St, Flushing, NY 11355) and its affiliated high risk clinics, and The Hypertension Center, located at 424 E 70th St, New York, NY 10021.

During the study, **as a part of standard prenatal care**, participants will be requested to have their blood pressure checked at each visit. In addition, lab work will be drawn at the initial screening visit and at 18-22 weeks, 24-28 weeks and 34-38 weeks gestation. If patients are scheduled to have their blood drawn for clinical purposes on that day, we will not perform an additional needle stick.

Data collection will be done through a secure RedCap database. Bio samples will be stored in a de-identified manner in the Nephrology research lab (Dr Suthanthiran's lab) in 1300 York Ave, 5th floor A building Lab A571.

5.1 Schedule of Evaluations

	Baseline Visit (<16 weeks)	Week 18-22 Visit	Week 24-28 Visit	Week 34-38 Visit	Study outcome	Delivery	Postpartum Visit
Performed by study personnel at study visits							
Informed consent	X						
Demographics	X						
Randomization	X						
Physical exam	X	X	X	X			X
Vital signs	X	X	X	X			X
Medication list	X	X	X	X			X
Adherence Questionnaire		X	X	X			
Obtained from chart review of information collected as part of standard clinical care							
Medical history	X						
Vital signs	X				X	X	
Physical exam	X				X	X	
CBC w/diff, platelets	X	X	X	X	X	X	X
Serum chemistry [a]	X	X	X	X	X	X	X
Urine studies [b]	X	X	X	X	X	X	X
Fetal testing results	X	X	X	X		X	X
Placental pathology report						X	
Operative and birth reports						X	
Neonatal records						X	
Outcome Evaluation (PEC)		X----------X					
Adverse event evaluation	X----------X						

a: Albumin, bicarbonate, BUN, chloride, creatinine, glucose, potassium, AST, ALT, sodium, uric acid and lactate dehydrogenase
b: Urinalysis, urine chemistry, urine protein, urine creatinine, urine albumin.

5.2. Treatment groups

At the initial visit, participants will be consented then randomized using the RedCap software to either Arm 1: 81mg ASA per day or Arm 2: 162mg ASA per day.

Adherence will be ascertained by questionnaire including a validated questionnaire: the simplified medication adherence questionnaire (SMAQ) (5).

Once funding can be obtained, we may further evaluate adherence by quantifying Thromboxane metabolite excretion in the collected and stored urine samples (urine samples will be stored in our BioBank at the Nephrology lab [Dr Suthanthiran's lab, 1300 York Avenue, 5th floor, A-571]). This method has previously been used in pregnancy to determine ASA activity/adherence (13, 14).

The patients will then be monitored throughout pregnancy for side effects, study endpoints and clinical data will be collected as described in section 5.1. Delivery records and postpartum course will also be considered to rule out delivery/postpartum complications including obstetric hemorrhage and postpartum PE.

5.3. Outcome Allocation

The major clinical outcome of PE will be determined by agreement of two co-investigators after the participant's chart is reviewed and adjudicated as: preterm PE (PE<37 weeks) or PE with severe features (Aim 1). If the two co-investigators do not agree, a third co-investigator will be asked to contribute to the adjudication of the case. The adjudicators will be blinded about the treatment groups in which the participants are.

Participants who do not have a documented pregnancy progressing beyond 20 weeks, cannot be adjudicated and will hence be excluded from analysis.

We will use the American College of Obstetricians and Gynecologists (ACOG) definition of PE to guide ascertainment of the outcome (1). PE will be diagnosed in women with new onset:

Hypertension documented before 20 weeks of gestation and defined as:

- Systolic blood pressure ≥ 140 mm Hg or diastolic ≥ 90 mm Hg at least 4 hours apart
- Systolic blood pressure ≥ 160 mm Hg or diastolic ≥ 110 mmHg confirmed within a short interval
- In addition to one of the following criteria:
- Proteinuria: ≥300 mg per 24-hour urine, Protein/creatinine ratio of 0.3 mg/dl or Protein on Dipstick ≥ 2+
- Thrombocytopenia: platelet count less than 100,000 $X10^9$/L
- Renal Insufficiency: serum creatinine > 1.1 mg/dl or doubling of serum creatinine
- Impaired liver function: elevated transaminases to twice normal
- Pulmonary edema
- Neurologic findings: New onset headache that does not respond to medication and that cannot be attributed to another etiology, seizure (which will prompt the diagnosis of Eclampsia).

In accordance with ACOG practice recommendations, superimposed PE can be diagnosed in women with underlying or preexisting hypertension, as a sudden increase in blood pressure in addition to the new onset of the criteria mentioned above (15). In women with a history of protein in their urine, the criterion of proteinuria for PE would be met if there is a significant change in the quantity of their proteinuria (15).

Severe features of preeclampsia are defined (as per ACOG 2019 guidelines) as follows (1):

- Systolic blood pressure ≥ 160 mm Hg or a diastolic≥ 110mmHg, measured ≥ twice in ≥ 4 hours (unless the patient is on anti-hypertensive medication)
- Thrombocytopenia: platelet count less than 100,000 $X10^9$/L
- Renal Insufficiency: serum creatinine > 1.1 mg/dl or doubling of serum creatinine
- Impaired liver function: elevated transaminases to twice normal

- Pulmonary edema
- New onset headache that does not respond to medication and that cannot be attributed to another etiology
- Changes in vision

Adverse event monitoring will be conducted throughout the course of the study and will be assessed at each visit. Adverse events will be reported to the IRB as indicated by their regulations.

5.4. Biobanking and cfRNA profiling

Blood and urine collected as per section 5.1 will be stored in our BioBank at the Nephrology lab (Dr Suthanthiran's lab, 1300 York Avenue, 5th floor, A-571). These samples would be used for future investigations of biomarkers of PE and for changes associated with different doses of ASA.

Once funding can be obtained, we will proceed with profiling of cell free mRNA in the collected plasma samples in order to investigate whether, in pregnant women at a high risk for PE, cfRNA profiles can anticipate PE or predict the ability of bASA to prevent (or not) this disease. The profiling of cfRNA will be done in collaboration with Dr Iwijn De Vlaminck (Cornell Ithaca).

Next generation sequencing.

cfRNA will be isolated from 1ml plasma according to a published protocol (8). We will use a Plasma/Plasma Circulating RNA and Exosomal Purification kit (Norgen, cat 42800) for RNA extraction. DNA contamination will be removed by Baseline-ZERO DNase (Epicentre) followed by RNA Clean and Concentrator-5 kit (Zymo). Library preparation will be performed using SMARTer Stranded Total RNAseq-Pico Input Mammalian kit (Clontech). cfRNA sequencing will be done using an Illumina based platform. Sequencing output will be processed through an in-house pipeline. cfRNA indicative of PE will be identified using the edgeR software by performing an exact test, a likelihood ratio test, and quasi-likelihood F-test.

Absolute quantification of cell-free RNAs using real time quantitative PCR assays (QPCR).

We will reverse transcribe the RNA isolated from the plasma samples to cDNA using the CellsDirect One-Step RT-qPCR kit (Thermo-Fisher) together with a pool of 96 primer pairs. If an informative cfRNA discovered by deep sequencing is not included in the standard primer/probe pool, we will design a specific primer/probe for the reverse transcription and preamplification of that cfRNA. A 20-cycle preamplification will then be done in Veriti® thermal cyclers. We will use Applied Biosystems' QuantStudio™ 7 Flex Real-Time PCR System for the absolute quantification of the cfRNAs discovered to be prognostic of PE at a false discovery adjusted p-value <0.01. The PCR reaction for each sample will be set up in duplicate.

Statistical analysis for cfRNA profiling:

Standard descriptive statistics (such as mean or median, range and percentage) will be computed for patient characteristics. Receiver-operating-characteristic (ROC) curve analysis of levels of cfRNAs will be performed and used to determine area under the curve (AUC) as the discrimination statistic, and the cutoff point that yields the highest combined sensitivity and specificity (Youden's index) for predicting PE vs. no PE. Logistic regression analysis with stepwise backward elimination will be used to develop parsimonious prognostic signatures based on linear combinations of cell-free RNAs and to compute the resulting AUC. Models will be

compared with likelihood ratio test (for nested models) and internally validated by 10-fold cross validation. Genes with an effect size threshold of 0.8 between the PE and No PE groups, and with false discovery rate (FDR) of 0.05 will be considered significantly different *(8).*

6. DOSING DELAYS/DOSE MODIFICATIONS

Patients will obtain their medications from their respective pharmacies. Women randomized to Arm 1, will be instructed to take one tablet of baby ASA 81mg per day while those in Arm 2, will be asked to simultaneously take 2 tablets per day for a total of 162mg.

Therapy will be prescribed at the baseline visit to be initiated before 16 weeks of gestation. Aspirin therapy will continue until a week prior to planned delivery or upon admission for unplanned/imminent delivery as per routine clinical care.

Aspirin is an over the counter medication and comes in very large pill containers thus rendering pill counts unpractical. Adherence will be assessed using a validated questionnaire.

Dose modifications are not planned. If for a clinical reason, the participant or their healthcare providers have a concern or issue with the randomized dose; then that participant can be taken off the study dose and, with their permission, would be observationally monitored in an intention to treat analysis.

7. ADVERSE EVENT REPORTING REQUIREMENTS

AE monitoring will be conducted throughout the course of the study and will be assessed at each visit. AEs will be reported to the IRB as indicated by their regulations.

8. PHARMACEUTICAL INFORMATION

Clinical trials evaluating the benefits of low-dose ASA have used a wide range of doses from 60mg to 150mg orally daily with low-dose being defined as less than 325mg per day. ASA is currently the only prophylactic therapy for PE in high-risk women to be recognized by the US Preventive Task Force (in accordance with the World Health Organization) and should be initiated early in the second trimester of pregnancy, before 16 weeks of gestation (4).

9. CORRELATIVE/SPECIAL STUDIES

There are no planned correlative or special studies.

10. MEASUREMENT OF EFFECT

The primary objective of this study is to evaluate the protective effect of ASA on the risk reduction of preterm PE and PE with severe features, and measure whether a dose-effect exists.

The secondary objectives of our study include to:

- Evaluate and compare the adherence of pregnant women to 81 mg and 162 mg of daily low-dose aspirin using a validated, Simplified Medication Adherence Questionnaire (SMAQ).
- Compare maternal and fetal outcomes in pregnant women at a high risk for preeclampsia who are treated with either 81 mg or 162 mg of daily aspirin during pregnancy, including preeclampsia ≥ 37 weeks, severe maternal hypertension, preterm delivery, fetal growth restriction, placental abruption and maternal/fetal mortality.
- Compare the time-to-event for developing PE across the 2 treatment groups.

11. DATA REPORTING / REGULATORY CONSIDERATIONS

11.1 Data Collection

Data collection will be done through a secure RedCap database. Data collected is described in section "5. Study Procedures". Data will only be exported without protected health identifiers for data analysis.

The name and identifiers (date of birth and medical record number) of patients who were screened for the study but were not eligible or did not consent to being in the study will be stored in a secure RedCap database until the end of the study and then, deleted. These identifiers will only be accessible to the study personnel in order to prevent re-screening and approaching again patients that are ineligible or unwilling to participate in our study. Laboratory personnel and other research personnel that is not involved in participant recruitment or follow up will not have access to identifiers.

Bio samples will be stored in a de-identified manner in the Nephrology research lab (Dr Suthanthiran's lab) in 1300 York Ave, 5th floor, A building Lab A571.

11.2 Regulatory Considerations

IRB approval will be obtained prior to the initiation of the study. IRB approval renewals will be sought as per routine for the duration of the study. All protocol amendments will be made by the Principal Investigator.

12. STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

Subjects will be recruited from the New York Presbyterian – Weill Cornell and Queens campuses' affiliated Obstetric Clinics and The Hypertension Center. Patients will be screened for eligibility and if eligible and consented, will be randomized to one of 2 arms: ASA 81mg daily vs ASA 162mg daily, to be started before 16 weeks' gestation. Randomization will be electronically performed though the RedCap study portal. This will be an open label pilot study. Those patients who are randomized to the 162mg group, will be instructed to take two 81mg tablets simultaneously. Patients will be instructed to continue ASA until delivery.

Subjects will be seen (as per routine clinical care) monthly in the second trimester and at least every 2 weeks thereafter. They will then be evaluated for antepartum complications and PEC. Study participants will be assessed by study personnel in synchrony with their routine obstetric appointments at baseline (<16 weeks), 18-22 weeks, 24-28 weeks, 34-38 weeks, delivery and postpartum (1-6 weeks after delivery). The schedule of evaluations is described in section “5.1. Schedule of Evaluations”.

Blood pressure, medication reconciliation with questionnaire, and chart review will be performed at each visit. Records will be reviewed to document laboratory tests and fetal testing. If the patient is diagnosed with PE at a time point other than the pre-set time points, we would collect the information as part of a “study outcome” visit. Delivery records will also be collected to include: blood pressure, medication regimen, laboratory tests, physical exam, imaging, placental pathology reports, operative records, birth and neonatal outcomes. Diagnosis of PE will be made by clinical chart review and ascertained by 2 independent adjudicators (co-investigators) (as detailed in section “5.3. Outcome Allocation”). If there is a disagreement, a 3rd adjudicator will be asked to review the chart.

The study will be done in 2 stages. The first stage will aim to recruit 50-100 patients within the first 6 months to a year in order to evaluate feasibility, safety and determine the incidence of preterm PE and PE with severe features in order to calculate/verify the adequate sample size needed for this study’s completion. The stages are further detailed in section “12.3 Sample Size/Accrual Rate”.

12.2 Treatment Regimens

Arm 1: 81mg oral ASA daily.

Arm 2: 162mg oral ASA daily.

Patients will obtain their prescriptions from their respective pharmacies. Women in Arm 1, will be instructed to take one table of 81mg aspirin per day; those in Arm 2, will be asked to take two tablets simultaneously orally once per day. Therapy will be initiated at the baseline visit and continue until 1 week before planned delivery or upon admission for unplanned/imminent delivery as per clinical routine.

12.3 Sample Size/Accrual Rate

A sample size of 394 participants (197 for each group) will allow us to detect at 7.1% difference in the incidence of PE between the 2 groups. This is based on prior data reporting an incidence of PE of 8.6% and 1.5% for 81mg and 150mg of ASA respectively (10, 12) with a target 85% power and a Type 1 error rate (alpha) of 5% (two-tailed). Assuming a response rate of 50%, we will aim to screen 788 women.

The above calculation is however only theoretical and very approximate. The incidence of PE in women on 162mg of ASA is not known and the incidence of PE in the 81mg of daily ASA that has been reported in the above-mentioned literature (10) appears to be far below the incidence of PE of 33.6% in

our patient population (16). Our elevated rate of PE likely stems from referral bias and from the fact that, at the time of this study, aspirin was still not widely prescribed as it was not standard of care yet.

The study will therefore be done in two stages:

Stage 1: Pilot stage to assess feasibility and ensure feasibility and safety with the randomization of 50-100 patients. The incidence of PE in the 2 groups will be evaluated and serve to calculate a sample size with a power of 85% to detect the observed difference between the two groups (adjusting for the interim analysis effect).
We will recruit 50-100 patients in the initial stage of this trial. We have obtained an approximate estimation for the number of patients we see in the Women's Health Practice and in J130 that would meet eligibility criteria for our study at <16 weeks gestation with USPTF high risk factors for the development of pre-eclampsia. We have sampled 3 separate months of the year so as to account for holidays, change in faculty coverage, and seasonality. In the month of January, there were 9 eligible patients; in October, there were 32 eligible patients; and in June, there were 29 eligible patients. Given the variability from month to month, and assuming a 50% response rate, 11 patients a month could potentially be randomized. Assuming a 75% adherence rate, we anticipate having approximately 50 patients for initial assessment of feasibility and safety by the end of 6-12 months, after which we would perform a more accurate power analysis and continue with the second stage of our study.

Stage 2: Larger, powered, clinical trial. Proceed with randomization of the remaining patients to the accrual ceiling as determined by Stage 1. The interim analysis that was used to establish an accurate sample size calculation will be taken into account.

12.4 Stratification Factors

A secondary objective of this study is to assess the impact of specific co-morbidities (diabetes, chronic hypertension, renal disease and autoimmune disease), blood pressure control, age and race on the relationship between treatment group and PE incidence.

12.5 Analysis of Secondary Endpoints

Standard descriptive statistics (such as mean or median, range and percentage) will be computed for patient characteristics. Baseline characteristics will be compared across treatment arms using Mann-Whitney U (for non-parametric variables), student's t-test (for normally distributed variables) or Chi-square tests (for categorical variables). Group differences in the incidence of preterm PE (<37 weeks) and PE with severe features (our primary outcome) will be tested using Chi-square.

As secondary analyses, multivariate logistic regression will be used to further assess the impact of co-morbidities (diabetes, chronic hypertension, renal disease and autoimmune disease), blood pressure, age and race on the relationship between treatment group and PE incidence. An exploratory analysis will be made to compare the time-to-event for PE across the 2 treatment groups using a log-rank test and a cox-regression model to adjust for the above-mentioned possible confounders. We will use Stata 15 (StataCorp. College Station, TX) for data analysis.

12.6 Reporting and Exclusions

12.6.1 Evaluation of toxicity.

Evaluation of toxicity will be monitored throughout the study period. AE will be assessed at each study visit.

12.6.2 Evaluation of response.

Evaluation of response will be monitored at each visit and the presence/absence of PE will be ascertained at the completion of the study.

13. DATA AND SAFETY MONITORING PLAN (DSMP)

Data and Safety Monitoring Plan (DSMP) is not applicable since both the intervention arms are considered standard of care.

The data will be stored in a secure RedCap database. The only other research personnel with access to the database will be the PI (Dr. Line Malha) and the study coordinator (Dr. Kathy Matthews). Laboratory personnel and other research personnel will not have access to identifiers.

HUMAN SUBJECTS CONCERNS

Patients will be recruited from New York Presbyterian – Weill Cornell and Queens campuses' affiliated Obstetric Clinics and The Hypertension Center. Institutional Review Board (IRB) approval for the Weill Cornell campus will be obtained before initiation of the trial. In accordance with IRB regulations, informed consent will be obtained at the enrollment visit, prior to randomization. We do not expect more than minimal risk to our subjects since both strategies tested are part of the current standard of care. Despite the absence of evidence for increased major bleeding associated with ASA (3, 4), we will inform participants of the risk of non-major bleeding associated with ASA in pregnancy and discomfort for additional time spent at each visit for research purposes. Risk for loss of confidentiality will be discussed and minimized by storing all identifiers in a password secured Redcap database. RedCap access will be limited to necessary research personnel. Data exported for analysis will be done in a de-identified manner. The potential benefit of this study to its participants include risk reduction of PEC. Consistently with the current equipoise, this benefit is considered to be similar for both groups and not distinct from standard of care.

NEW AND/OR UNIQUE ASSAY/TECHNOLOGY: Not applicable.

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Appendix

Validated Aspirin Adherence Questionnaire

Simplified medication adherence questionnaire (SMAQ)

1	Do you ever forget to take your aspirin? Yes No
2	Are you careless at times about taking your aspirin? Yes No
3	Sometimes if you feel worse, do you stop taking your aspirin? Yes No
4	Did you not take any of your aspirin over the past weekend? Yes No
5	Thinking about the last week, how often have you not taken your aspirin? Never 1x 2-3x 4-5x 6-7x
6	Over the past 3 months, how many days have you not taken any aspirin at all? ≤2 days >2 days