A PHASE II STUDY OF NICOTINAMIDE (VITAMIN B3 AMIDE) SUPPLEMENTATION IN WOMEN WITH EARLY ONSET PREECLAMPSIA

NCT number NCT03419364
Document Date 10/03/2019

A PHASE II STUDY OF VITAMIN B_3 AMIDE SUPPLEMENTATION IN WOMEN WITH EARLY ONSET PREECLAMPSIA

Coordinating Investigator: Kim Boggess MD
Dept of Obstetrics and Gynecology
Maternal Fetal Medicine
University of North Carolina School of Medicine
CB 7570
3010 Old Clinic Bldg
919-966-1601

Co-Investigators: Noboyo Maeda PhD
Dept of Pathology and Laboratory Medicine
University of North Carolina School of Medicine

S. Ananth Karumanchi MD
Dept of Medicine
Harvard University

Matthew Laughon MD, PhD
Dept of Pediatrics
Neonatal-Perinatal Medicine
University of North Carolina School of Medicine

Craig Lee Pharm D
Division of Pharmacotherapy and Experimental Therapeutics
University of North Carolina School of Pharmacy

Study Product: Nicotinamide, the amide form of vitamin B_3

Protocol Number: *NicPhaseIIv.7*

IND Number: 118832

Confidential and Proprietary
This document contains confidential information and is intended solely for use by the specified clinical investigator and must not be disclosed to any other party. Acceptance of this document constitutes agreement that no unpublished information contained herein will be disclosed without prior written approval of the coordinating investigator, except that this document may be

disclosed to the appropriate Human Ethics Committees who will agree to maintain confidentiality.
Version Date: October 3, 2019

Table of Contents

List of Abbreviations

ACRONYM	DESCRIPTION
ACOG	American College of Obstetrics and Gynecology
AE	Adverse event
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
APGAR	APGAR score
AST	Aspartate amniotransferase
B_3A	B_3-amide
BP	Blood pressure
BPP	Biophysical profile
CBC	Complete blood count
CRF	Case report form
CT	Computerized tomography
CTCA v3.0	Common terminology criteria for adverse events v3.0
DSMB	Data Safety and Monitoring Board
EOP	Early onset preeclampsia
EFW	Estimated fetal weight
ET	Endothelin
GI	Gastrointestinal
HELLP	Hemolysis, elevated liver enzymes, low platelets
HIV	Human immunodeficiency virus
INH	Isoniazid
LFTs	Liver function tests (includes AST and ALT)
MRI	Magnetic resonance imaging
MVP	Maximum vertical pocket
MWF	Monday, Wednesday, Friday
NIH	National institutes of health
PHI	Personal health information
NICU	Neonatal intensive care unit
NIH	National institutes of health
NST	Non-stress test
PHI	Protected health information
pK	Pharmacokinetics
PlGF	Placental growth factor
PI	Principal investigator
P/C	Protein to creatinine ratio
RDA	Recommended daily allowance
sFlt-1	Soluble fms-like tyrosine kinase-1
SD	Standard deviation
TID	Three times per day
UA	Umbilical artery
ULN	Upper limit of normal
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

Study Summary

Title	A PHASE II STUDY OF VITAMIN B_3 AMIDE DIETARY SUPPLEMENTATION IN WOMEN WITH EARLY CONSET PREECLAMPSIA
Short Title	NicPreDose
Protocol Number	001v7
Phase	Phase II
Methodology	Open label
Study Duration	24 months
Study Center(s)	University of North Carolina Women's Clinic/Hospital
Objectives	1. To test the effects of a novel oral agent, nicotinamide, on mean arterial pressure (MAP) after 48 hours and 7 days of treatment; 2. To measure safety of nicotinamide in women with early-onset preeclampsia (n=25). Safety defined as development of maternal liver toxicity, defined as $\geq$ 3x ULN of ALT or AST while on treatment, defined as within 24 hours of dose; 3. To determine maternal tolerability of nicotinamide 2.5 gm/day, administered as 1000mg at 8AM and 12MN; 500mg at 4PM 4. To measure pharmacokinetics (pK) of nicotinamide 1000 mg in normal healthy reproductive age women (n=6) and healthy, normotensive pregnant women (n=6).
Number of Subjects	37 (25 preeclamptic; 6 non-pregnant; 6 healthy, normotensive, pregnant)

Diagnosis and Main Inclusion/Exclusion Criteria	Diagnosis and Inclusion Criteria for women with severe preeclampsia
	• Maternal age 18-45 years
	• Singleton or twin pregnancy with no known fetal anomalies
	• Early-onset preeclampsia defined as:
	  o Early-onset: between 24 weeks 0 days and -33 weeks 3 days, based on menstrual dating confirmed by first or second trimester ultrasound OR second trimester ultrasound if menstrual dating unavailable;
	  o Preeclampsia:
	  ▪ New onset hypertension and proteinuria, with systolic BP ≥ 140 mm Hg and/or diastolic BP ≥ 90 mm Hg on two occasions 6 hours apart and > 300 mg proteinuria on 24 hour urine collection OR urine P/C ratio >0.3;
	  ▪ New onset hypertension and NO proteinuria, with systolic BP ≥ 140 mm Hg and/or diastolic BP ≥ 90 mm Hg on two occasions 6 hours apart and one or more of the following: serum creatinine >1.1 mg/dL or doubling from baseline ,or central nervous system symptoms or visual changes
	  ▪ Severe gestational hypertension defined as new onset systolic BP ≥ 160 mm Hg and/or diastolic BP ≥ 105.
	  o Candidate for expectant management for at least 48 hours
	  o Deemed clinically stable by primary clinician and candidate for expectant management (delayed delivery) for at least 48 hours;
	• Maternal liver function tests < 2x ULN
	• Maternal platelet count ≥ 100,000 mm^3
	• Planned expectant management
	• Pre-existing medical diseases such as hypertension, diabetes, endocrine disorders, gastrointestinal diseases, are well controlled
	• Fetal well-being established by estimated fetal weight ≥ 5^{th} %tile; normal amniotic fluid volume (MVP ≥ 2 cm); normal UA Dopplers; or reactive NST or BPP > 6
	• Delivery not anticipated within 48 hours of enrollment
	
	Exclusion Criteria for women with severe preeclampsia
	• Pre-existing renal disease (creatinine ≥ 1.5 mg/dL)
	• Any pre-existing medical condition that would increase risk for liver toxicity (e.g. hepatitis B or C; HIV; INH use)
	• Eclampsia; cerebral edema on CT/MRI; headache unrelieved by analgesics
	• Evidence of liver dysfunction (LFTs ≥ 2x ULN)
	• Thrombocytopenia (platelets < 100,000 mm^3)
	• Pulmonary edema
	• HELLP syndrome
	• Evidence of fetal compromise: EFW < 5^{th} percentile; or BPP < 6; or absent or reverse diastolic UA blood flow; or oligohydramnios (MVP < 2 cm)

	• Placental abruption defined as unexplained vaginal bleeding • Preterm labor defined as regular contractions and cervical change • Any condition deemed by the investigator to be a risk to mother or fetus in completion of the study • Any condition deemed by the investigator to require delivery within 48 hours Inclusion Criteria for healthy, normotensive, pregnant women • Maternal age 18-45 years • Singleton or twin pregnancy with no known fetal anomalies (24 weeks to 33 weeks 3 days) • No known medical complications Exclusion Criteria for healthy, normotensive, pregnant women • Current cigarette smoker • Hypertension requiring medical treatment • Diabetes requiring medical treatment • Pre-existing renal disease (creatinine $\geq$ 1.5 mg/dL) • Any pre-existing medical condition that would increase risk for liver toxicity (e.g. hepatitis B or C; HIV; INH use) • Currently taking medication for a chronic medical condition • Currently taking an oral or IV antibiotic or antifungal medication Diagnosis and Inclusion Criteria for healthy nonpregnant women • Age 18-45 years • No known medical complications or disease (eg hypertension, diabetes, liver or kidney disease) Exclusion Criteria for healthy nonpregnant women • Current cigarette smoker • Hypertension requiring medical treatment • Diabetes requiring medical treatment • Pre-existing renal disease (creatinine $\geq$ 1.5 mg/dL) • Any pre-existing medical condition that would increase risk for liver toxicity (e.g. hepatitis B or C; HIV; INH use) • Currently taking medication for a chronic medical condition (excluding oral contraceptives) • Currently taking an oral or IV antibiotic or antifungal medication

Study Product, Dose, Route, Regimen	Vitamin B_3-amide; 2.5 mg daily, administered as 1000mg at 8AM and 12MN; 500mg at 4PM, provided by Major Pharmaceuticals **Wendy Ernst** Advisor, Contracts and Billing 17177 N Laurel Park Dr., Suite 233 Livonia, MI 48152 Office: (734) 743-6232 | Fax: (734) 743-7232 Email: wernst@major-pharm.com
Duration of administration	Until delivery or 34 weeks' gestation
Statistical Methodology	Primary Outcome • Mean arterial blood pressure before, 48 hours, and 7 days after daily dosing Secondary Outcomes • Maternal liver toxicity: the proportion of women who develop elevated liver function tests (ALT and/or AST $\geq$ 3x ULN within 24 hours of study drug) will be determined and compared to expected proportion of 20% • Maternal side effects (nausea, vomiting, rash, headache) • Optional: Plasma nicotinamide and 2-pyridone levels; sFlt-1 and PlGF levels

1. Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Preeclampsia is a hypertensive disorder unique to pregnancy that occurs in approximately 5 – 8% of pregnancies in the United States.[1] The disease is mild in 75% of cases, and 10% of cases occur before 34 weeks' gestation.[2] Despite its relatively rare prevalence, preeclampsia is the **leading cause** of maternal morbidity and mortality in the United States. Worldwide, 10 to 15% of direct maternal deaths (i.e., resulting from obstetric complications of pregnancy) are associated with preeclampsia/eclampsia.[3] Women who develop preeclampsia are at risk for pulmonary edema [4], coagulation defects, hepatic and/or renal failure, seizures, cerebral hemorrhage, and blindness.[5-7]

In 2013 ACOG published an Executive Summary from the Task Force on Hypertension in Pregnancy. They proposed the following for the diagnosis of preeclampsia:

- Blood pressure greater than or equal to 140 mm Hg systolic or greater than or equal to 90 mm Hg diastolic on two occasions at least 4 hours apart after 20 weeks of gestation in a woman with a previously normal blood pressure; or greater than or equal to 160 mm Hg systolic or greater than or equal to 110 mm Hg diastolic, within a short interval (minutes) to facilitate timely antihypertensive therapy

AND

- Proteinuria, defined as $\geq$ to 300 mg per 24-hour collection (or this amount extrapolated from a timed collection) or protein/creatinine ratio $\geq$ 0.3, or urine dipstick reading of 1+ (if other quantitative methods not available)

OR

- New-onset hypertension and no proteinuria with the new onset of any of the following: platelet count less than 100,000/microliter, creatinine > 1.1 mg/dL or doubling from baseline, elevated LFTs > 2xULN, pulmonary edema, CNS symptoms, or visual changes.

Preeclampsia is also a significant contributor to neonatal morbidity and mortality. Fetal complications are not uncommon and include poor fetal growth from chronic placental hypoperfusion[8], especially in cases of early-onset preeclampsia.[9, 10] Placental abruption, premature separation of the placenta with disruption of blood flow to the fetus, is an uncommon but life-threatening complication that occurs in up to 3% of pregnancies complicated by severe preeclampsia.[11]

There are no proven measures to treat preeclampsia; resolution occurs only with delivery. In early onset preeclampsia (< 34 weeks), delaying delivery and prolongation of pregnancy is of benefit to the fetus but potentially detrimental to the mother. Medically indicated preterm birth as a secondary result of fetal or maternal complications contributes significantly to neonatal morbidity and mortality associated with preeclampsia.[12] As shown in Figure 1, **preeclampsia is**

one of the leading causes of medically indicated preterm birth. Conservatively estimated, > 20,000 preterm births < 34 weeks occur annually in the United States due to complications of preeclampsia. A therapeutic approach to early-onset preeclampsia to prolong pregnancy that will do no harm to both the mother and fetus is an important and unmet med.

The pathophysiology of preeclampsia is complex and incompletely understood. Activation of several different pathways is thought to be operative in the development of preeclampsia. Evidence exists to support excessive maternal inflammation[13] maternal immunologic responses [14], placental hypoxia and oxidative stress[15], generalized vasospasm, and alteration of growth factor production, as part of the pathophysiologic process in preeclampsia. The sentinel paper by Roberts et. al.[16] implicated angiogenic imbalance with endothelial activation and generalized maternal endothelial dysfunction as the explanation for most of the clinical aspects of preeclampsia. These pathways are all believed to culminate in endothelial activation and dysfunction[17], characterized by an imbalance between circulating pro- and anti-angiogenic factors.

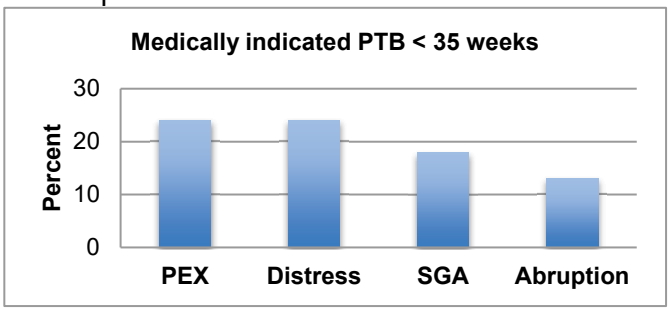


Figure 1. Percent of medically indicated preterm birth (PTB) due to preeclampsia (PEX), fetal distress, small for gestational age (SGA), and abruption

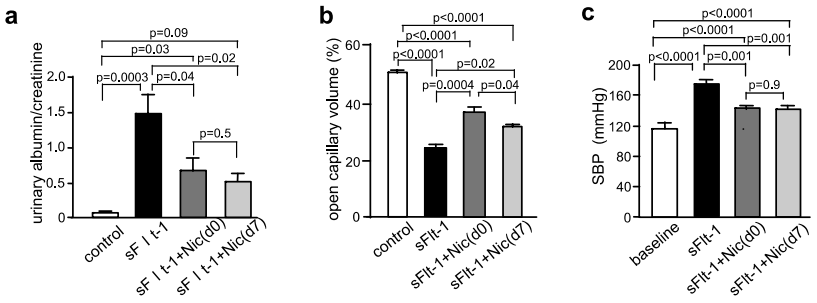
Angiogenesis requires the complex interplay between vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and their receptors, soluble fms-like tyrosine kinase-1 (sFlt-1) and VEGFR-1. The placenta is a rich source of these factors, and in addition to regulating vascular homeostasis, VEGF, PlGF, and sFlt-1 are key components in regulating trophoblast cell function and survival.[18-20] sFlt-1 acts as an anti-angiogenic factor by neutralizing PlGF and VEGF.[21]

Animal and laboratory data exist to support the theory of angiogenesis imbalance as the root cause of preeclampsia. Overexpression of sFlt-1 induces hypertension and fetal growth restriction in pregnant mice, and the time course of blood pressure increase mirrors the increase in sFlt-1.[22] The preeclampsia-like clinical phenotype associated with sFlt-1 expression occurs via activation of the endothelin (ET) system.[23, 24] In humans, low plasma vascular endothelial growth factor (VEGF) is the first trimester of pregnancy is a predictive marker for preeclampsia. In addition, sFlt-1/PlGF ratios are increased in women who subsequently develop preeclampsia compared to normotensive women.[25] ~90% of women with EOP have elevated sFlt-1.[26] An sFlt-1/PlGF ratio >0.85 is associated with adverse pregnancy outcomes: placental abruption, elevated liver enzymes and/or low platelets, and small-for-gestational-age birth weight and/or absent/reversed umbilical artery Doppler, and significantly shorter latency period, among women with EOP. 75% of women with sFlt-1/PlGF >0.85 deliver within one week and 85% within two weeks, compared to only 10% and 15% of women with sFlt-1/PlGF <0.85. sFlt-1/PlGF is a useful discriminator for women with EOP who are at risk for adverse outcome and need for delivery prior to 34 weeks.[27] The natural history of circulating sFlt-1 levels among women with preeclampsia has been described in several longitudinal studies.[28, 29] sFlt-1 levels are elevated prior to the development of clinical symptoms of preeclamspsia, and remain elevated until delivery.[17] Based on this data, we believe that elevated circulating sFlt-1 levels identify those women at greatest risk for adverse outcome, particularly if managed expectantly, and thus the women most likely to yield benefit from treatments that allow for longer latency.

To address the angiogenic imbalance believed to be in the pathophysiology of preeclampsia, we[24] and others[30-33] have demonstrated that ET receptor antagonists greatly ameliorate the preeclampsia-like phenotypes induced by sFlt-1 in animal models. While this data is promising, ET receptor antagonists are teratogenic and contraindicated in pregnant

women. However, this does not preclude the use of substances that block the action of factors downstream of the ET receptor that would mediate sFlt-1 expression and action.

Following this reasoning, we tested the effects of nicotinamide, the amide of vitamin B_3 (B_3A) a potent inhibitor of ADP ribosyl cyclase (ADPR cyclase). ADPR cyclase is activated by endothelin, and mediates an increase in cytosolic Ca2+ and vasoconstriction. Using our mouse model of sFlt-1 overexpression, we found that nicotinamide ameliorates the increased BP and urinary albumin excretion induced by increased sFlt-1. In our mouse studies we used 500mg/kg/day, equivalent to 41mg/kg/day in humans, which would be 2.7g/day for an average adult with a weight of 60kg. As shown in the Figure on the following page,we found a significant lowering of urinary albumin excretion, increase in open capillary volume on renal histopathology, and lowering of blood pressure, when vitamin B_3A was given to mice as preventive (Nic (d0) group) or as therapeutic (Nic (d7) group) treatment. **Panel a** shows urinary excretion of albumin normalized to urinary creatinine concentration. **Panel b** shows open capillary volume on renal histopathology. **Panel c** shows systolic blood pressure (SBP) measured by telemetry.



Legend: [control]: mice receiving neither virus nor vitamin B_3. [sFlt-1]: mice receiving sFlt-1 virus but not given vitamin B_3 (n=5); [sFlt-1 + Nic (d0)]: mice given vitamin B_3 daily starting on the same day as they received the virus (n=6); [sFlt-1 + Nic (d7)]: mice treated with vitamin B_3 starting 7 days after they received the virus.

1.2 Investigational Agent

The investigational agent for this protocol is Vitamin B_3 Amide (B_3A; Nicotinamide) **Chemical Formula:** C6-H6-N2-O. Metabolites include N1-methylnicotinamide and its oxidation products.

Niacin (nicotinic acid, vitamin B_3) is an essential vitamin. Vitamin B_3 and the amide form have identical vitamin functions, but vitamin B_3-amide does not have the same pharmacological and toxic effects as vitamin B_3. Thus it does not reduce cholesterol or induce flushing.[34] In cells, vitamin B_3 is incorporated into nicotinamide adenine dinucleotide (NAD) and NAD-phospate, which are coenzymes in a wide variety of oxidation-reduction reactions.[35]

The U.S. Recommended Daily Allowance (RDA) for niacin is 18 mg/day during pregnancy and 17 mg/day during lactation. The recommended upper limits for pregnant and lactating women are 30 – 35 mg/day. [36] Prenatal vitamins contain 14-18 mg of niacin.

1.3 Preclinical Data

The LD50 of vitamin B_3-amide in rodents is 3-7g/kg when given orally[37], and its therapeutic index is correspondingly wide.

Ieraci et al.[38] have shown that a single high dose of vitamin B_3-amide protects mice from developing the neurological damage and behavioral abnormalities that occur in adults that were exposed to high doses of alcohol perinatally at the time when the developing nervous system is most susceptible to chemical damage. The control mice given vitamin B_3-amide but no alcohol were indistinguishable neurologically and in behavior from the mice given saline. Thus vitamin B_3-amide is harmless to mice exposed to it at a dose level and at a time when it is highly protective against damage from alcohol.[38]

Vitamin B_3-amide has also been shown to protect rat pups from long-term neurologic effects of perinatal asphyxia.[39]

1.4 Clinical Data to Date

Vitamin B_3-amide has several properties that may be of clinical benefit to patients. Vitamin B_3-amide has been used clinically to treat acne vulgaris[40], anxiety and schizophrenia[41], Alzheimer's disease[42, 43] and cancer.[44] Vitamin B_3A has been used for long periods without significant side effects at doses of 50mg/kg/day for a variety of conditions, including type 1 diabetes and schizophrenia.[45, 46] Use of high dose vitamin B_3-amide to treat schizophrenia was not associated with any embryonic or fetal toxicity among women who experienced pregnancy while on treatment[46], based on exposure of 3 pregnant women. Human data demonstrate a risk for jaundice of 1 in 2000 following high dose vitamin B_3-amide treatment for psychosis.[47]

Studies in children have been conducted to prevent insulin-dependent diabetes. In a population-based diabetes prevention trial, 173 school age children with islet cell antibodies received vitamin B_3-amide treatment (500 mg BID) for 2.7 years. Side effects, liver function abnormalities, and growth were recorded. There were no reactions or toxicity ascribed to vitamin B_3-amide treatment.[48] In another study, vitamin B_3-amide at a dose of 1.2 g/m^2/day or placebo was given to children age 3-12 years who were at risk for Type I diabetes. While vitamin B_3-amide failed to reduce the cumulative diabetes incidence at 3 years, no side effects were observed in the children receiving vitamin B_3-amide. Body growth was compared and decrease in growth was not observed in children receiving vitamin B_3-amide compared to placebo.[49]

In a clinical trial of vitamin B_3-amide versus placebo for diabetes prevention, 276 subjects were randomized to 1.2 g/m^2 per day of vitamin B_3-amide.[50] Regular safety analyses done throughout the trial showed no differences between the treatment groups with respect to adverse events. 35 serious adverse events were reported in 18 participants in the active treatment group and 15 in the placebo group. Increased LFTs (> 3x ULN) were noted in nine participants during the trial. Of these, five were taking active treatment group and four were on placebo. Six women became pregnant during the trial, four in the vitamin B_3A group and two in placebo. Trial medication was stopped as soon as pregnancy was confirmed. No congenital abnormalities were reported in the offspring.[50]

According to Reprotox®, possible pregnancy effects of pharmaceutical doses of vitamin B_3-amide have not been studied[51], and the WHO Working Group on Human Lactation did not find available data on vitamin B_3A excretion in human milk to be sufficient to comment on its safety.[52]

We recently completed a Phase I study of 10 women receiving nicotinamide supplementation. Five women received 500 mg/day for up to two weeks; 5 women received 1000 mg/day for up to two weeks. One subject (#5, on 500 mg/day) experienced an adverse event, acute renal failure, which was not believed to be related to study medication or procedures. There were no cases of liver toxicity. All subjects tolerated nicotinamide without significant side effects.

Nicotinamide pharmacokinetics (pK) are well described in healthy, predominantly male, adults. [53-55] However, metabolite levels following our dosing regimen, and metabolite pK have not been reported and directly compared in healthy female adults or healthy pregnant women. We will address these gaps by enrolling six nonpregnant healthy and six pregnant healthy women and measuring pK of the main and secondary metabolites following a single 1000 mg dose.

Dose Rationale and Risk/Benefits

Overall, vitamin B_3A rarely causes side effects, and the FDA labels it Generally Regarded as Safe (GRAS) when used as a direct food additive and nutrient and/or dietary supplement. [56] This means that there is no evidence in the available information on vitamin B_3A that

demonstrates, or suggests reasonable grounds to suspect that it is a hazard when used at levels that are now current or that might reasonably be expected in the future, which the FDA places as 3-9 gm/day. We propose 2.5 gm/day which is below the dose expected to result in significant toxicity (3 gm/day).

2 Study Objectives

The **objectives** of this phase II study are to 1) study the effect of vitamin B_3 amide dietary supplementation on mean arterial pressure during expectant management of early-onset preeclampsia; and 2) further evaluate its safety in this context. We also aim to measure pharmacokinetics (pK) of nicotinamide 1000 mg in normal healthy reproductive age women and healthy, normotensive pregnant women.

3 Study Design

3.1 General Design

This is an open labeled phase II study of vitamin B_3-amide dietary supplementation in pregnant women with early-onset preeclampsia, per ACOG guidelines. We will enroll 24 pregnant women at 24-33 weeks 3 days with EOP and anticipated treatment by expectant observation. If the woman is anticipated to remain undelivered for 48 hours after diagnosis she will receive vitamin B_3-amide, 2.5 mg/day dosed in the following manner: administered as 1000mg at 8AM and 12MN; 500mg at 4PM, continuing until delivery or 34 weeks, whichever occurs first. Optional research blood samples will be collected at times of clinically indicated blood draws (baseline and 2-3 times per week) to measure nicotinamide and its metabolite 2-pyridone, sFlt-1 and PlGF. A sample of cord blood will be collected (6ml) following delivery.

All study subjects will receive standard surveillance and treatment for expectant management of EOP, which may include but is not limited to:

- Daily prenatal vitamin
- 24-hr urine collection for protein excretion at admission
- Blood sampling for hematologic, liver, and kidney function at time of diagnosis and every 2-3 days thereafter
- Magnesium sulfate for seizure prophylaxis
- Betamethasone to accelerate fetal lung maturity
- Antihypertensive agents
- Twice weekly fetal surveillance by NST (30 minutes of continuous fetal heart rate monitoring) or BPP (30 minutes of ultrasound to monitor for fetal breathing, movement, fluid)
- Weekly ultrasound to measure amniotic fluid and UA Dopplers

In addition to above standard care for EOP, subjects will receive surveillance and treatment solely for study purposes, including:

- Daily administration of vitamin B_3-amide, 2.5 Gm/day in 3 divided doses; “on treatment” is defined as within 24 hours of dose
- Daily (M-F) study staff visit to query side effects if in-patient; with each outpatient visit (typically twice a week) if discharged from the hospital
- Optional blood sampling to coincide with clinical blood draws to measure sFlt-1, nicotinamide and 2-pyridone levels; chart abstraction to review liver function testing results (done clinically)
- Chart abstraction for maternal and delivery data (up until maternal discharge) and neonatal data (until 30 days of age or until discharge, whichever occurs first). We will

also perform a phone call at 30 days post stopping the study medication and 30 days following infant delivery to determine the occurrence of any adverse events.

In addition to enrolling women with early-onset severe preeclampsia we will enroll 12 healthy control women (6 non pregnant, and 6 pregnant between 24-33 weeks 3 days). Study staff will interview control women and confirm medical history consistent with no exclusions. Once enrolled, healthy participants will have a baseline blood sampling for nicotinamide levels and LFTs. They will then ingest 1000 mg nicotinamide, and have blood drawn 2, 8 and 24 hours following the dose of nicotinamide. Urine samples will also be collected at baseline, and at 8 and 24 hours following the dose of nicotinamide. Participants will be given the option to remain in the clinic for the 2- and 8-hour blood collection and then return the following day for the 24 hour blood collection, or to be admitted to the UNC Clinical and Translational Research Center for all blood collection.

3.2 Study Outcomes

PRIMARY

- Maternal mean arterial blood pressure, defined as the highest MAP within 24 hour period

SECONDARY-SAFETY AND TOLERABILITY

- Liver toxicity, defined as 3x ULN of ALT or AST within 24 hours of study drug
- Maternal report of side effects
- Clinical parameters of maternal and fetal well being
- Composite neonatal outcome

Maternal or Fetal Parameter	Safety Endpoint
History	Headache unrelieved by oral analgesics
Physical examination	Abdominal tenderness, (right upper quadrant or epigastric)
Vaginal bleeding	Fall in hematocrit of $\geq$ 3%
Urine output	Oliguria (< 500 cc/24 hr)
Serum LFTs	AST and/or ALT $\geq$ 3x ULN within 24 hours of study drug
Fetal NST (or BPP)	Category III (or < 6)

SECONDARY-PHARMACOKINETICS

- Nicotinamide and metabolite concentrations in plasma at baseline, 2, 8, and 24 hours
- Peak plasma drug and metabolite concentration and time to reach peak
- Nicotinamide and metabolite elimination half-life
- Nicotinamide and metabolite area under the curve (integral of the plasma concentration-time curve)
- Nicotinamide and metabolite concentrations in urine at baseline, 8 and 24 hours

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria for participants with early-onset preeclampsia

1. Maternal age 18-45 years
2. Singleton or twin pregnancy with no known fetal anomalies
3. Early-onset preeclampsia OR early-onset severe gestational hypertension
    a. Early onset defined as 24-33 weeks 3 days gestation
    b. Preeclampsia defined as new onset hypertension and proteinuria: systolic BP $\geq$ 140 mm Hg and/or diastolic BP $\geq$ 90 mm Hg on two occasions 6 hours apart and > 300 mg proteinuria on 24 hour urine collection OR urine P/C ratio >0.3;

   c. Or BP ≥ 160 mm Hg and/or diastolic BP ≥ 105
   d. Dating criteria based on menstrual dating confirmed by first or second trimester ultrasound OR second trimester ultrasound if menstrual dating unavailable;
   e. Deemed clinically stable by primary clinician and candidate for expectant management (delayed delivery) for at least 48 hours;
   f. Dating criteria based on menstrual dating confirmed by first or second trimester ultrasound OR second trimester ultrasound if menstrual dating unavailable;
4. Deemed clinically stable by primary clinician and candidate for expectant management (delayed delivery);
5. Pre-existing medical diseases such as hypertension, diabetes, endocrine disorders, gastrointestinal diseases, are well controlled
6. Maternal liver function tests < 2x ULN
7. Maternal platelet count ≥ 100,000 mm^3
8. Fetal well-being established by estimated fetal weight ≥ 5^{th} %tile; normal amniotic fluid volume (MVP ≥ 2 cm); normal UA Dopplers; AND reactive NST or BPP > 6
9. Plan for expectant management until delivery
10. Delivery not anticipated within first 48 hours

4.2 Exclusion Criteria for participants with early-onset preeclampsia

1. Preeclampsia < 24 or ≥ 33 weeks 4 days gestation;
2. Multiple gestation;
3. Suspected fetal structural or chromosomal abnormality;
4. Pre-existing renal disease (creatinine ≥ 1.5 mg/dL)
5. Plan for delivery within 48 hours
6. Any pre-existing medical condition that would increase risk for liver toxicity (e.g. hepatitis B or C; HIV)
7. Eclampsia; cerebral edema on CT/MRI; headache unresolved with analgesics
8. Pulmonary edema
9. HELLP syndrome
10. Evidence of liver dysfunction (LFTs ≥ 2x ULN)
11. Thrombocytopenia (platelets < 100,000 mm^3)
12. Evidence of fetal compromise: EFW < 5^{th} percentile; BPP < 6; absent or reverse diastolic UA blood flow; oligohydramnios (MVP < 2 cm)
13. Placental abruption defined as unexplained vaginal bleeding
14. Preterm labor defined as regular contractions and cervical change
15. Any condition deemed by the investigator to be a risk to mother or fetus in completion of the study
16. Any condition deemed by the investigator to require delivery within 48 hours

4.1a Inclusion Criteria for healthy, normotensive, pregnant women

- Maternal age 18-45 years
- Singleton or twin pregnancy with no known fetal anomalies (24 weeks to 33 weeks 3 days)
- No known medical complications

4.2a Exclusion Criteria for healthy, normotensive, pregnant women

- Current cigarette smoker
- Hypertension requiring medical treatment
- Diabetes requiring medical treatment
- Pre-existing renal disease (creatinine ≥ 1.5 mg/dL)

- Any pre-existing medical condition that would increase risk for liver toxicity (e.g. hepatitis B or C; HIV; INH use)
- Currently taking medication for a chronic medical condition
- Currently taking an oral or IV antibiotic or antifungal medication

4.1b Diagnosis and Inclusion Criteria for healthy nonpregnant women
- Age 18-45 years
- No known medical complications or disease (eg hypertension, diabetes, liver or kidney disease)

4.2b Exclusion Criteria for healthy nonpregnant women
- Current cigarette smoker
- Hypertension requiring medical treatment
- Diabetes requiring medical treatment
- Pre-existing renal disease (creatinine $\geq$ 1.5 mg/dL)
- Any pre-existing medical condition that would increase risk for liver toxicity (e.g. hepatitis B or C; HIV; INH use).
- Currently taking medication for a chronic medical condition (excluding oral contraceptives)
- Currently taking an oral or IV antibiotic or antifungal medication

4.3 Subject Recruitment and Screening

Study personnel will review in-patient records or prenatal records under a limited waiver of HIPAA granted by the Institutional Review Board to determine if a patient meets eligibility requirements. If eligible, study personnel will approach medical provider to determine appropriateness of patient to serve as a study subject. If appropriate, study personnel will invite the patient to serve as a study subject.

Study personnel will describe the study in detail and review the study protocol with the patient. Women agreeing to participate will sign a consent form; one copy will be placed in their medical record, and a copy will be given to the patient for her records.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects may withdraw at any time and for any reason. Subjects may be withdrawn if they no longer meet eligibility criteria; or if in the opinion of the investigator it is not in the subject's best interest to continue. A subject may also be withdrawn if she experiences an unmanageable or irreversible adverse Grade 3 or 4 adverse event using the CTCA v3.0 criteria as defined in section on safety monitoring. The date of withdrawal from study participation will be recorded in the CRF. Subjects who either complete or are removed from the treatment phase at any time will have complete data chart abstracted and recorded for study purposes.

Specific study stopping rules include:
- Any maternal or fetal death deemed to be related or possibly related to study participation
- More than expected prevalence of liver toxicity within 24 hours of study drug, thus > 20% of subjects experience liver toxicity (more than 1 subject for every 5 subjects enrolled)
- More than expected prevalence of fetal side effects (defined as BPP < 6 OR UA Dopplers show absence or reversal of diastolic blood flow), (more than 1 delivery for fetal reasons per every 5subjects enrolled)

Specific rules to stop study intervention for individual subject

- Delivery, or two weeks of treatment ("on-treatment" defined as within 24 hours of study drug)
- Liver toxicity (LFTs ≥ 3x ULN within 24 hours of study drug)
- Maternal side effects that cannot be managed with oral medication

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Withdrawn subjects will have data collected through delivery events. The full data set will include subjects who enroll and receive at least 72 hours of vitamin B_3A dietary supplementation, as the primary outcome will be measured at 72 hours. Every effort will be made to obtain data on all enrolled subjects, even if they are withdrawn and deliver elsewhere.

5 Study Agent

5.1 Description

The investigational agent is
Vitamin B_3-amide; 2.5 Gm daily, provided as 500mg oral capsules in three divided doses: administered as 1000mg at 8AM and 12MN; 500mg at 4PM, provided by Major Pharmaceuticals

Wendy Ernst
Advisor, Contracts and Billing
17177 N Laurel Park Dr., Suite 233
Livonia, MI 48152
Office: (734) 743-6232 | Fax: (734) 743-7232
Email: wernst@major-pharm.com

5.2 Treatment Regimen

Subjects will receive vitamin B_3-amide dietary supplementation, 2.5Gm/day daily until delivery or 34 weeks' gestation whichever occurs first. "On-treatment" is defined as 24 hours of study drug.

5.3 Preparation and Administration of Study Agent

Major Pharmaceuticals will supply the vitamin B_3-amide (study agent). The study agent will be stored by either the UNC or the WakeMed pharmacy and dispensed via pharmacy services following enrollment.

5.4 Subject Compliance Monitoring

Medication admission records will be examined by study staff to confirm dispensing of the study agent. Subjects will be queried daily by study staff regarding compliance with study regimen. If the patient goes home on therapy, she will be given a weeks' supply of the study medication and her compliance monitored by study staff.

5.5 Prior and Concomitant Therapy

Subjects will have clinical treatment decisions made by the primary physician, independent of study participation. All subjects will receive standard care as noted below:

- Daily prenatal vitamin
- Blood sampling for hematologic, liver, and kidney function at enrollment and every 2-7

days thereafter
- Magnesium sulfate for seizure prophylaxis
- Betamethasone to accelerate fetal lung maturity
- Antihypertensive agents as needed, determined by primary clinician
- At least biweekly fetal surveillance by NST (30 minutes of continuous fetal heart rate monitoring) or BPP (30 minutes of ultrasound to monitor for fetal breathing, movement, fluid)
- Weekly ultrasound to measure amniotic fluid and UA Dopplers

5.6 Receiving, Storage, Dispensing and Return

5.6.1 Receipt of Supplies

Triangle Compounding Pharmacy will ship the vitamin B_3-amide directly to the Pharmacy. Upon receipt, an inventory will be performed and an investigational agent receipt log filled out and signed by the person accepting the shipment. Designated study staff will count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study agent in a given shipment will be documented in the study files.

5.6.2 Storage

The study agent will be stored in a cool, well-ventilated area at the Pharmacy. The vitamin B_3-amide should be kept away from heat, sources of ignition, and from incompatibles such as oxidizing agents, moisture. Vitamin B_3-amide is hydroscopic, air and light sensitive and should be stored in light-resistant containers**.**

5.6.3 Dispensing of Study Agent

The Pharmacy will dispense vitamin B_3-amide. Regular reconciliation will be performed to document study agent assigned, consumed, and remaining. This reconciliation will be logged on the study log.

5.6.4 Return or Destruction of Study Agent

At the completion of the study, there will be a final reconciliation of study agent shipped, consumed, and remaining. This reconciliation will be logged on the reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused investigational agent. Vitamin B_3-amide destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Eligibility

All study procedures will be conducted in the UNC Women's Clinic or Hospital. Study staff will review records on a daily basis. Eligibility for participation is outlined above. Briefly, women with suspected hypertensive complications of pregnancy at 24-33 3/7 weeks' gestation and no exclusion criteria are eligible. Eligible women will be approached after study staff obtains verbal consent to do so from the primary provider (study staff will confirm with primary provider that the woman is a candidate for expectant management/delayed delivery and there is a viable fetus).

Women who agree to participate will sign written consent and be assigned a unique study ID that will be used to identify all study data collection forms and research specimens. The master

list linking the unique study ID with subject PHI will be kept on a locked computer file in a password protected file.

Healthy women will be recruited from the UNC Women's Clinic at the time of a routine prenatal appointment or well-woman gynecologic visit. Eligible women will be approached after study staff obtains verbal consent to do so from the primary provider. Women who agree to participate will sign written consent and be assigned a unique study ID that will be used to identify all study data collection forms and research specimens. The master list linking the unique study ID with subject PHI will be kept on a locked computer file in a password protected file.

6.2 Study intervention and procedures for participants with early onset severe preeclampsia

The respective pharmacy will dispense vitamin B_3-amide, which will be administered given to the subject by study staff if out-patient or by in-patient nursing staff if in-patient. Study staff will meet with subjects at each prenatal visit or daily [(M-F) if in the hospital] to discuss compliance and side effects.

Clinical care will continue per recommendations of primary obstetrician caring for the patient. Standard procedures may include:

- 24-hr urine collection for protein excretion at admission
- Blood sampling for hematologic, liver, and kidney function at enrollment and every 2-3 days thereafter
- Physical examination by primary clinical provider
- Weight and intake/output measurement as ordered by primary clinical provider
- Magnesium sulfate for seizure prophylaxis
- Betamethasone to accelerate fetal lung maturity
- Antihypertensive agents
- Daily fetal surveillance by NST (30 minutes of continuous fetal heart rate monitoring) or BPP (30 minutes of ultrasound to monitor for fetal breathing, movement, fluid)
- Weekly ultrasound to measure amniotic fluid and UA Dopplers

The primary obstetrician will make decisions regarding indication and timing of delivery. Clinical management of the infant after delivery will be per standard treatment dictated by the neonatologist caring for the baby.

Study procedures include:

- Daily administration of vitamin B_3-amide, 2.5Gm given as administered as 1000mg at 8AM and 12MN; 500mg at 4PM
- Daily (if in-patient) or with each prenatal visit (if out-patient) study nurse visit to query side effects
- Optional blood sampling to coincide with clinical blood draws to measure nicotinamide and 2-pyridone, sFlt-1 and PlGF levels
- Optional blood sampling for Nicotinamide levels at the following times:
    - Day 1 - Baseline – pre 1st dose; Peak – 2 hrs post 1st dose; Trough – pre 2nd dose
    - Day 3 – Trough – pre AM dose; Peak – 2 hrs post AM dose
    - Day 7 - Trough – pre AM dose; Peak – 2 hrs post AM dose
    - Cord blood following delivery.
- Chart abstraction to review maternal liver function testing results, delivery data (up until discharge) and neonatal data (until 30 days of age or until discharge, whichever occurs

first)

6.2a Study intervention and procedures for healthy pregnant and non-pregnant controls

- Administration of a single dose (1000mg) of Nicotinamide at 8AM.
- Blood sampling at baseline (pre-dose), 2 hours post dose, 8 hours post dose and 24 hours post dose.
- Urine sample at baseline (pre-dose), 8 hours post dose and 24 hours post dose.
- This will be done either in the clinic or the CTRC

6.3 Laboratory specimen handling procedures

Blood for research purposes will be collected at the time of clinically indicated blood draws. Specimens will be labeled with the subject's unique study ID and processed.

Specimens to be tested for circulating sFlt-1 and PlGF levels will be stored and shipped as a single batch on dry ice to the research laboratory at Harvard University. The interassay coefficient of variance for sFlt1 and PlGF immunoassays ranges from 2.6% to 3.0%.

Specimens collected for nicotinamide and 2-pyridone measurements will be sent to the UNC School of Pharmacy Lab (Dr. Craig Lee). Dr. Lee developed a LC-MS/MS method using protein precipitation (acetonitrile) to measure nicotinamide and 2-pyridone during our previous phase I study.

7 Statistical Plan

7.1 Statistical Methods

Sample size: The study sample size was calculated to the primary outcome of MAP. We stipulated *a priori* that α =.05 and β = 0.2. Using the highest BP measured within each 24-hr period and applying the following formula we will calculate daily MAP:

$$\boldsymbol{mean\ arterial\ BP} = [\mathbf{2}(\boldsymbol{diastolic\ BP}) + \boldsymbol{systolic\ BP}] \div \mathbf{3}$$

Assuming that the mean (±sd) MAP at enrollment of our subjects is 107 (±10) mm Hg, to detect a difference from enrollment to 48 hours after treatment of 7 mm Hg in MAP requires that 20 subjects be enrolled. We will enroll 24 to allow for 20% attrition due to delivery prior to 48 hours of treatment.

- We will use a repeated measurement ANOVA to compare baseline MAP to MAP at 48 hours, 7 days and weekly thereafter until delivery or 34 weeks.
- Maternal liver toxicity: the proportion of women who develop elevated liver function tests (ALT and/or AST $\geq$ 3x ULN within 24 hours of study drug) will be determined and compared to expected proportion of 20%.
- Descriptive report of proportion of women with
    - Side effects
    - Fetal effects

7.2 Subject Population(s) for Analysis

All subjects will be included in the analysis, regardless of whether they received investigational agent. Subject demographic and baseline characteristics will be summarized. Descriptive summaries will include the number of subjects, mean, standard deviation, median, minimum and maximum for continuous parameters, frequency and percentages for categorical parameters.

8 Safety and Adverse Events

The PI and/or study staff will conduct daily medical monitoring for unanticipated problems, AEs, and serious AEs and record and report them to the UNC or WakeMed (respectively-dependent on where they are cared for) IRB and FDA as outlined below (Sections 8.2, 8.3, 8.5). The FDA will be notified after 1 case of liver toxicity and after every 1 case of delivery within 48 hours of enrollment, and will conduct ad-hoc review as they deem appropriate.

Study stopping rules and subject withdrawal for safety reasons are described previously (Section 4.4) and below (Section 8.4).

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research,

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Adverse Event Grading

Adverse events will be graded using the Common Terminology for Criteria for Adverse Events (CTCAE) version 4.03 (published June 14, 2010).

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated; limiting. (e.g., GI distress, nausea, vomiting; flushing)

Grade 2: Moderate; minimal, local or noninvasive intervention indicated. (e.g. GI distress requiring IV therapy)

Grade 3: Severe or medically significant but no immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

Adverse Event Reporting Period

The study period during which adverse events must be reported is defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up for the mother is defined as until discharge from the hospital and the infant as until discharge or 30 days of life, whichever occurs first.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality will be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

Post-study Adverse Event

The investigator will follow all unresolved adverse events until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator will notify the IRB of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The IRB will also be notified if the investigator becomes aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality will be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. discontinuation of the study agent, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery will be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required for an efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.
- Surgery is undertaken for delivery of the infant (cesarean section).

Potential Risks and/or Toxicities of Study Agent

The most serious risk of vitamin B_3-amide is liver toxicity. Potential risks of vitamin B_3-amide at doses $\underline{\leq}$ 3 gm/day include:

Rare (< 1%)
Liver toxicity
Facial erythema
Hives
Sore mouth
Dry hair
Fatigue

Infrequent (1-10%)
Flushing (< 2%)
Headache (1%)
Nausea (< 2%)
Heartburn (< 2%)

8.2 Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document, grouped under one diagnosis.

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators will conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others

If the report is supplied as a narrative, the minimum necessary information to be provided includes

Study Identifier	Current status
Study Center	Whether study treatment was discontinued
Subject number	Reason why event classified as serious
Date and description of the event	Investigator determination of association between event and treatment

Investigator reporting:

For reportable deaths, the initial submission to the UNC IRB may be made by contacting the IRB Director or Associate Director. The AE/Unanticipated Problem Form is required as a follow up to the initial submission. AEs are reported to the IRB if they are unexpected in nature, severity or frequency and related or possibly related to administration of study intervention.

Other Reportable events:

For clinical drug trials, the following events are also reportable to the UNC IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the PI to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.3.1 Sponsor reporting: Notifying the FDA

The study sponsor (in this case, the PI) is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study agent
  - unexpected,
  - fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  - associated with the use of the study agent,
  - unexpected, and
  - serious, but not fatal or life-threatening

  -or-

– a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

Any finding from tests in laboratory animals that:

– suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements

Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process

Adverse events may be submitted on FDA Form 3500A or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3. The contact information for submitting IND safety reports is noted below:

Charlene Williamson at Charlene.Williamson@fda.hhs.gov

8.4 Stopping Rules

The study will be stopped under the following circumstances:

- Any maternal or fetal death deemed to be related or possibly related to study participation
- >20% subjects experience liver toxicity (defined as AST or ALT ≥ 3x ULN within 24 hours of study drug)

Specific rules to stop study intervention for individual subject

- Delivery, or 34 weeks, which ever occurs first
- Liver toxicity (LFTs ≥ 3x ULN within 24 hours of study drug)
- Maternal side effects that cannot be managed with oral medication

8.5 Medical Monitoring

The PI will oversee the safety of the study at her site. This safety monitoring will include careful assessment and appropriate reporting of AEs as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious AEs.

The PI will monitor subject data for safety concerns. Laboratory data will be reviewed daily to determine if abnormalities occur.

8.6 Data Safety Monitoring Board

The NC TraCS Data and Safety Monitoring Board with serve as the DSMB of record for this single center clinical study. The master protocol has been reviewed and approved. The DSMB monitor safety using *a priori* defined study stopping rules and rules for withdrawal of subjects. The DSMB will review data after every 6 enrolled subjects; the PI will notify the DSMB after every 2 cases of liver toxicity and after every 2 cases of delivery within 48 hours of beginning nicotinamide. The NC TraCS DSMB Charter is available for review.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) will be the primary data collection instrument for the study. All data requested on the CRF will be recorded and all missing data will be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, "N/D" will be filled. If the item is not applicable to the individual case, "N/A" will be filled. All entries will be printed legibly in black ink. If any entry error has been made, to correct such an error, we will draw a single straight line through the incorrect entry and enter the correct data above it. All such changes will be initialed and dated. To clarify illegible or uncertain entries, the clarification will be printed above the item, initialed and dated.

9.4 Records Retention

The investigator will retain study essential documents and specimens for up to 10 years following study completion.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The PI will monitor the study to ensure quality and integrity of data collected. She will review study files, regulatory documents, and consent forms. The PI will allocate adequate time for such monitoring activities. The PI will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The PI will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.). Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, will be obtained before that subject undergoes any study procedure. The subject will sign the consent form, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

The funding source is NIH.

12.2 Conflict of Interest

All investigators will follow the University conflict of interest policy. None of the investigators have a conflict of interest.

12.3 *Subject Incentives*

Subjects will be provided compensation for participation. Each subject will receive $20 for the enrollment and baseline blood draw and $20 for each subsequent study blood draw (up to 3) then $20 for each week (or part of a week) of participation following the 2nd week which will include taking daily vitamin B_3-amide dietary supplement, up to 10 weeks ($240 maximum total)

Baseline (includes blood draw)	$20	
Day 3 trough	$20	
Day 3 Peak		$20
Day 7 blood draw and start of wk 2		$20
Week 3-10	$20 each week on study med	

References

1. Saftlas A, Olson, DR, Franks, AL, Atrash, HK, Pokras, R. Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986. . Am J Obstet Gynecol 1990;163:460-5.
2. Sibai BM. Preeclampsia: an inflammatory syndrome? Am J Obstet Gynecol 2004;191(4):1061-2.
3. Maternal mortality--United States, 1982-1996. MMWR Morb Mortal Wkly Rep 1998;47:705-7.
4. Benedetti T, Kates, R, Williams, V. Hemodynamic observations in severe preeclampsia complicated by pulmonary edema. Am J Obstet Gynecol 1985;152:330-4.
5. Drislane F, Wang, AM. Multifocal cerebral hemorrhage in eclampsia and severe pre-eclampsia. J Neurol 1997;244:194-8.
6. Morriss M, Twickler, DM, Hatab, MR, Clarke, GD, Peshock, RM, Cunningham, FG. Cerebral blood flow and cranial magnetic resonance imaging in eclampsia and severe preeclampsia. Obstet Gynecol 1997;89:561-8.
7. Cunningham F, Fernandez, CO, Hernandez, C. Blindness associated with preeclampsia and eclampsia. Am J Obstet Gynecol 1995;172:1291-8.
8. Xiao R, Sorensen, TK, Williams, MA, Luthy, DA. Influence of pre-eclampsia on fetal growth. J Matern Fetal Neonatal Med 2003;13:157-62.
9. Rasmussen S, Irgens, LM. Fetal growth and body proportion in preeclampsiaObstet Gynecol 2003;101:575-83.
10. Odegård R, Vatten, LJ, Nilsen, ST, Salvesen, KA, Austgulen, R. Preeclampsia and fetal growth. Obstet Gynecol 2000;96:950-5.
11. Sibai BM, Kustermann L, Velasco J. Current understanding of severe preeclampsia, pregnancy-associated hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, hemolysis, elevated liver enzymes, and low platelet syndrome, and postpartum acute renal failure: different clinical syndromes or just different names? Curr Opin Nephrol Hypertens 1994;3(4):436-45.
12. Friedman S, Schiff, E, Kao, L, Sibai, BM. Neonatal outcome after preterm delivery for preeclampsia. Am J Obstet Gynecol 1995; 172:1788-92.
13. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol 1999;180(2 Pt 1):499-506.
14. Dong M, He J, Wang Z, Xie X, Wang H. Placental imbalance of Th1- and Th2-type cytokines in preeclampsia. Acta Obstet Gynecol Scand 2005;84(8):788-93.
15. Harsem NK, Braekke, K., Staff, A.C. Augmented oxidative stress as well as antioxidant capacity in maternal circulation in preeclampsia. Eur J Obstet Gynecol Reprod Biol 2006;128:209-15.
16. Roberts JM, Taylor RN, Musci TJ, et al. Preeclampsia: an endothelial cell disorder. Am J Obstet Gynecol 1989;161(5):1200-4.
17. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350(7):672-83.
18. Crocker IP, Strachan BK, Lash GE, et al. Vascular endothelial growth factor but not placental growth factor promotes trophoblast syncytialization in vitro. J Soc Gynecol Investig 2001;8(6):341-6.
19. Desai J, Holt-Shore V, Torry RJ, Caudle MR, Torry DS. Signal transduction and biological function of placenta growth factor in primary human trophoblast. Biol Reprod 1999;60(4):887-92.
20. Shore VH, Wang TH, Wang CL, et al. Vascular endothelial growth factor, placenta growth factor and their receptors in isolated human trophoblast. Placenta 1997;18(8):657-65.

21. Clark DE, Smith SK, He Y, et al. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. Biol Reprod 1998;59(6):1540-8.
22. Lu F, Longo M, Tamayo E, et al. The effect of over-expression of sFlt-1 on blood pressure and the occurrence of other manifestations of preeclampsia in unrestrained conscious pregnant mice. Am J Obstet Gynecol 2007;196(4):396 e1-7.
23. Murphy SR, LaMarca BB, Cockrell K, Granger JP. Role of endothelin in mediating soluble fms-like tyrosine kinase 1-induced hypertension in pregnant rats. Hypertension 2010;55(2):394-8.
24. Li F, Hagaman JR, Kim HS, et al. eNOS deficiency acts through endothelin to aggravate sFlt-1-induced pre-eclampsia-like phenotype. J Am Soc Nephrol 2012;23(4):652-60.
25. Kim SY, Ryu HM, Yang JH, et al. Increased sFlt-1 to PlGF ratio in women who subsequently develop preeclampsia. J Korean Med Sci 2007;22(5):873-7.
26. Masuyama H, Nakatsukasa H, Takamoto N, Hiramatsu Y. Correlation between soluble endoglin, vascular endothelial growth factor receptor-1, and adipocytokines in preeclampsia. J Clin Endocrinol Metab 2007;92(7):2672-9.
27. Rana S, Powe CE, Salahuddin S, et al. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. Circulation 2012;125(7):911-9.
28. McElrath TF, Lim KH, Pare E, et al. Longitudinal evaluation of predictive value for preeclampsia of circulating angiogenic factors through pregnancy. Am J Obstet Gynecol 2012;207(5):407 e1-7.
29. McKeeman GC, Ardill JE, Caldwell CM, Hunter AJ, McClure N. Soluble vascular endothelial growth factor receptor-1 (sFlt-1) is increased throughout gestation in patients who have preeclampsia develop. Am J Obstet Gynecol 2004;191(4):1240-6.
30. Costantine MM, Tamayo E, Lu F, et al. Using pravastatin to improve the vascular reactivity in a mouse model of soluble fms-like tyrosine kinase-1-induced preeclampsia. Obstet Gynecol 2010;116(1):114-20.
31. Fox KA, Longo M, Tamayo E, et al. Effects of pravastatin on mediators of vascular function in a mouse model of soluble Fms-like tyrosine kinase-1-induced preeclampsia. Am J Obstet Gynecol 2011;205(4):366 e1-5.
32. Kumasawa K, Ikawa M, Kidoya H, et al. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. Proc Natl Acad Sci U S A 2011;108(4):1451-5.
33. Bergmann A, Ahmad S, Cudmore M, et al. Reduction of circulating soluble Flt-1 alleviates preeclampsia-like symptoms in a mouse model. J Cell Mol Med 2010;14(6B):1857-67.
34. Jaconello P. Niacin versus niacinamide. CMAJ 1992;147(7):990.
35. Belenky P, Bogan KL, Brenner C. NAD+ metabolism in health and disease. Trends Biochem Sci 2007;32(1):12-9.
36. National Research Council: Recommended Dietary Allowences. 10th ed: National Academy Press; 1989.
37. Hoffer A. Biochemistry of nicotinic acid and nicotinamide. Psychosomatics 1967;8(2):95-100.
38. Ieraci A, Herrera DG. Nicotinamide protects against ethanol-induced apoptotic neurodegeneration in the developing mouse brain. PLoS Med 2006;3(4):e101.
39. Morales P, Simola N, Bustamante D, et al. Nicotinamide prevents the long-term effects of perinatal asphyxia on apoptosis, non-spatial working memory and anxiety in rats. Exp Brain Res 2010;202(1):1-14.
40. Shalita AR, Smith JG, Parish LC, Sofman MS, Chalker DK. Topical nicotinamide compared with clindamycin gel in the treatment of inflammatory acne vulgaris. Int J Dermatol 1995;34(6):434-7.

41. Hoffer A, Prousky J. Successful treatment of schizophrenia requires optimal daily doses of vitamin B3. Altern Med Rev 2008;13(4):287-91.
42. Birkmayer JG. Coenzyme nicotinamide adenine dinucleotide: new therapeutic approach for improving dementia of the Alzheimer type. Ann Clin Lab Sci 1996;26(1):1-9.
43. Demarin V, Podobnik SS, Storga-Tomic D, Kay G. Treatment of Alzheimer's disease with stabilized oral nicotinamide adenine dinucleotide: a randomized, double-blind study. Drugs Exp Clin Res 2004;30(1):27-33.
44. Kaanders JH, Pop LA, Marres HA, et al. Accelerated radiotherapy with carbogen and nicotinamide (ARCON) for laryngeal cancer. Radiother Oncol 1998;48(2):115-22.
45. Vague P, Picq R, Bernal M, Lassmann-Vague V, Vialettes B. Effect of nicotinamide treatment on the residual insulin secretion in type 1 (insulin-dependent) diabetic patients. Diabetologia 1989;32(5):316-21.
46. Hoffer A. Use of nicotinic acid and/or nicotinamide in high doses to treat schizophrenia. Can J Psychiatr Nurs 1966;7(8):5-6.
47. Hoffer A. Adverse effects of niacin in emergent psychosis. JAMA 1969;207(7):1355.
48. Elliott RB, Pilcher CC, Fergusson DM, Stewart AW. A population based strategy to prevent insulin-dependent diabetes using nicotinamide. J Pediatr Endocrinol Metab 1996;9(5):501-9.
49. Lampeter EF, Klinghammer A, Scherbaum WA, et al. The Deutsche Nicotinamide Intervention Study: an attempt to prevent type 1 diabetes. DENIS Group. Diabetes 1998;47(6):980-4.
50. Gale EA, Bingley PJ, Emmett CL, Collier T. European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. Lancet 2004;363(9413):925-31.
51. Reprotox. "http://www.reprotox.org/Members.AgentDetail.aspx?a=1722". Accessed February 21 2013.
52. Bennet P, editor. Drugs and Human Lactation. Amsterdam, NY: Oxford; 1988. The WHO Working Group.
53. Dragovic J, Kim SH, Brown SL, Kim JH. Nicotinamide pharmacokinetics in patients. Radiother Oncol 1995;36(3):225-8.
54. Stratford MR, Dennis MF, Hoskin PJ, et al. Nicotinamide pharmacokinetics in normal volunteers and patients undergoing palliative radiotherapy. Acta Oncol 1996;35(2):213-9.
55. Stratford MR, Rojas A, Hall DW, et al. Pharmacokinetics of nicotinamide and its effect on blood pressure, pulse and body temperature in normal human volunteers. Radiother Oncol 1992;25(1):37-42.
56. Knip M, Douek IF, Moore WP, et al. Safety of high-dose nicotinamide: a review. Diabetologia 2000;43(11):1337-45.