NIAGEN® **in Paclitaxel-induced Peripheral Neuropathy**

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CLINICAL STUDY PROTOCOL

NIAGEN® in Paclitaxel-induced Peripheral Neuropathy

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Study Number -

Investigational Product - Nicotinamide riboside

Clinical Phase - Phase II

Sponsor - National Cancer Institute

Protocol Version – March 24, 2021

Confidentiality Statement:

Synopsis

Primary Objective

The purpose of this single-arm phase II trial is to determine whether nicotinamide riboside (NR) prevents the progression of peripheral sensory neuropathy in patients receiving paclitaxel or nab-paclitaxel for treatment of stage IV breast cancer, or platinum-resistant recurrent ovarian, peritoneal, endometrial, or fallopian tube cancer. A decision to propose a large multi-center phase III trial of nicotinamide riboside for the relief of sensory neuropathy and the experimental design of that trial will be based on these findings.

Secondary Objectives

The secondary objective of this single-arm phase II trial is to determine whether treatment with nicotinamide riboside can prevent reductions in the dose of taxane therapy due to the incidence or severity of neuropathy.

Exploratory Objective

The utility of the Total Neuropathy Score – clinical (TNS-c) questionnaire to monitor the progression of peripheral sensory neuropathy will be explored.

Primary Outcome Variables

The primary outcome variable is binary (yes/no) and defined as no worsening of the grade of peripheral sensory neuropathy, scored according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 guidelines, from the initiation of NIAGEN® treatment to the conclusion of NIAGEN® treatment 7 to 14 days after the final taxane infusion.

Secondary Outcome Variables

Secondary outcome variables for this trial include (1) the percentage of patients that experience a dose reduction of taxane therapy due to neuropathy; (2) the incidence of dose reduction events due to neuropathy (each occasion of dose reduction is a separate event); (3) the total cumulative dose of taxane administered, and (4) the score on the 11-item NTX subscale of the FACT&GOG-NTX questionnaire determined at the conclusion of NIAGEN® treatment 7 to 14 days after the final taxane infusion.

Study Duration

Study duration is expected to be no longer than three years from enrollment of the first subject to completion of followup on the last subject.

Study Design

The study design is an open-label, prospective non-randomized, single-arm phase II trial.

Study Population

This trial will be conducted in English-speaking patients who are 18 to 85 years old with a diagnosis of stage IV (metastatic) breast cancer, platinum-resistant recurrent ovarian, peritoneal, endometrial, or fallopian tube cancer, or platinum-resistant recurrent or metastatic head and neck cancer who are receiving intravenous infusions of paclitaxel or nab-paclitaxel as treatment. To enroll, the patient must report at least a grade 1 peripheral neuropathy after initiation of paclitaxel or nab-paclitaxel therapy or present with a residual CIPN ≤ 2 from prior therapy, scored according to the CTCAE guidelines for peripheral sensory neuropathy.

Number of Participants

We anticipate that 39 patients will develop grade 1 neuropathy and meet eligibility requirements for enrollment in this study during the three years of the study.

Number of Study Sites

There will be two study sites: University of Iowa Hospitals and Clinics, Iowa City, IA and Wake Forest Baptist Medical Center, Winston-Salem, North Carolina.

Study Calendar	Study Treatment Days [a] (± 2 days)													EOT	Follow - up		
Study Procedures	Screening[b]	1[j]	8	15	22	29	36	43	50	57	64	71	78	7-14 days after last taxane dose	30-37 days after last dose of NIAGEN [h]	12-14 week Post treatment follow up [h]	24-26 week Post treatment follow up [h]
Scoring of Peripheral Neuropathy by CTCAE [f]	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X
Complete NTX subscale of FACT&GOG	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X
Progress Note [e]	X				X			X			X			X			
Recording of Dairy Intake	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X
Collect Drug Diary			X	X	X	X	X	X	X	X	X	X	X				
Recording of NIAGEN® Self-Medication																X	X
Vital Signs	X		X	X	X	X	X	X	X	X	X	X	X	X			
Physical Exam, including weight [e]; height only at screening	X				X			X			X			X			
ECOG Performance Status [e]	X				X			X			X			X		X	X
Review of AEs and SAEs [c/d]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pretreatment NAD level [g]		X	X	X	X	X	X	X	X	X	X	X	X				
Taxane infusion [a]		X	X	X	X	X	X	X	X	X	X	X	X				
Post-treatment taxane level [g]		X[i]	X	X	X	X	X	X	X	X	X	X	X				
Pregnancy (serum or urine)	X																

Dispense 75 mg capsules of NIAGEN® [k]		X								-							
Dispense 250 mg capsules of NIAGEN® [k]			X				X				X						
Chemistry [e]	X		X	X	X	X	X	X	X	X	X	X	X	X			
CBC, differential, Platelets	X		X	X	X	X	X	X	X	X	X	X	X	X			
AST, ALT, Alk-Phos, Bili, Albumin	X		X	X	X	X	X	X	X	X	X	X	X	X			
Scoring of neuropathy by Total Neuropathy Score – clinical [l]	X		X				X				X			X			

[a] The calendar is based a treatment program of 12 weekly infusions. However, for any one patient, weeks on therapy may range from as few as 1 to as many as12. The actual number and timing of planned taxane infusions is at the physician's discretion in accordance with standard of care for that disease. For those treatment programs in which infusions may occur every other week, or several weeks in a row followed by one week "off", all indicated measures will be taken at the time of visit.

[b] All screening assessments may be collected up to 3 days prior to the screening visit.

[c] To use CTCAE version 4.03 guidelines .

[d] All serious adverse events will be reported within 24 hours of the discovery of the event. See Appendix B.

[e] Include testing of Creatinine, NA, K, Co and BUN.

[f] Scoring of Peripheral Neuropathy by CTCAE can be done by oncologist or nurse.

[g] See Appendix C for lab procedures.

[h] This may be collected per phone interview.

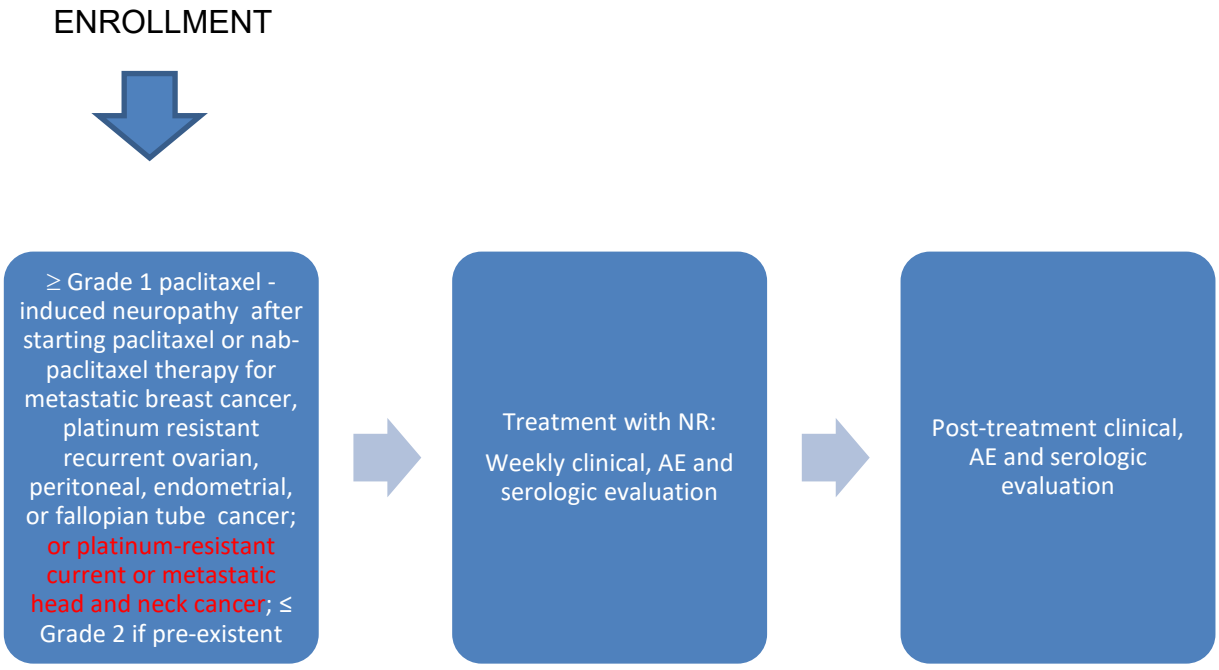
[i] On Day 1, this level needs to be collected 30 ± 10 minutes after the paclitaxel dose, prior to starting NR. Thereafter, this blood sample needs to be collected 30 ± 10 minutes after the paclitaxel dose. For patients receiving nab-paclitaxel, the blood sample needs to be collected between 15 and 30 min after infusion ends.

[j] Screening and Treatment Day 1 visits may be combined.

[k] On day 1, a one week supply (or enough to get patient to next visit) of the 75 mg capsules will be dispensed. Thereafter, a one-month supply or (on the last visit) enough of the 250 mg capsules to supply the patient through day 7 after the final taxane infusion will be dispensed. Treatment with NIAGEN® will conclude 7 days after the last, planned infusion of taxane. Treatment with NIAGEN will not exceed 12 weeks.

[l] Assessment of neuropathy by a clinician trained in the neurological assessment procedures using the Total Neuropathy Score - clinical

Study Schema



Abbreviations

Abbreviation	Explanation
FACT&GOG-NTX	Functional Assessment of Cancer Therapy Gynecologic Oncology Group – Neurotoxicity questionnaire
CIPN	Chemotherapy-induced peripheral neuropathy
CTCAE	Common Terminology Criteria of Adverse Events
FDA	Food and Drug Administration
TNS-c	Total neuropathy score – clinical questionnaire
GMP	Good manufacturing practices
NAD	Nicotinamide adenine dinucleotide
IRB	Institutional Review Board
NR	Nicotinamide Riboside
NMNAT1	Nicotinamide nucleotide adenylyl transferase 1
SARM	sterile-motif containing and armadillo-motif containing protein
UIHC	University of Iowa Hospitals and Clinics
HCCC	Holden Comprehensive Cancer Center
AE	Adverse event
SAE	Serious adverse event
1-MeNAM	1-methyl-nicotinamide
2-PY	N-methyl-2-pyridone-5-carboxamide

Glossary of Terms

Glossary	Explanation
Allodynia	Pain due to a stimulus that does not normally evoke pain.
dysesthesias	An unpleasant abnormal sensation, whether spontaneous or evoked.
hyperalgesia	Increased pain produced by a stimulus that does not normally cause pain

Table of Contents

1 - Introduction

This document is a protocol for the conduct of research in human subjects. The purpose of this document is to ensure that the research protocol is conducted in accordance with all institutional and governmental regulations, and with the approval of the Institutional Review Board.

2 - Background

2.1 Background/prevalence of research topic

2.1.1. Chemotherapy-induced peripheral neuropathy (CIPN) is a significant health care problem.

Peripheral neuropathies are a dose-limiting, disabling, and debilitating side effect of virtually every known class of chemotherapeutic agent. When assessed within 30 days of completing therapy, 68% of patients have CIPN [1]. Although the specific characteristics of CIPN are a function of the agent, the incidence and severity of CIPN increase as the cumulative dose, frequency of administration, and the number of therapeutic cycles increase. Patients experience paresthesias, dysesthesias (an unpleasant abnormal sensation, whether spontaneous or evoked), hyperalgesia (increased pain from a stimulus that normally provokes pain), allodynia (pain due to a stimulus that does not normally provoke pain), numbness or loss of sensation, or ongoing pain that is burning, lancinating or electric shock-like in nature. These signs occur first in the distal hands and feet and progress proximally in a stocking and glove distribution as far as the knees and elbows, reflecting the selective vulnerability of the longest nerves. CIPN can seriously diminish a patient's quality of life, and can interfere with self-care and activities of daily living. The severity of CIPN may also necessitate reducing the dose of chemotherapeutic agent, delaying the next cycle of chemotherapy or terminating treatment entirely [2-5]. Less well appreciated is the persistence of CIPN for months to years after completion of chemotherapy in as many as 30% of cancer survivors [1, 2]. Given that advances in cancer diagnosis and treatment of cancer have increased the number of survivors to nearly 14.5 million in 2014 [6], up to 4.5 million survivors may suffer from CIPN after their treatment is completed.

2.1.2. Therapeutic options for CIPN remain an unmet medical need.

CIPN can no longer be considered a toxic side effect of chemotherapy to be "endured" as the price for longer survival or managed simply by reducing the dose or frequency of chemotherapy. The American Society of Clinical Oncology considers the development of adjuncts for the prevention and relief of CIPN as essential for patient care [7]. Much effort has gone into testing drugs like gabapentin, lamotrigine, and amitriptyline (used to treat other sensory neuropathies) only to learn that they are largely ineffective against CIPN. Perhaps this is not surprising given that the number needed to treat to demonstrate benefit by these drugs in those neuropathies is 4 to 5 [8-10]. At present, duloxetine is the only evidence-based treatment for the prevention or relief of CIPN in patients [4, 11, 12]. However, while duloxetine

ameliorated CIPN produced by platinum compounds, it was no better than placebo in relieving CIPN produced by taxanes [13]. Moreover, its efficacy was subsequently linked to the emotional health of the patient [14]. Given that the peripheral neuropathies induced by the different classes of chemotherapeutic agent are mechanistically distinct [15], it is unlikely that one agent will uniformly relieve CIPN produced by different classes of chemotherapy. Thus, effective treatment for CIPN remains a significant unmet need in healthcare.

2.1.3. Bioenergetic deficits in CIPN and the protective role of nicotinamide adenine dinucleotide (NAD^+)

Multiple mechanisms are implicated in the development and maintenance of CIPN [15-18]. In the case of paclitaxel, these mechanisms include bioenergetic deficits arising from mitochondrial dysfunction and nitro-oxidative stress that may contribute to the axonal degeneration, slowing of nerve conduction velocity, and epidermal nerve fiber loss observed in CIPN. In recent years, evidence has accrued that nicotinamide adenine dinucleotide (NAD^+), an essential redox coenzyme required for cell viability, basic bioenergetics and fast axonal transport [19, 20], plays an important role in protection against axonal injury from either mechanical or neurotoxic injury [21-25]. Maintenance of NAD^+ has also been shown to be protective in mitochondrial disease [26].

The neuroprotective role of NAD^+ first came to light with the Wallerian degeneration slow mouse, in which axons degenerate much more slowly after transection. This effect is attributed to a chromosomal aberration that causes overexpression of a biosynthetic enzyme for NAD^+ (NMNAT1, Nicotinamide nucleotide adenylyl transferase-1) in axons and mitochondria [27], and enhances NAD^+ biosynthesis. Conversely, axonal injury is accompanied by rapid degradation of another isoform of this NAD^+ biosynthetic enzyme (NMNAT2) [21-24, 28]. Injury or chemotherapeutic agents can also lead to decreased NAD^+ in axons due to activation and polymerization of sterile-motif-containing and armadillo-motif containing protein (SARM), which is required for Wallerian degeneration [25]. Surgically transected nerves can be rescued from degeneration in vitro by lentiviral introduction of a NAD^+ biosynthetic gene and the addition of NAD^+ precursors (e.g. nicotinic acid or nicotinamide) [23].

2.1.4. Nicotinamide riboside is a source of NAD^+.

Any process that consumes NAD^+ demands compensatory de novo synthesis from dietary tryptophan or synthesis from one of three vitamin precursors: nicotinic acid, nicotinamide, or the more recently discovered vitamin nicotinamide riboside (Fig. 1). Nicotinamide riboside is a naturally occurring precursor of NAD^+; small quantities are present in whey and milk [29, 30]. Now considered a third major form of vitamin B3 [31], it is readily available to consumers commercially as NIAGEN® [25].

2.2 Preclinical Experience

The efficacy of NIAGEN® was tested in a rodent model of CIPN in which female Sprague-Dawley rats received three intravenous injections of paclitaxel of a span of five days for a total dose of 19.8 mg/kg [32]. As per FDA guidelines for scaling doses among species [33], this dose of paclitaxel scales to 120 mg/m^2 body surface area and approximates that used in humans to treat breast cancer [34, 35]. Paclitaxel-treated rats develop tactile hypersensitivity and exhibit place escape-avoidance behaviors to repetitive stimulation of the hindpaw with a filament that is not noxious when used in untreated rats. When administered prophylactically for seven days before injection of paclitaxel and continuing for 11 days thereafter, once daily oral administration of 200 mg/kg NIAGEN® prevented the development of tactile hypersensitivity and blunted escape-avoidance behaviors in these rats. When administered therapeutically 14 days after paclitaxel, when tactile hypersensitivity was well-established, once daily oral administration of 200 mg/kg NIAGEN® progressively alleviated tactile hypersensitivity in 50% of the rats and blunted place escape-avoidance behaviors. The prophylactic dose regimen increased levels of NAD^+ in the blood of rats by 40% when assessed after three weeks administration. This dose of NIAGEN® scales to 32 mg/kg in human.

The efficacy of NIAGEN® was also tested in a mouse model of prediabetes and Type 2 diabetes [36]. Dietary supplementation with ~185 mg/kg nicotinamide riboside for eight weeks decreased weight gain, hepatic steatosis, circulating cholesterol levels and alanine aminotransferase levels. It improved non-fasting glucose levels and tended to normalize hemoglobin A1c. It also prevented the slowing of sensory nerve conduction velocity, protected against the loss of intraepidermal nerve fibers, and reversed heat insensitivity that are hallmarks of Type 2 diabetes.

A study was conducted to determine whether the beneficial effect of NIAGEN® was due to an inhibition of the actions of paclitaxel. As myelosuppression is a hallmark of paclitaxel treatment, this study determined whether NIAGEN® treatment interfered with the myelosuppressive effects of paclitaxel. Female rats were dosed with 200 mg/kg/day NIAGEN® orally or with water for one week prior to i.v. injection of three divided doses of paclitaxel (cumulative dose of 19.8 mg/kg) and continuing for 6 days after the first dose of paclitaxel. Blood samples were drawn at the end of the study for a complete blood count. In NR-treated rats, paclitaxel reduced numbers of white blood cells, red blood cells, hemoglobin and hemocrit to the same extent as in vehicle-treated rats. These data suggest that NIAGEN® will not interfere with the chemotherapeutic actions of paclitaxel.

Complementary *in vitro* studies were conducted to determine whether NIAGEN® would induce transcript for or inhibit the activity of the P450 enzymes involved in metabolism of paclitaxel. No induction of transcript for CYP1A2, CYP2B6, CYP3A4, or CYP2C8 occurred in primary hepatocytes isolated from three different human donors following incubation with 0.34, 3.4 or 34 μM NIAGEN®. Assay of lactate dehydrogenase release following three days' incubation of primary hepatocytes from three different donors did not identify any cytotoxic effects of 0.34,

3.4 or 34 μM NIAGEN®. Concentrations of NIAGEN® as high as 33 μM did not inhibit CYP2C8 or CYP3A4 activity in human liver microsomes. To further test for possible inhibition, a time-dependent assay was conducted in which NIAGEN® was incubated in human liver microsomes for 30 minutes with and without NADPH. Unlike the positive controls, concentrations as high as 50 μM NIAGEN® did not inhibit CYP2C8 or CYP3A4, and no leftward shift was observed in the presence of NADPH. These data provide further support for the expectation that NIAGEN® will not interfere with the chemotherapeutic actions of paclitaxel.

Additional in vivo studies were conducted to determine whether NIAGEN® would promote tumorigenesis or interfere with the chemotherapeutic actions of paclitaxel. For these studies, the carcinogen N-methyl-nitrosourea was injected in female rats to induce cancer in the chain of mammary glands. This is a well-established rodent model of breast cancer [37]. The first set of studies examined whether NIAGEN® would facilitate tumorigenesis. For these studies, groups of 14 rats received vehicle or 200 mg/kg NIAGEN® once daily beginning 6 weeks after injection of MNU to assess the effects of NIAGEN® on the natural progression of tumor development. At this time-point, only a few rats exhibited palpable tumors. These rats continued treatment with vehicle or NIAGEN® for another 5 weeks, at which time they were euthanized. Dependent measures included number of palpable tumors, size of tumors, number of tumors/rat and total tumor load for the cohort. This experiment was replicated twice. The results indicated that five weeks treatment with NIAGEN® did not alter tumor growth compared to vehicle-treated rats. The second set of studies examined whether NIAGEN® would interfere with the anti-tumor effects of paclitaxel. For these studies, 25 rats were treated with MNU and upon the appearance of a palpable tumor received a cumulative dose of 19.8 mg/kg paclitaxel over five days. Daily treatment with 200 mg/kg NIAGEN® did not interfere with the ability of paclitaxel to decrease in tumor growth and did not interfere with tumor growth in rats as compared to values in vehicle treated rats. These data indicate that NIAGEN® will neither interfere with the effects of paclitaxel, nor facilitate tumor growth.

Preclinical studies concluded that NIAGEN® was not cytotoxic as determined by its lack of activity in a bacterial reverse mutagenesis assay, an in vitro chromosome aberration assay, and an in vivo micronucleus assay. Acute toxicity testing of a single oral dose of 5000 mg/kg in male and female Sprague-Dawley rats identified no gross pathological changes in the subsequent 14 days. A 14-day repeat dose study [38] of 2500 and 5000 mg/kg/day in male and female Sprague Dawley rats identified an 8% reduction in weight and feed consumption at the highest dose in male rats. No adverse effects were observed in female rats. A 90-day subchronic toxicity study was conducted with 300, 1000 and 3000 mg/kg/day in male and female rats. There was no mortality at any dose. In male rats, the two lowest doses caused a < 10% loss in weight and the highest dose caused a 17% decrease in body weight. Female rats exhibited no weight loss. Significant changes in hematological and clinical chemistry parameters occurred at the 1000 and 3000,

but not 300 mg/kg, doses in male and female rats. All three doses produced significant decreases in organ weights in male rats, but only marginal effects in female rats. At the 3000 mg/kg/day dose, histopathological findings included centrilobular hepatocellular hypertrophy in both male and female rats, as well as degeneration or atrophy of seminiferous tubules in male rats and hypertrophy of corpora lutea in female rats

An OECD-compliant rat developmental toxicity study was conducted in which pregnant Sprague-Dawley rats were administered 0, 325, 750 or 1500 mg /kg/day NIAGEN® by gavage from gestational day 5 – 19. Although no morbidity/mortality or adverse clinical signs were reported in the dams at any dose level, the NOAEL for dams and fetuses was determined to be 350 mg/kg/day and 750 mg/kg/day, respectively. These values were established due to observations that dams (1) exhibited treatment-related significant reductions in maternal body weight, corrected for body weight gain, and feed consumption at 750 and 1500 mg/kg/day and (2) a significant decrease in maternal uterine weights and an increase in late resorptions in dams occurred at 1500 mg/kg/day. Fetuses exhibited a significant decrease in fetal weights and an increase in the incidence of fetal anasarca at 1500 mg/kg/day. Fetuses also exhibited significant increases in minor anomalies, such as extra, accessory and rudimentary ribs at 1500 mg/kg/day, which were attributed to maternal stress evidenced by decrease in body weight and food consumption in the dams.

An OECD-compliant one-generation reproduction study in was conducted in rats. NIAGEN® was mixed in an experimental diet at 0, 3000, 6000, and 12000 ppm and provided (ad libitum) to male and female rats (n=25 rats/sex/group). No treatment-related clinical signs or mortalities were observed at any of the doses tested. At 12,000 ppm, a significant treatment-related decrease in body weight (4.6 to 6.5%) was observed in males from treatment Day 43 to 92 (measured at weekly intervals), when compared to control group. The decrease was <10% and therefore considered to be not adverse. The food consumption was unaffected in males at all of the doses tested. In females, body weights and food consumption were unaffected during pre-mating, gestation and lactation periods. With respect to fertility parameters, the duration of gestation (gestation length), pre-coital time, and fertility indices were not affected by the treatment at all doses tested, when compared to the control group. The survival indices of the pups were unaffected by the treatment at all the doses tested, when compared to the control group. At first observation on Day 1 of littering, no abnormalities were observed in live and dead pups at all the doses tested. Finally, there were no test item-related changes in terminal body weights/organ weights, gross and histopathology endpoints in adult rats of either sex. The pups sacrificed at weaning did not reveal any gross abnormalities. The above results support a NOAEL for fertility and reproductive performance of 12,000 ppm (equal to 675.21 mg/kg/day in males and 1,088.38 mg/kg/day in females) under the testing conditions and doses employed.

A safety pharmacology screening study was conducted to quantify the *in vitro* effects of NIAGEN® on the potassium-selective I_{Kr} current generated in normoxic conditions in stably transfected Human Embryonic Kidney cells (HEK 293 cells). The hERG assay is used to assess the potential of a compound to interfere with the rapidly activating delayed-rectifier potassium channel. NIAGEN®, over the range of concentrations tested in this study (0.034 to 1.03 mM), caused no inhibition of hERG tail current density.

2.3 Clinical Experience

2.3.1. Single-dose, double-blind, randomized, cross-over pharmacokinetic study in healthy adults [39]

The safety, pharmacokinetics and efficacy of single administration of three dosages of NIAGEN® was studied in healthy human subjects (ages 30 – 55 years) in a randomized, double-blind three-arm crossover trial [39]. Results indicate that NIAGEN® is metabolized similarly to nicotinamide in healthy humans and can be utilized as a form of Vitamin B3. Urine and plasma concentrations of nicotinamide riboside metabolites nicotinamide, *N*-methyl-nicotinamide, *N*-methyl-4-pyridone-5-carboxamide and *N*-methyl-2-pyridone-5-carboxamide exhibited a dose dependent relationship for each collection time (0-6 hr, 6-12 hr, and 12-24 hr) following treatment with 100 mg, 300 mg, or 1000 mg NIAGEN®. Increased levels of nicotinamide, *N*-methyl-nicotinamide, *N*-methyl-4-pyridone-5-carboxamide and *N*-methyl-2-pyridone-5-carboxamide are indicative of underlying increases in cellular NAD^+ concentrations.

No clinically adverse effects on hematology, clinical chemistry, urinalysis or liver or kidney function parameters were noted. Evaluation of vital sign measures showed that there were no differences between 100 mg, 300 mg or 1000 mg dosages of NIAGEN® in systemic blood pressure, diastolic blood pressure, or heart rate. All values for blood pressure and heart rate were at a normal and acceptable range for healthy adults. There were no between group differences in hemoglobin, hematocrit, numbers of white blood cells or red blood cells, mean corpuscular volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelets, lymphocytes, or monocytes in 100 mg, 300 mg and the 1000 mg dosages of NIAGEN®, except for neutrophils. Participants administered 300 mg of NIAGEN® showed an increase while participants administered with 1000 mg of NIAGEN® showed a decrease in neutrophils resulting in a significant difference between these two groups ($p=0.04$). However, this difference is most likely due to one individual with a low level of neutrophils in the NIAGEN® 1000 mg dosage group that was not considered clinically significant. Levels of aspartate aminotransferase, alanine transaminase, γ-glutamyl transaminase, total bilirubin, creatinine, and estimated glomerular filtration rate, as well as the electrolytes sodium, potassium, and chloride

were not different at 24 hour post-dose between the 100 mg, 300 mg or the 1000 mg dosage groups of NIAGEN®.

A total of 18 adverse events (AE) were reported by 10 participants during the study. All AEs ranged in severity from mild to moderate. A total of 5 AE's were reported by 5 participants receiving the 100 mg dose of NIAGEN®. Two of these were categorized as General Disorders and Administration Site Conditions (feeling of warmth and tiredness). Because these were not seemingly dose-dependent, both AE's were interpreted by the contract research organization as unlikely related to the 100 mg dose of NIAGEN®. One AE was categorized as Skin and Subcutaneous Disorder and another as Nervous System Disorder. Both were considered to be "unlikely" related to the 100 mg dose of NIAGEN®. One AE categorized as Investigations (decrease in hemoglobin) was classified as "possibly" related to the study product and the participants were withdrawn from the study. Seven participants receiving the 300 mg dose of NIAGEN® reported 9 AEs. Four of these AEs were categorized as Nervous System Disorders (headache) and were classified as "unlikely" related to the study product. Two AEs were categorized as General Disorders and Administration Site Conditions (flushing and feeling warm) and were categorized as "unlikely" related to the study product. Two AEs were classified as Gastrointestinal disorders (soft/loose stools) and were considered "unlikely" related to the study product. One AE was categorized as Investigations (decrease in white blood cell count) and was considered "possibly" related to test item administration. Four participants reported 4 AEs after receiving 1000 mg NIAGEN®. Two of these were categorized as "General Disorders and Administration Site Conditions" (feeling hot) and were categorized as unlikely related to study product, one AE was categorized as a "Nervous System Disorder" (headache) and unlikely related to study product, and one AE was categorized as "Investigations" (low hemoglobin) and unlikely related to study product, however, this participant was withdrawn from the study. All AEs were resolved without the need for intervention and there was no association between AE severity and dose. No SAEs were reported during the study.

2.3.2. Six-week randomized, placebo-controlled, double blind, crossover study in healthy midlife and older adults [40]

In a 6-week randomized, placebo-controlled, double blind, crossover study, after preliminary screening for inclusion/exclusion criteria, 30 healthy midlife and older adult men and women (ages 55 – 80 years) were randomized to one of two groups (Group A or Group B) [40]. Group A received placebo for 6 weeks followed by NIAGEN® (500 mg, 2x/day) for 6 weeks [40] (NCT02921659). Group B received the same treatments in the opposite order. Due to the length of the placebo period and relatively short half-life of NAD^+, a carryover effect between phases was not expected; therefore, a washout period between phases was not included. The dosing regimen with NIAGEN® produced a 60% increase in levels of NAD^+ in peripheral blood mononuclear cells (PBMC). All AEs were rated as mild-to-moderate in severity. The majority of AEs occurred during the placebo intervention, and were

classified as unrelated to NIAGEN® supplementation. No effect of NIAGEN® was observed on body mass at any time point during the study. Heart rate stayed within normal ranges at all time points and no treatment by time interaction was observed. Blood pressure remained stable and within normal physiological values at all time points under both treatment conditions. All hematological, metabolic (e.g., liver and kidney function), and lipid profiles remained within the normal reference range during both treatment conditions. In summary, findings suggest that 6 weeks of 1,000 mg/day oral NIAGEN® supplementation is safe and well tolerated and does not alter blood chemistry or markers of renal and liver function in middle-aged and older adults.

2.3.3 An eight-week, repeat-dose, randomized, placebo-controlled, double blind, parallel group pharmacokinetic study in healthy adults.

A randomized, double-blind, placebo-controlled, parallel group study was conducted in which healthy subjects ingested placebo, 100, 300, or 1000 mg NIAGEN®/day (n=30/group) for eight weeks [ChromaDex study 15NRHC]. Plasma and urine samples were obtained for assessment of metabolites including the primary metabolite 1-methyl-nicotinamide (1-MeNAM), and the secondary metabolite N-methyl-2-pyridone-3/5-carboximide (2-PY). Levels of NAD^+ in red blood cells and of nicotinamide in plasma were also determined. Additional exploratory analyses of resting energy expenditure, C-reactive protein, and amino acid metabolism were conducted. The accompanying safety assessment included hematology and clinical chemistry panels, as well as kidney and liver function tests.

Compared to values in the placebo control group; daily ingestion of either 300 or 1000 mg/day NIAGEN® resulted in a (1) dose-dependent increase in levels of 1-meNAM in plasma and urine, and (2) dose-dependent increase in levels of 2-PY in plasma and urine. Levels of nicotinamide in plasma were significantly increased only after ingestion of 1000 mg/day NIAGEN®; urine levels were not determined. Doses of 300 mg or 1000 mg/day NIAGEN® increased NAD^+ levels in red blood cells by 44% and 132%, respectively. This increase was evident within one week and remained unchanged for the duration of the study. These findings document the absorption and metabolism of NIAGEN®, and the ability of both 300 and 1000 mg/day to achieve the desired increase in NAD^+ levels. They also suggest that 1-meNAM or 2-PY can be used to document drug exposure given the difficulty in measuring nicotinamide riboside in blood.

Exploratory analysis of resting energy expenditure (i.e. energy utilization while awake but at rest, not actively digesting food and at thermoneutrality) did not identify any significant differences for any dose compared to placebo. Exploratory analysis of amino acid levels identified no significant effects of any dose of NIAGEN® on amino acid metabolism. Finally, no dose of NIAGEN® affected high sensitivity C-reactive protein.

With respect to safety, a total of 95 adverse events (AE) among 61 unique participants were reported in this study. All were classified as mild to moderate and resolved by the end of the study. Of the 26 AE documented in the 100 mg NIAGEN®

group, 24 were reported as being unlikely or not related to the study product. The 2 AEs reported as being possibly related (leg pain, high blood pressure) were both mild in intensity. Of the 27 AEs documented in the 300 mg NIAGEN® group, 25 were reported as being unlikely or not related to the study product. The 2 AEs reported as being possibly related (nausea, muscle pain) were both mild in intensity. Of the 22 AEs reported in the 1000 mg NIAGEN® group, 19 were reported as being unlikely or not related to the study product. The 3 AEs reported as being possibly related (sore back, muscle soreness, nausea) were all mild in intensity. Of the 20 AEs reported in the placebo group, 16 were reported as being unlikely to the study product. Of the 4 AEs reported as being possible related, 3 were mild in intensity (rash, raised liver function tests, nausea) and 1 was moderate in intensity (upset stomach).

With respect to hematology, significant differences were identified in (1) participants' white blood cell count between the 300 mg NIAGEN® and placebo group at week 8; (2) red cell distribution width between the 1000 mg NIAGEN® group and placebo group from screening to week 8; and (3) eosinophil count between the 1000 mg NIAGEN® group and 100 mg NIAGEN®, 300 mg NIAGEN®, and placebo group were identified. However, these remained within healthy clinical values for all groups. As such, these results were not deemed to be clinically significant. No other significant between-group differences were found in the hematology parameters of participants.

With respect to clinical chemistry, the significant difference in participants' ALT between the 1000 mg NIAGEN® group and the 100 mg NIAGEN® and placebo group at baseline to week 1, and between the 1000 mg NIAGEN® group and 100 mg NIAGEN® group and placebo group from baseline to week 2, as well as a significant between-group difference in AST between the 1000 mg NIAGEN® group and 100 mg NIAGEN® and placebo group at baseline to week 2 remained within healthy clinical values for all groups. As such, these results were not deemed to be clinically significant. No other significant between-group differences were found in the clinical chemistry parameters of participants.

No significant difference between-groups in mean systolic blood pressure, mean diastolic blood pressure, mean heart rate, weight, or body mass index were identified among the 140 participants.

2.3.4. Open label escalating dose trial in healthy adults

Eight healthy adult men and women (ages 21 – 50 years) were entered into an open label study of the safety and pharmacokinetics of escalating doses of NIAGEN® [41]. Subjects received 250 mg daily on days 1 and 2, 250 mg twice daily on days 3 and 4, 500 mg twice daily on days 5 and 6, and 1000 mg twice daily on Days 7 and 8. On day 9, subjects received a single dose of 1000 mg. No AEs attributable to NIAGEN® were reported during dose escalation of NIAGEN® to 1000 mg orally twice daily. Serum levels of creatine kinase, glucose, uric acid and alanine aminotransferase were unchanged. Serum potassium decreased by an average of 0.4 mEq/L. Although this small decrease was statistically significant, the individual levels remained in normal range and the decrease was not considered clinically

significant. Serum levels of sodium, chloride, urea nitrogen, creatinine, lactate dehydrogenase or aspartate aminotransferase were unchanged. There were no significant changes in blood pressure, body temperature, body weight, white blood cell differential or white blood cell count. However, very small, albeit statistically significant, decreases in hemoglobin, hematocrit and platelet count were observed.

This dose regimen increased levels of NAD^+ in PBMC by two-fold. Of note, despite the instability of NIAGEN® in blood, this study also attempted to quantitate plasma levels of nicotinamide riboside. Following the final dose of 1000 mg NIAGEN®, plasma levels of nicotinamide riboside were increased in four of the eight participants with a C_{max} of 3 hr and a half-life of 2.7 hr. However, these values were at the limit of detection of the assay.

2.3.5 Phase II studies in patients with skin cancer

Two placebo-controlled phase II studies [42] of patients with a prior history of skin cancer and current actinic keratoses, which are predictive of non-melanoma skin cancers, demonstrated a 30% reduction in new actinic keratoses in patients taking 1 g/day of the NAD^+ precursor nicotinamide. A recent phase III study [43] of 386 patients with a prior history of skin cancers demonstrated that daily oral administration of up to 1 g/day nicotinamide for one year decreased the rate of new nonmelanoma skin cancers by 23% and that of new actinic keratoses by 12%.

3 - Rationale/Significance

3.1 Problem Statement

Peripheral neuropathies are a dose-limiting, disabling, and debilitating side effect of virtually every known class of chemotherapeutic agent. Relatively few patients escape CIPN. Patients experience paresthesias, dysesthesias, hyperalgesia (increased pain produced by a stimulus that does not normally cause pain), allodynia (pain due to a stimulus that does not normally evoke pain), numbness or loss of sensation, or ongoing pain that is burning, lancinating or electric shock-like in nature. CIPN can seriously diminish a patient's quality of life, and can interfere with self-care and activities of daily living. The severity of CIPN may also necessitate reducing the dose of chemo-therapeutic agent, delaying the next cycle of chemotherapy or terminating treatment entirely. Given that advances in cancer diagnosis and treatment of cancer have increased the number of survivors to nearly 14.5 million in 2014 [6], up to 4.5 million survivors may suffer from CIPN after their treatment is completed.

The American Society of Clinical Oncology considers the development of adjuncts for the prevention and relief of CIPN as essential for patient care. Much effort has gone into testing drugs like gabapentin, lamotrigine, and amitriptyline (used to treat other sensory neuropathies) only to learn that they are ineffective against CIPN. Thus, effective treatment for CIPN remains a significant unmet need in healthcare.

3.2 Purpose of Study/Potential Impact

The purpose of this study is to test the hypothesis that nicotinamide riboside (NIAGEN®) will prevent the progression of sensory neuropathy in breast cancer patients or patients with platinum-resistant recurrent ovarian, peritoneal, endometrial, or fallopian tube cancer receiving paclitaxel or nab-paclitaxel. These findings will dictate whether there is adequate evidence to support the conduct of a placebo-controlled, phase III trial and drive the design of that study.

3.3 Potential Risks and Benefits

3.3.1 Potential Benefits

At present, there are no evidence-based efficacious treatments for the prevention or relief of CIPN in patients. The only possible exception is duloxetine [8], for which the effect was not large. The development of a therapeutic agent that can prevent CIPN or reverse established CIPN will be transformative for the patient, the oncologist, and for cancer treatment. It will permit patients to obtain the full benefit of chemotherapy and dramatically improve their quality of life both during therapy and in the years that follow. A finding that nicotinamide riboside is efficacious would also support further phase II and phase III trials of efficacy in treating peripheral neuropathies induced by other chemotherapies and for other types of cancers. In addition, it will provide data to support or refute the hypothesis that an elevation of NAD+ is a mechanism by which neuroprotection occurs, leading to the proposal of more mechanistic studies in patients. Identification of the mechanism of action will guide the development of an entirely new class of therapeutic agent to address a significant unmet need in cancer care.

3.3.2 Potential Risks

There is a risk of a small amount of brief pain during venipuncture to obtain a blood sample. The first blood sample will be obtained during a standard of care blood draw at the start of the visit. The second sample will be obtained at the end of the infusion of taxane.

Either an increase or decrease in paclitaxel concentrations could confound interpretation of the efficacy of NIAGEN®. A false negative finding could occur if peripheral sensory neuropathy worsens or there is a further decrease in white blood cells, red blood cells and hematocrit should the levels or bioavailability of free paclitaxel increase due to displacement from plasma proteins. A false positive finding could occur if plasma concentrations of paclitaxel decrease. Therefore, although paclitaxel concentrations are not monitored as standard of care for treatment of breast cancer, blood levels of paclitaxel will be measured at the end of each taxane infusion. With enrollment of the 10th patient, enrollment will pause so that an interim analysis of paclitaxel levels in all 10 patients receiving NIAGEN® can be conducted.

There is a small risk that NIAGEN® may enhance oncogenesis. Published data indicate that increasing NAD^+ levels, either by administration of nicotinamide or NIAGEN®, decreases metastases, increases survival, and interferes with cancer progression [44-46]. Two phase II studies [42] and one phase III study [43] have demonstrated that oral administration of up to 1 g of nicotinamide daily for one year, significantly decreased the rate of new nonmelanoma skin cancers by 23% and the number of new actinic keratoses by 12%.

There is minimal risk that NIAGEN® will cause flushing. Unlike niacin or nicotinic acid, NIAGEN® does not activate GPR109A and therefore does not cause flushing [47].

4 - Study Objectives

4.1 Hypothesis

We hypothesize that daily administration of NIAGEN® will prevent the progression of sensory neuropathy in stage IV breast cancer patients, platinum-resistant recurrent ovarian, peritoneal, endometrial, or fallopian tube cancer patients, or platinum-resistant recurrent or metastatic head and neck cancer patientsreceiving paclitaxel or nab-paclitaxel.

4.2 Primary Objective

The purpose of this single-arm phase II trial is to determine whether nicotinamide riboside prevents the progression of peripheral sensory neuropathy in patients receiving paclitaxel or nab-paclitaxel for treatment of stage IV breast cancer, platinum-resistant recurrent ovarian, peritoneal, endometrial, or fallopian tube cancer, or platinum-resistant recurrent or metastatic head and neck cancer. A decision to propose a large multi-center phase III trial of nicotinamide riboside for the relief of sensory neuropathy and the experimental design of that trial will be based on these findings.

4.3 Secondary Objectives

The secondary objective of this single-arm pilot phase II trial is to determine whether treatment with nicotinamide riboside can prevent reductions in the dose of taxane therapy due to the incidence or severity of neuropathy.

4.4 Exploratory Analysis

An exploratory analysis will be conducted to determine whether the Total Neuropathy Score – clinical version (TNS-c) [48] is an instrument can be used in subsequent studies to detect changes in neuropathy over time.

5 - Study Design

5.1 General Design

This unblinded, prospective non-randomized and single-arm pilot phase II trial is designed to obtain preliminary data to determine whether a phase III randomized controlled trial should be proposed and to drive the experimental design of that trial. The trial will include a pause in enrollment after the 10th patient to enable a safety assessment to ensure that NIAGEN® does not adversely affect the plasma concentrations of paclitaxel.

5.1.1 Study Duration

The study will be open for enrollment for 3 years or until 39 patients have completed the study. Patients will receive NIAGEN® for approximately 12 weeks concomitant with their scheduled taxane infusions, with the final followup scheduled 24 to 26 weeks after the end of treatment.

5.1.2 Number of Study Sites

There will be two study sites: University of Iowa Hospitals and Clinics, Iowa City, IA and Wake Forest Baptist Medical Center, Winston-Salem, North Carolina.

5.2 Outcome Variables

5.2.1 Primary Outcome Variable

The primary outcome variable is binary (yes/no) and defined as no worsening of the grade of peripheral sensory neuropathy, scored according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 guidelines, from the initiation of NIAGEN® treatment to the conclusion of NIAGEN® treatment. It will be determined on an end-of-treatment visit scheduled 7 to 14 days after the final taxane infusion.

5.2.2 Secondary Outcome Variables

The secondary outcome variables are:

- Percentage of patients that experience a neuropathy-driven dose reduction. Dose reduction is defined as a 15% decrease from initial intended dose prescribed at baseline (including discontinuation of therapy) due to the peripheral neuropathy.
- Incidence of dose reduction (each reduction is a single event).
- Cumulative dose of paclitaxel administered after NIAGEN® therapy is started.
- Change in the score on the11-item NTX subscale of the FACT&GOG-NTX questionnaire determined from baseline to follow-up.

5.3 Study Population

This trial will be conducted in patients who are 18 to 85 years old with a diagnosis of stage IV breast cancer, platinum-resistant recurrent ovarian, peritoneal, endometrial, or fallopian tube cancer, or platinum-resistant recurrent or metastatic head and neck cancer who are receiving intravenous infusions of paclitaxel or nab-paclitaxel at the University of Iowa Hospitals and Clinics or the Wake Forest Baptist Medical Center. To enroll, the patient must have metastatic breast cancer, platinum-

resistant recurrent ovarian, peritoneal, endometrial, or fallopian tube cancer, or platinum-resistant recurrent or metastatic head and neck cancer and report at least a grade 1 peripheral neuropathy after initiation of paclitaxel therapy, scored according to the CTCAE version 4.03 guidelines for peripheral sensory neuropathy. Patients with recurrent disease may also be enrolled if they have residual CIPN from prior chemotherapy that is ≤ grade 2.

5.3.1 Number of Participants

We estimate that 72 new patients with metastatic breast cancer or platinum-resistant recurrent ovarian, peritoneal, endometrial, or fallopian tube cancer will be placed on paclitaxel or nab-paclitaxel at either the University of Iowa Hospitals and Clinic or Wake Forest Baptist Medical Center over the course of three years. The incidence of CIPN in patients receiving taxanes ranges from 57% to 83% [52, 53]. In a large study of breast cancer patients receiving 12 weekly infusions of 80 mg/m^2 paclitaxel, 38%, 21%, and 9% developed a sensory neuropathy of grade 1, 2, or 3 [54]. Finally, a recent study of weekly taxanes in breast cancer patients documented that 28% of patients developed grade 2 and 17% developed grade 3 neuropathy when placed on weekly paclitaxel [55]. Thus, we expect that 60% (43 patients) will develop grade 1 neuropathy or greater within 9 weeks, most likely after the 5th or 6th infusion, and become eligible for enrollment.

Of the 43 patients with neuropathy, we anticipate that 90% will meet eligibility requirements and consent to participate. We anticipate minimal, if any, loss to follow-up as they will meet with their physician within 14 days of the final infusion of taxane. Thus, we expect to have 39 patients complete the 12 weeks of paclitaxel therapy. Our primary outcome variable will be binary (yes/no) for each patient. A "favorable outcome" is defined as no worsening of the grade of sensory neuropathy, as defined by the CTCAE guidelines for scoring peripheral sensory neuropathy, from the initiation of NIAGEN® treatment to the visit that occurs within 14 days of the final infusion of taxane. An "unfavorable outcome" is defined as otherwise.

5.3.2 Eligibility Criteria

Decisions about eligibility will be made by the research nurse and verified by the treating physician. To be eligible, patients must:

- Be able to give written informed consent and HIPAA authorization
- Be 18 to 85 years old
- Have been diagnosed with stage IV breast cancer of any type, platinum-resistant recurrent ovarian, peritoneal, endometrial, or fallopian tube cancer, or platinum-resistant recurrent or metastatic head and neck cancer and are anticipated to survive for at least three months
- Have an ECOG Performance Status of 0-2
- Able to take medication orally – up to four capsules in the morning (am) and four capsules in the evening (pm).

- Be undergoing infusions of paclitaxel or nab-paclitaxel for treatment of breast cancer, platinum-resistant recurrent ovarian, peritoneal, endometrial, or fallopian tube cancer, or platinum-resistant recurrent or metastatic head and neck cancer and be determined to have at least a grade 1 neuropathy based on the CTCAE version 4.03 guidelines for peripheral sensory neuropathy. Breast cancer patients may also be treated concomitantly with monoclonal antibodies to HER2 such as trastuzumab (Herceptin) and pertuzumab (Perjeta). Patients with platinum-resistant ovarian, peritoneal, endometrial, or fallopian tube cancer or platinum-resistant recurrent or metastatic head and neck cancer may also be treated concomitantly with a vascular endothelial growth receptor 2 inhibitor such as bevacizumab (Avastin) or a checkpoint inhibitor.
- Females must be either postmenopausal for at least 1 year or surgically sterile for at least 6 weeks. Females of childbearing potential must have a negative pregnancy test at screening to be eligible for study participation, and agree to take appropriate precautions to avoid pregnancy from screening through follow-up.
- Males must agree to take appropriate precautions to avoid fathering a child from screening through follow-up. The following methods have been determined to be more than 99% effective (<1% failure rate per year when used consistently and correctly) [56] and are permitted under this protocol for use by the patient and his/her partner:
    - Complete abstinence from sexual intercourse when this is in line with the preferred and usual lifestyle of the patient
    - Double barrier methods
        - Condom with spermicide in conjunction with use of an intrauterine device
        - Condom with spermicide in conjunction with use of a diaphragm
    - Surgical sterilization (bilateral oopherectomy with or without hysterectomy, tubal ligation or vasectomy) at least 6 weeks prior to taking study treatment. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow-up levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and/or estradiol

  Non-hormonal intrauterine device used as directed by provider placing this is also acceptable.

5.3.3 Exclusion Criteria

- Pre-existent peripheral neuropathy that is unrelated to chemotherapy
- Pre-existent chemotherapy-induced peripheral neuropathy greater than grade 2
- Known metastases to the brain, spinal cord or peripheral nerves, or leptomeningeal disease
- Concurrent administration of a poly (ADP-Ribose) polymerase inhibitor (e.g. olaparib, rucaparib)

- Concurrent administration of a platinum-based chemotherapy
- Diabetes managed by medication
- Neutrophils < 1,000 cells/m^3
- Hemoglobin < 8.0 g/dcl
- Platelets < 100,000 cells/m^3
- Creatinine clearance < 30 ml/min
- AST or ALT values > 2.5 X upper limits of normal
- Total bilirubin > 2.0 X upper limits of normal
- Heavy alcohol use defined at > 8 drinks/week by women or 12 drinks/week by men
- Chronic pain greater than 3 months duration within the past year.
- Severe psychiatric illness
- Pregnancy
- Current imprisonment
- Limitations of self-expression, defined as an inability to answer questions posed by physicians, nurses, care-givers, or other members of the investigative team or an inability to describe somatosensations.
- Known HIV, not on therapy
- Regular use of nutritional supplements that contain nicotinamide or nicotinamide riboside within the previous 30 days
- Use of duloxetine (Cymbalta®) within the previous 30 days
- Pancreatic insufficiency requiring exocrine enzyme replacement therapy
- GI conditions where malabsorption of B complex vitamins is known to occur.
- Breastfeeding

6 - Methods

6.1 Treatment - Drug

6.1.1 Identity of Investigational Product/New Drug

NIAGEN® manufactured by ChromaDex and provided as capsules.

6.1.2 Dosage, Admin, Schedule (if applicable)

Dosing will begin upon documentation of at least a grade 1 peripheral neuropathy and conclude one week after the 12th or last infusion of paclitaxel or nab-paclitaxel therapy. An initial total daily dose of 300 milligrams taken orally during the first week of therapy will escalate to a total daily dose of 1 gram taken orally for all subsequent weeks of the trial. The daily dose will be taken as two divided doses at roughly 12 hr intervals. If a patient vomits or misses a dose they will be directed to resume with the next scheduled dose.

The physician may choose not to escalate to the 1 gram dose. If, following dose escalation, the treating physician believes that the 1 gram dose has significant adverse effects, they may return the patient to the 300 mg dose.

6.1.3 Method of Assignment/Randomization

Not applicable. This is a single-arm, unblinded, and non-randomized study.

6.1.4 Blinding and Procedures for Unblinding

Not applicable

6.1.5 Packaging/Labelling

ChromaDex will provide NIAGEN® as bottles of capsules containing either 75 mg or 250 mg of the product. NIAGEN® capsules will be dispensed by the Investigational Pharmacy at each site. A four week supply will be dispensed to patients every four weeks while on study. When less than four weeks of treatment remains, a quantity will be dispensed that is sufficient to complete treatment through one week after the last taxane infusion.

6.1.6 Storage Conditions

Drug should be stored at room temperature, protected from light. Drug will be under the control of the Investigational Pharmacy at each site. Unused drug will be returned to ChromaDex in Irvine, CA.

6.1.7 Concomitant therapy

Patients will be treated in accordance with the standard of care for metastatic breast, or platinum-resistant ovarian, peritoneal, endometrial, or fallopian tube cancer. After enrollment in the study and for the duration of the study, they will be requested not to supplement the dose of nicotinamide riboside with nicotinamide riboside that can be purchased over the counter or on the internet. Compliance will be determined through discussion and documented in the review of concomitant medications at each visit. Dairy intake will be queried at each visit. ~1 mg of NIAGEN® is present in four 8 oz glasses of milk [30].

6.2 Assessments

6.2.1 Efficacy

The efficacy of NIAGEN® to prevent the progression of CIPN will be determined by the grade of peripheral sensory neuropathy, scored according to the CTCAE guidelines, and patient responses to the 11-item NTX subscale of the FACT&GOG-NTX questionnaire. Exposure to NIAGEN® will be monitored by measurement of NAD^+ in red blood cells and N-methyl-nicotinamide in the blood. These assessments will be made at each visit.

6.2.2 Safety

Paclitaxel is highly bound to protein. While unlikely, NIAGEN® could compete for plasma protein binding sites and as a result increase the concentration of free paclitaxel and potentially worsen the sensory neuropathy in patients as well as exacerbate paclitaxel-induced myelosuppression. Conversely, NIAGEN® could induce the P450 (CYP3A and CYP28A) enzymes that metabolize paclitaxel,

decreasing its plasma concentrations and compromising therapy. The latter is of minimal concern given that in vitro studies in human liver microsomes concluded that NIAGEN® neither inhibits nor induces these enzymes. Nonetheless, paclitaxel concentrations and complete blood counts will be monitored on each visit.

6.2.2.1 Adverse Events Definition and Reporting

Adverse effects will be monitored at each visit by the Principal Investigator. The Data Safety and Monitoring Plan (Appendix B) describes the HCCC requirements for Adverse Event (AE) reporting and Serious Adverse Event (SAE) reporting.

6.2.3 Pharmacokinetics of Taxanes

Two 5 ml blood samples will be collected at each time point indicated on the schedule of events. Each set of blood will be drawn within 30 ± 10 min of the end of each infusion of paclitaxel and between 15 and 30 minutes after each infusion of nab-paclitaxel, and submitted for measurement of paclitaxel concentration. See Appendix C.

6.2.4 Biomarkers

Although a method that diminishes the instability of NR in blood has recently been identified [41], NR levels in blood will not be measured. The published method has not been validated as reproducible and sufficiently sensitive. In addition, due to clinic scheduling constraints patients may be sampled as soon as the first hour or as late as 9 hours after their morning dose. The results are anticipated to be quite variable as a result. In its stead, the levels of NAD^+ and N-methyl-nicotinamide will serve as biomarkers to ascertain exposure levels to NIAGEN®. The increase in levels of NAD^+ and N-methyl-nicotinamide are very consistent and achieve steady state that is independent of time of dosage. The methods of quantitation are also validated. Levels of NAD^+ and N-methyl-nicotinamide will be measured via blood obtained prior to therapy along with standard of care labs when the patient arrives for their visit. Two 3-ml samples will be collected. See Appendix C.

6.3 Study Procedures

6.3.1 Study Schedule

See Study Calendar in Synopsis.

6.3.2 Informed Consent

After the physician has confirmed the presence of grade 1 or higher CIPN or the existence of a preexisting CIPN of no greater than grade 2, he or she will inform the patient of the possibility of participation in the study. Any patient who expresses interest will be referred to the research study nurse for further description of the trial. The research study nurse or treating physician will obtain informed consent.

6.3.3 Screening and Enrollment

The research study nurse will review the patient's electronic medical record to determine the patient's eligibility.

Laboratory assessments completed within 3 days of enrollment will include serum or urine pregnancy test for females of childbearing potential, as well as renal and hepatic function. See Study Calendar for detailed list of screening items to be collected.

When all inclusion/exclusion criteria have been met, the patient will be considered enrolled.

6.3.4 On Study Visits

Patients will be scheduled to receive an intravenous infusion of paclitaxel or nab-paclitaxel in accordance with standard of care for that disease as determined by the physician. The schedule of taxane infusion may include a periodic scheduled week "off" from infusion. Daily dosing with NIAGEN® will begin on Treatment Week 1, Day 1 following completion of the infusion of paclitaxel or nab-paclitaxel. It will continue for one week after the last scheduled infusion of taxane. At each visit, the grade of the patient's peripheral sensory neuropathy will be determined per the CTCAE guidelines and the patient will complete the 11-item NTX subscale of the FACT&GOG questionnaire. Review of drug administration, dairy intake, collection of vital signs, liver function tests, complete blood counts, and adverse events will occur at each visit. Clinical chemistries will be assessed at each visit. Scoring of sensory peripheral neuropathy by CTCAE guidelines and the FACT&GOG NTX subscale, as well as the ECOG Performance Status can be determined by either the oncologist or the research nurse on the weeks when the oncologist is not seeing the patient. In addition, a clinician trained in the neurological assessment procedures will assess the neuropathy on enrollment, after the first week of NIAGEN®, at monthly intervals thereafter, and at the end of treatment visit using the TNS-c questionnaire. Patients will not be required to come in for measurement on their scheduled week "off."

At each visit, blood from a sample drawn prior to initiation of the scheduled infusion of paclitaxel or nab-paclitaxel will be sent for analysis of the levels of NAD^+ and N-methyl-nicotinamide, and a sample drawn at the end of the taxane infusion will be sent for analysis of paclitaxel levels. Also, patients will be asked to bring their drug diary and any remaining NIAGEN® in at each visit. Seven to 14 days after the last taxane infusion and again 12 to 14 weeks and 24 to 26 weeks after the last taxane infusion the study research nurse will contact the patients during their scheduled follow-up visit and ask them to complete the CTCAE and NTX subscale questionnaire.

6.3.5 End of Study and Follow-up

Seven to 14 days after the last infusion of taxane, and again 12 to 14 weeks and finally 24 to 26 weeks after the last infusion of paclitaxel or nab-paclitaxel, the research study nurse will contact the patients during their scheduled follow-up visit, score their peripheral sensory neuropathy as per the CTCAE guidelines for peripheral sensory neuropathy, and obtain their response on the 11-item NTX subscale of the FACT&GOG questionnaire. Patients will be specifically asked if they have continued nicotinamide riboside of their own volition at the follow-up visits at

12-14 weeks and 24-26 weeks. Patients will also be contacted 30-37 days after the last dose of NIAGEN® to assess for any adverse events. This contact can be made by phone contact if the patient is not scheduled for a standard of care office visit during this timeframe.

6.3.6 Removal of subjects (Stopping rules)

Subjects will be removed from the study if they develop a hypersensitivity reaction at the time of the taxane infusion. In this event, NIAGEN ® administration will cease, and an end of study visit to assess the primary outcome will be scheduled one week later. Further followup will not occur as the patients will be placed on a different chemotherapy.

Subjects will also be removed from the study should any hematological or non-hematological laboratory values meet criteria for exclusion from the study (section 5.3.3). For the first 10 subjects enrolled, subjects will also be removed if plasma levels of paclitaxel increase or decrease more than 2 S.D. of the group mean pre-NIAGEN® levels. Patients enrolled thereafter will be removed if plasma levels of paclitaxel fall outside the bounds established by iterative refinement of the predictive probability of paclitaxel levels. Finally, subjects will be removed for any other unacceptable toxicity in the opinion of the attending physician.

6.4 Statistical Method

6.4.1 Statistical Design

The purpose of this study is to determine whether NIAGEN® prevents the progression of peripheral sensory neuropathy in patients receiving paclitaxel or nab-paclitaxel for treatment of breast cancer or platinum-resistant recurrent ovarian, peritoneal, endometrial, or fallopian tube cancer. The study is designed as a single-arm, observational phase II trial. A decision to propose a phase III trial of nicotinamide riboside for the relief of sensory neuropathy at the end of the proposed study will be based on the findings of this phase II trial with predictive probability approach using the Bayesian analysis.

6.4.2 Sample Size Considerations

We estimate that 72 new patients at the University of Iowa Hospitals and Clinics will be placed on paclitaxel or nab-paclitaxel in the course of 24 months. An additional number of new patients will be placed on this therapy at Wake Forest Baptist Medical Center. The incidence of CIPN in patients receiving taxanes ranges from 57% to 83% [52, 53]. In a larger study of breast cancer patients receiving 12 weekly infusions of 80 mg/m^2 paclitaxel, 38%, 21%, and 9% developed a sensory neuropathy of grade 1, 2, or 3 [54]. Finally, a recent study of weekly taxanes in breast cancer patients documented that 28% of patients developed grade 2 and 17% developed grade 3 neuropathy when placed on weekly paclitaxel [55]. Thus, we expect that 60% (43 patients) will develop grade 1 neuropathy or greater within 9 weeks, most likely after the 5th or 6th infusion, and become eligible for enrollment.

Of the 43 patients with neuropathy, we anticipate that 90% will meet eligibility requirements and consent to participate. We anticipate minimal, if any, loss to

follow-up as they will meet with their physician within 14 days of the final infusion of taxane. Thus, we expect to have 39 patients at the University of Iowa Hospitals and Clinics complete the 12 weeks of paclitaxel therapy, and the maximum sample size of the study is assumed as 39 patients.

6.4.3 Planned Analyses

6.4.3.1 Primary Analyses

The primary analysis will be based on the predictive probability approach using the Bayesian analysis for this phase II trial [57]. Compared to the traditional Simon's optimal or MiniMax design [58] with two pre-determined time points for evaluation of efficacy, the Bayesian design allows more frequent monitoring. Based on the historical data, it was assumed that 50% of the patients' grade of peripheral neuropathy would not worsen if they do not receive NIAGEN® treatment. We also assumed the NIAGEN® treatment would increase the favorable outcome rate to 70%. Bayesian inference interprets the probability as a degree of belief, and unknown parameters are random variables with prior probability distributions. Therefore, instead of a fixed favorable outcome rate of 50% for the no NIAGEN® treatment group, a weakly informative (i.e. very large standard deviations) [59] prior distribution centered on the 50% favorable outcome estimate was used. A vague Beta prior distribution with parameters 0.5 and 0.5 (Beta (α=0.5, β= 0.5)) provides a mean 50% and standard deviation 35%, and was used. As is typical for phase II trials, both type I and type II error rates were assumed to be 10%. In addition, the maximum sample size was assumed 39, and the trial will be monitored continuously after enrolling the first 10 patients. Under these assumptions, according to the Bayesian predictive probability design, the following rejection regions are calculated: (favorable outcome/current sample size) 1/10, 2/11, 3/13, 4/15, 5/16, 6/18, 7/20, 8/21, 9/23, 10/24, 11/26, 12/27, 13/29, 14/30, 15/31, 16/32, 17/33, 18/34, 19/35, 20/36, 21/37, 22/38, 23/39. The trial will be stopped and the NIAGEN® treatment will be deemed ineffective the first time the number of favorable outcomes falls into the rejection region. Accordingly, the trial will be stopped for futility if only 1 favorable outcome is observed out of the first 10 patients, or 2 favorable outcomes are observed out of the first 11 patients, and so on. Otherwise, the trial will continue until enrolling the maximum sample size of 39 patients. If at the end of the trial, NIAGEN® treatment prevents the progression of CIPN in 24 or more patients, out of 39, NIAGEN® will be considered effective and we will recommend proceeding to the phase III trial.

The determination of futility will be based on the Bayesian predictive probability approach, as defined above. Such determination will be informed by verification that (1) each patient in the analysis had adequate exposure to NIAGEN® as validated by levels of NAD^+ in red blood cells and N-methyl-nicotinamide in plasma and (2) plasma levels of paclitaxel were within the 95% confidence limits of the baseline values determined for the patients enrolled.

If a patient discontinues NIAGEN® treatment or supplements their own during the protocol, follow-ups will continue until the 8^{th} day after the last infusion of taxane

for the primary analysis. The primary outcome of change in grade of sensory neuropathy will be assessed at that time regardless of the patient's discontinuation or supplementation of the NIAGEN® treatment.

6.4.3.2 Secondary Objectives Analyses

The secondary endpoints are the percentage of patients experiencing a neuropathy-driven dose reduction, the incidence of dose reduction (each reduction is a single event), the cumulative dose of taxane administered after NIAGEN® therapy is started, and the change in the score on the11-item NTX subscale of the FACT&GOG-NTX questionnaire determined from baseline to follow-up. First, the Shapiro-Wilks test will be used to determine if the change in the score on the11-item NTX subscale of the FACT&GOG-NTX questionnaire and actual and planned cumulative doses of paclitaxel are normally distributed. Then, based on the normality assumption, paired t-test or Wilcoxon sign rank test will be used to compare whether differences in the actual vs. planned cumulative dose of paclitaxel and the change on the score on the11-item NTX subscale of the FACT&GOG-NTX questionnaire are different from zero. A one-sample test of proportions (Binomial test) will be used to determine if the proportions of patients experiencing a dose-reduction differs from 17% and if the incidence of individual dose reductions differs from 16%.

6.4.3.3 Safety

Studies of the pharmacokinetics of Taxol® indicate that plasma levels of paclitaxel vary greatly among a population of patients receiving a standardized dose. Efforts to model a personalized dose to achieve a defined plasma level of paclitaxel yielded doses that differed by as much as four-fold in the population of patients *[60]*. Of note, the plasma levels of paclitaxel also vary greatly week-to-week for individual patients receiving a standardized dose *[61, 62]*. Therefore, a hybrid of frequentist and Bayesian approaches will be used to monitor plasma levels of paclitaxel in patients. For patients 1 through 10, paclitaxel levels that fall outside 2 S.D. of the group mean of baseline (pre-NIAGEN®) values will be considered aberrant and this finding communicated to the treating oncologist and DSMC. With the enrollment of patient 11, and concordant with initiation of the Bayesian analysis of the primary outcome measure, a Bayesian model of predictive probability for paclitaxel levels in this patient population will be developed. This model will be iteratively refined as additional data on paclitaxel levels are obtained with continued enrollment of new patients. The attending oncologist and the DSMC will be notified of patients whose paclitaxel levels lie outside these bounds.

6.4.3.4 Analysis of Subject Characteristics

- Age
- Gender
- Race
- Ethnicity

- Breast cancer receptor status
- Type and stage of breast cancer
- Type and stage of ovarian cancer
- Type and stage of endometrial cancer
- Type and stage of fallopian tube cancer
- Type and stage of peritoneal cancer
- Type and stage of head and neck cancer

6.4.3.5 Interim Analysis

Interim analysis is not applicable to the design of this phase II trial, which will use a Bayesian model to evaluate outcome on a patient by patient basis after enrollment of the first ten patients. An interim analysis of paclitaxel levels in the first 10 patients will be conducted as part of ongoing safety assessment.

6.4.3.6 Health economic evaluation

Not applicable

6.4.4 Subsets and Covariates

Not applicable

6.4.5 Handling of Missing Data

Given that these patients routinely have several clinic appointments each month and this is not a randomized study, the loss to follow-up rate is expected to be low in this study. To prevent missing data, every effort, including phone call reminders to patients will be made. Loss to follow-up, missing data and the reasons leading to loss of follow-up or data will be recorded. In case of missing data, we will impute missing data using different methods. Because of the repeated measures nature of the data, the 'last observation carried forward' method will be utilized [63]. As a sensitivity analysis for the missing data, study results will be compared for the following two conditions: 1) patients with missing primary outcome variable are excluded from analyses; and 2) results with last observation carried forward. Robustness of the results under both scenarios will be assessed.

7 - Trial Administration

7.1 Institutional Review Board (IRB) Review

This study will be reviewed and overseen by the Holden Comprehensive Cancer Center, and also by the University of Iowa Institutional Review Board (IRB). As patients will also be enrolled at Wake Forest Baptist Health Center, the enrollment and treatment of patients at that site will also be reviewed and overseen by the Wake Forest University IRB.

7.2 Subject Confidentiality

Discussions with the patient, by the physician or the research nurse, will occur in a private room to minimize the risk of loss of privacy. Personal information about the patient will not be discussed with individuals outside the study team. The risk of loss of confidentiality will be minimized by use of data collection forms that contain only a study ID, and not the subject's name or medical record number. The patient will provide a preferred method of communication for follow-up about the study.

7.3 Unanticipated Problems

Unanticipated problems will be communicated by letter and email to all members of the study team. A response and plan of action will be developed through consultation among team members, the Data and Safety Monitoring Committee and the IRB. The study team will communicate the problem and the proposed resolution to the FDA (Food and Drug Administration) as applicable.

7.4 Data Quality Assurance

Quality of data collection will be ensured by training the study dedicated research nurses in administration of the questionnaires and completion of the data collection forms. Protocols will be developed and validated for measurement of paclitaxel concentrations and levels of NAD+ and its metabolites. Measurements of paclitaxel or of NAD+ and its metabolites will be made by a single laboratory. Collection of blood samples will be done by a trained phlebotomist. All samples will be blinded for analysis. The investigational drug will be prepared by the manufacturer according to GMP (Good Manufacturing Practice) standards, and dispensed by the Investigational Pharmacy at each site. . Additional information on monitoring by the HCCC is provided in the Data Safety and Monitoring Plan (Appendix B).

7.4.1 Data Collection

The Methods section describes data collection in more detail. Briefly, the electronic medical record will be the source of research materials about the age, gender, racial and ethnic identity, type of breast cancer, treatment, and doses of paclitaxel administered to the patients. The results of the CTCAE sensory neuropathy, the score on the11-item NTX subscale of the FACT&GOG-NTX questionnaire, and the score on the TNS-c questionnaire will be recorded on data collection forms, will be recorded on data collection forms, which will document the presence and severity of peripheral sensory neuropathy. Blood levels of NAD+ and N-methyl-nicotinamide will be determined using tandem mass spectrometry by Keystone Bioanalytical, a GLP-compliant CRO specializing in quantitative LC/MS/MS method development, validation and analysis, and the data returned to the research team for entry on the data collection form and analysis. Levels of paclitaxel after infusion will also be determined by the Department of Pathology at Wake Forest using a laboratory developed test that has been validated, and results communicated to the designated research study nurse for entry on the data collection form and analysis. All data will be de-identified. Data will be collected at each visit and again

7-14 days, 12-14 weeks, and 24-26 weeks after the last visit. These forms will be retained for at least 7 years after completion of the study in compliance with NIH guidelines for data retention, and then destroyed by shredding.

7.4.1.1 Access to Source

The data collection form, questionnaires, telephone survey, laboratory analyses of paclitaxel and NAD+ and its metabolites, and the patients' electronic medical record will serve as sources of data. These materials will be filed at the treating physician's site. Access to the electronic medical record will be restricted to health professionals involved in the clinical care of the individual, as well as to Dr. Thomas, Dr. Hammond, the research coordinator, and the research nurse. The data collection forms will be accessible to all members of the investigative team, with the identity of the individual, access date and time logged. Study nurses at each site will abstract data from the data collection forms and enter the data into the study database for analysis. Drs. Hammond and Thomas will permit trial related monitoring, audits, IRB review and regulatory inspection, providing direct access to all related source documents.

7.4.1.2 Data Storage/Security

The study identification number and the patient's name will be stored in a password protected file on a password protected computer within a locked office. This file will be stored separately from the data collection forms and database. Only Dr. Hammond, Dr. Thomas and the research coordinator will have access to that file. Paper forms will be stored in locked cabinets in locked offices. Electronic files will be kept in password protected files on a password protected computer in a locked office, and a backup copy placed on a remote server configured to secure sensitive health care information. Only patient identification numbers will be associated with the data, there will be no direct patient identifiers stored within the study database. If laptop computers are used, they will be encrypted.

7.5 Study Records

The following are considered study records and will be maintained for the duration of the study and retained for at least 7 years after completion of the study:

- informed consent form
- electronic medical record
- data collection form
- CTCAE questionnaire
- 11-item NTX subscale of the FACT&GOG-NTX questionnaire
- TNS-c questionnaire
- chromatograms of NAD and N-methyl-nicotinamide in blood
- chromatograms of paclitaxel in blood
- follow-up survey

- all IRB correspondence

7.5.1 Retention of Records

All study records and documents, whether arising at the University of Iowa or Wake Forest University, that pertain to the conduct of the study including: data collection forms, source documents, and IRB correspondence will be retained by the Principal investigator for at least 7 years following the completion of the study. Movement or destruction of records will require consent of Dr. Hammond.

7.6 Study Monitoring

The Holden Comprehensive Cancer Center will assist with monitoring of the study to ensure compliance and quality of data collection. Review of records will occur at each study site and will be conducted by a Clinical Research Auditor/Monitor not affiliated with the study. The Medical Monitor for this study will be Daniel Vaena, M.D.

7.7 Data Safety Monitoring Plan

The Data Safety and Monitoring Committee of the Holden Comprehensive Cancer Center will be used (refer to Appendix B).

7.8 Study Modification

All study modifications will only be made by the principal investigators after consultation with the investigative team and the Holden Comprehensive Cancer Center. All protocol modifications will be submitted to the local IRB for information and approval in accordance with local requirements. IRB approval will be obtained before any changes are implemented, except for changes necessary to eliminate an immediate hazard to a study participant. If recommended, modifications to the dose or frequency of administration of NIAGEN® will be communicated by phone to the patient within 24 hrs of approval and confirmed with the patient at the time of the next clinic visit.

7.9 Study Discontinuation

This study will be discontinued if the Bayesian analysis of favorable outcomes, defined as a failure of CIPN to worsen, does not support continued enrollment of patients. According to the Bayesian predictive probability design, the following rejection regions are calculated: (favorable outcome/current sample size) 1/10, 2/11, 3/13, 4/15, 5/16, 6/18, 7/20, 8/21, 9/23, 10/24, 11/26, 12/27, 13/29, 14/30, 15/31, 16/32, 17/33, 18/34, 19/35, 20/36, 21/37, 22/38, 23/39. For example, if only 1 favorable outcome is obtained in the first 10 patients enrolled, the trial will be halted for futility. It will also be discontinued if NIAGEN® is determined to worsen the progression of CIPN in an unacceptable number of patients in the opinion of the physician investigators.

7.10 Study Completion

The study completion will be defined as the completion of the data collection and the final analysis. The principal investigator will notify the IRB of the study completion.

7.12 Funding Source

This study is funded by the National Cancer Institute.

7.13 Publication Plan

Primary responsibility for publication of the results lies with the Principal Investigators, Dr. Alexandra Thomas and Dr. Donna Hammond and the investigative team. There are no restrictions on publication by the National Cancer Institute. ChromaDex, as supplier of the compound, will have 3 months to review the manuscript prior to submission, but will be unable to restrict publication of the findings or modify the findings or conclusions of the study.

References

1. Seretny M, Currie GL, Sena ES, Ramnarine S, Grant R, MacLeod MR, Colvin LA, Fallon M. Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: A systematic review and meta-analysis. Pain. 2014;155:2461-70.
2. Argyriou AA, Bruna J, Marmiroli P, Cavaletti G. Chemotherapy-induced peripheral neurotoxicity (CIPN): an update. Crit Rev Oncol Hematol. 2012;82:51-77.
3. Argyriou AA, Kyritsis AP, Makatsoris T, Kalofonos HP. Chemotherapy-induced peripheral neuropathy in adults: a comprehensive update of the literature. Cancer Manag Res. 2014;6:135-47; PMCID: 3964029.
4. Miltenburg NC, Boogerd W. Chemotherapy-induced neuropathy: A comprehensive survey. Cancer Treat Rev. 2014;40:872-82.
5. Park SB, Goldstein D, Krishnan AV, Lin CS, Friedlander ML, Cassidy J, Koltzenburg M, Kiernan MC. Chemotherapy-induced peripheral neurotoxicity: a critical analysis. CA Cancer J Clin. 2013;63:419-37.
6. National Cancer Institute Cancer Survival Statistics. Cancer Statistics. http://www.cancer.gov/about-cancer/what-is-cancer/statistics: 2015 Contract No.: June 19, 2015.
7. Hershman DL, Lacchetti C, Dworkin RH, Lavoie Smith EM, Bleeker J, Cavaletti G, Chauhan C, Gavin P, Lavino A, Lustberg MB, Paice J, Schneider B, Smith ML, Smith T, Terstriep S, Wagner-Johnston N, Bak K, Loprinzi CL, Oncology ASoC. Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol. 2014;32:1941-67.
8. Finnerup NB, Otto M, McQuay HJ, Jensen TS, Sindrup SH. Algorithm for neuropathic pain treatment: an evidence based proposal. Pain. 2005;118:289-305.
9. Moore RA, Derry S, Aldington D, Cole P, Wiffen PJ. Amitriptyline for neuropathic pain and fibromyalgia in adults. Cochrane Database Syst Rev. 2012;12:CD008242.
10. Quilici S, Chancellor J, Löthgren M, Simon D, Said G, Le TK, Garcia-Cebrian A, Monz B. Meta-analysis of duloxetine vs. pregabalin and gabapentin in the treatment of diabetic peripheral neuropathic pain. BMC Neurol. 2009;9:6; PMCID: PMC2663537.
11. Cavaletti G, Alberti P, Frigeni B, Piatti M, Susani E. Chemotherapy-induced neuropathy. Curr Treat Options Neurol. 2011;13:180-90.
12. Wolf S, Barton D, Kottschade L, Grothey A, Loprinzi C. Chemotherapy-induced peripheral neuropathy: prevention and treatment strategies. Eur J Cancer. 2008;44:1507-15.
13. Lavoie Smith EM, Pang H, Cirrincione C, Fleishman S, Paskett ED, Ahles T, Bressler LR, Fadul CE, Knox C, Le-Lindqwister N, Gilman PB, Shapiro CL, Alliance for Clinical Trials in O. Effect of duloxetine on pain, function, and quality of life among patients with chemotherapy-induced painful peripheral neuropathy: a randomized clinical trial. JAMA. 2013;309:1359-67; PMCID: 3912515.
14. Smith EM, Pang H, Ye C, Cirrincione C, Fleishman S, Paskett ED, Ahles T, Bressler LR, Le-Lindqwister N, Fadul CE, Loprinzi C, Shapiro CL, Alliance for Clinical Trials in O. Predictors of duloxetine response in patients with oxaliplatin-induced painful chemotherapy-induced peripheral neuropathy (CIPN): a secondary analysis of randomised controlled trial - CALGB/alliance 170601. Eur J Cancer Care (Engl). 2015; PMCID: PMC4879099.

15. Fehrenbacher JC. Chemotherapy-induced peripheral neuropathy. Prog Mol Biol Transl Sci. 2015;131:471-508.
16. Carozzi VA, Canta A, Chiorazzi A. Chemotherapy-induced peripheral neuropathy: What do we know about mechanisms? Neurosci Lett. 2015;596:90-107.
17. Flatters SJ. The contribution of mitochondria to sensory processing and pain. Prog Mol Biol Transl Sci. 2015;131:119-46.
18. Han Y, Smith MT. Pathobiology of cancer chemotherapy-induced peripheral neuropathy (CIPN). Front Pharmacol. 2013;4:156; PMCID: 3866393.
19. Ruggieri S, Orsomando G, Sorci L, Raffaelli N. Regulation of NAD biosynthetic enzymes modulates NAD-sensing processes to shape mammalian cell physiology under varying biological cues. Biochim Biophys Acta. 2015;1854:1138-49.
20. Yang Y, Sauve AA. NAD^+ metabolism: Bioenergetics, signaling and manipulation for therapy. Biochim Biophys Acta. 2016;1864:1787-800.
21. Araki T, Sasaki Y, Milbrandt J. Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. Science. 2004;305:1010-3.
22. Sasaki Y, Araki T, Milbrandt J. Stimulation of nicotinamide adenine dinucleotide biosynthetic pathways delays axonal degeneration after axotomy. J Neurosci. 2006;26:8484-91.
23. Sasaki Y, Vohra BP, Baloh RH, Milbrandt J. Transgenic mice expressing the Nmnat1 protein manifest robust delay in axonal degeneration in vivo. J Neurosci. 2009;29:6526-34; PMCID: 2697066.
24. Sasaki Y, Vohra BP, Lund FE, Milbrandt J. Nicotinamide mononucleotide adenylyl transferase-mediated axonal protection requires enzymatic activity but not increased levels of neuronal nicotinamide adenine dinucleotide. J Neurosci. 2009;29:5525-35; PMCID: 3162248.
25. Gerdts J, Brace EJ, Sasaki Y, DiAntonio A, Milbrandt J. SARM1 activation triggers axon degeneration locally via NAD^+ destruction. Science. 2015;348:453-7.
26. Khan NA, Auranen M, Paetau I, Pirinen E, Euro L, Forsstrom S, Pasila L, Velagapudi V, Carroll CJ, Auwerx J, Suomalainen A. Effective treatment of mitochondrial myopathy by nicotinamide riboside, a vitamin B3. EMBO Mol Med. 2014;6:721-31; PMCID: PMC4203351.
27. Conforti L, Gilley J, Coleman MP. Wallerian degeneration: an emerging axon death pathway linking injury and disease. Nat Rev Neurosci. 2014;15:394-409.
28. Di Stefano M, Nascimento-Ferreira I, Orsomando G, Mori V, Gilley J, Brown R, Janeckova L, Vargas ME, Worrell LA, Loreto A, Tickle J, Patrick J, Webster JR, Marangoni M, Carpi FM, Pucciarelli S, Rossi F, Meng W, Sagasti A, Ribchester RR, Magni G, Coleman MP, Conforti L. A rise in NAD precursor nicotinamide mononucleotide (NMN) after injury promotes axon degeneration. Cell Death Differ. 2015;22:731-42; PMCID: 4392071.
29. Bieganowski P, Brenner C. Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a Preiss-Handler independent route to NAD^+ in fungi and humans. Cell. 2004;117:495-502.
30. Trammell SA, Yu L, Redpath P, Migaud ME, Brenner C. Nicotinamide riboside is a major NAD^+ precursor vitamin in cow milk. J Nutr. 2016;146:957-63.
31. Chi Y, Sauve AA. Nicotinamide riboside, a trace nutrient in foods, is a vitamin B3 with effects on energy metabolism and neuroprotection. Curr Opin Clin Nutr Metab Care. 2013;16:657-61.
32. Hamity MV, Walder RY, White SR, Schmidt M, Brenner C, Hammond DL. Nicotinamide riboside, a form of vitamin B3 and NAD^+ precursor, relieves the

nociceptive and aversive dimensions of paclitaxel-induced peripheral neuropathy in female rats. Pain. 2017;158:962-72; PMCID: PMC Journal - in process.
33. Food and Drug Administration. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. In: Center for Drug Evaluation and Research (CDER), editor. Washington, D.C.: U.S. Department of Health and Human Services,; 2005.
34. Luck HJ, Roche H. Weekly paclitaxel: an effective and well-tolerated treatment in patients with advanced breast cancer. Crit Rev Oncol Hematol. 2002;44 Suppl:S15-30.
35. Sparano JA, Wang M, Martino S, Jones V, Perez EA, Saphner T, Wolff AC, Sledge GW, Jr., Wood WC, Davidson NE. Weekly paclitaxel in the adjuvant treatment of breast cancer. The New England journal of medicine. 2008;358:1663-71; PMCID: PMC2743943.
36. Trammell SA, Weidemann BJ, Chadda A, Yorek MS, Holmes A, Coppey LJ, Obrosov A, Kardon RH, Yorek MA, Brenner C. Nicotinamide riboside opposes type 2 diabetes and neuropathy in mice. Sci Rep. 2016;6:26933.
37. Thompson HJ. Mammary Cancer in Rats. In: Teicher BA, editor. Tumor Models in Cancer Research. New York: Humana Press; 2010. p. 245-58.
38. Conze D, Crespo-Barreto J, Kruge C. Safety assessment of nicotinamide riboside, a form of vitamin B3. Human and Exper Toxicol. 2016:1-12.
39. Trammell SAJ, Schmidt MS, Weidemann BH, Redpath P, Jaksch F, Dellinger RW, Migaud ME, Brenner C. Nicotinamide riboside is uniquely bioavailable in mouse and man. Nature Comm. 2016;7:14298.
40. Martens CR, Denman BA, Mazzo MR, Armstrong ML, Reisdorph N, McQueen MB, Chonchol M, Seals DR. Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD^+ in healthy middle-aged and older adults. Nat Commun. 2018;9:1286; PMCID: PMC5876407.
41. Airhart SE, Shireman LM, Risler LJ, Anderson GD, Nagana Gowda GA, Raftery D, Tian R, Shen DD, O'Brien KD. An open-label, non-randomized study of the pharmacokinetics of the nutritional supplement nicotinamide riboside (NR) and its effects on blood NAD+ levels in healthy volunteers. PLoS One. 2017;12:e0186459; PMCID: PMC5718430.
42. Surjana D, Halliday GM, Martin AJ, Moloney FJ, Damian DL. Oral nicotinamide reduces actinic keratoses in phase II double-blinded randomized controlled trials. J Invest Dermatol. 2012;132:1497-500.
43. Chen AC, Martin AJ, Choy B, Fernandez-Penas P, Dalziell RA, McKenzie CA, Scolyer RA, Dhillon HM, Vardy JL, Kricker A, St George G, Chinniah N, Halliday GM, Damian DL. A Phase 3 Randomized Trial of Nicotinamide for Skin-Cancer Chemoprevention. N Engl J Med. 2015;373:1618-26.
44. Santidrian AF, LeBoeuf SE, Wold ED, Ritland M, Forsyth JS, Felding BH. Nicotinamide phosphoribosyltransferase can affect metastatic activity and cell adhesive functions by regulating integrins in breast cancer. DNA Repair (Amst). 2014;23:79-87; PMCID: PMC4252795.
45. Tummala KS, Gomes AL, Yilmaz M, Grana O, Bakiri L, Ruppen I, Ximenez-Embun P, Sheshappanavar V, Rodriguez-Justo M, Pisano DG, Wagner EF, Djouder N. Inhibition of de novo NAD^+ synthesis by oncogenic URI causes liver tumorigenesis through DNA damage. Cancer Cell. 2014;26:826-39.
46. Santidrian AF, Matsuno-Yagi A, Ritland M, Seo BB, LeBoeuf SE, Gay LJ, Yagi T, Felding-Habermann B. Mitochondrial complex I activity and NAD+/NADH

balance regulate breast cancer progression. J Clin Invest. 2013;123:1068-81; PMCID: PMC3582128.
47. Canto C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, Fernandez-Marcos PJ, Yamamoto H, Andreux PA, Cettour-Rose P, Gademann K, Rinsch C, Schoonjans K, Sauve AA, Auwerx J. The NAD^+ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab. 2012;15:838-47; PMCID: 3616313.
48. Cavaletti G, Cornblath DR, Merkies IS, Postma TJ, Rossi E, Frigeni B, Alberti P, Bruna J, Velasco R, Argyriou AA, Kalofonos HP, Psimaras D, Ricard D, Pace A, Galie E, Briani C, Dalla Torre C, Faber CG, Lalisang RI, Boogerd W, Brandsma D, Koeppen S, Hense J, Storey D, Kerrigan S, Schenone A, Fabbri S, Valsecchi MG, Group CI-P. The chemotherapy-induced peripheral neuropathy outcome measures standardization study: from consensus to the first validity and reliability findings. Ann Oncol. 2013;24:454-62; PMCID: 3551481.
52. Kuroi K, Shimozuma K, Ohashi Y, Hisamatsu K, Masuda N, Takeuchi A, Aranishi T, Morita S, Ohsumi S, Hausheer FH. Prospective assessment of chemotherapy-induced peripheral neuropathy due to weekly paclitaxel in patients with advanced or metastatic breast cancer (CSP-HOR 02 study). Support Care Cancer. 2009;17:1071-80.
53. Stubblefield MD, Burstein HJ, Burton AW, Custodio CM, Deng GE, Ho M, Junck L, Morris GS, Paice JA, Tummala S, Von Roenn JH. NCCN task force report: management of neuropathy in cancer. J Natl Compr Canc Netw. 2009;7 Suppl 5:S1-S26; quiz S7-8.
54. Perez EA, Vogel CL, Irwin DH, Kirshner JJ, Patel R. Multicenter phase II trial of weekly paclitaxel in women with metastatic breast cancer. J Clin Oncol. 2001;19:4216-23.
55. Rugo HS, Barry WT, Moreno-Aspitia A, Lyss AP, Cirrincione C, Leung E, Mayer EL, Naughton M, Toppmeyer D, Carey LA, Perez EA, Hudis C, Winer EP. Randomized Phase III Trial of Paclitaxel Once Per Week Compared With Nanoparticle Albumin-Bound Nab-Paclitaxel Once Per Week or Ixabepilone With Bevacizumab As First-Line Chemotherapy for Locally Recurrent or Metastatic Breast Cancer: CALGB 40502/NCCTG N063H (Alliance). J Clin Oncol. 2015;33:2361-9; PMCID: PMC4500830.
56. Trussell J. Contraceptive failure in the United States. Contraception. 2004;70:89-96.
57. Lee JJ, Liu DD. A predictive probability design for phase II cancer clinical trials. Clin Trials. 2008;5:93-106.
58. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials. 1989;10:1-10.
59. Gelman A. Prior distributions for variance parameters in hierarchical models. Bayesian Anal 2006;6:1-19.
60. Kraff S, Nieuweboer AJ, Mathijssen RH, Baty F, de Graan AJ, van Schaik RH, Jaehde U, Joerger M. Pharmacokinetically based dosing of weekly paclitaxel to reduce drug-related neurotoxicity based on a single sample strategy. Cancer Chemother Pharmacol. 2015;75:975-83.
61. Fidias P, Supko JG, Martins R, Boral A, Carey R, Grossbard M, Shapiro G, Ostler P, Lucca J, Johnson BE, Skarin A, Lynch TJ. A phase II study of weekly paclitaxel in elderly patients with advanced non-small cell lung cancer. Clin Cancer Res. 2001;7:3942-9.

62. Nokihara H, Yamamoto N, Ohe Y, Hiraoka M, Tamura T. Pharmacokinetics of Weekly Paclitaxel and Feasibility of Dexamethasone Taper in Japanese Patients with Advanced Non-small Cell Lung Cancer. Clin Ther. 2016;38:338-47.
63. Little RJA, Rubin DB. Statistical analysis with missing data. 2nd ed. Hoboken, N.J.: Wiley; 200. 381 p.

APPENDIX A - PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B - Trial-Specific Data and Safety Monitoring Plan

Type of Clinical Trial:

☑ Investigator-initiated (UI/HCCC)
❑ Pilot study
☑ phase I/II
❑ phase III
❑ Interventional Treatment
❑ Non-Interventional
❑ Investigator-initiated, participating site
❑ phase I
❑ phase II
❑ Compassionate-use/Expanded Access
☑ Interventional Non-Treatment

Study risk-level:

❑ Level 1—low risk of morbidity or death, * <1% of death or any adverse event
❑ Level 2—risk of death* <1% or any adverse event 1% – 5%
❑ Level 3—risk of death* 1% – 5% or grade 4 – 5 SAE 1% – 5%
☑ Level 4—risk of death* >5% or grade 4 – 5 SAE >5%
❑ Drugs being used on a "compassionate" basis

***** *Risk of death" refers specifically to 100-day treatment-related mortality*

Reporting and Monitoring Requirements:

All institutional investigator initiated trials (IITs), regardless of assigned risk level are subject to routine DSMC monitoring activities which may include but are not limited to review of signed consent documents, eligibility and adverse event reporting.

All institutional IITs have the following **reporting requirements** as part of their DSMP:

- Provide an annual progress report to the DSMC and PRMC
- Register subjects in HCCC's Clinical Trial Management System, OnCore
- Document Adverse Events
- Document protocol deviations

Selected monitoring strategy based on risk-level: Risk Level 4

Interventional treatment trials involving investigational agents or devices with a risk of death* (>5% or grade 4 – 5 SAE >5%), e.g. all investigator initiated INDs, most Phase I/II trials, gene therapy, gene manipulation or viral vector systems high-risk clinical procedures if performed solely for research purposes. The use of a new chemical or drug for which there is limited or no available safety data in humans.

Study Safety Review

The DSMC Chair (or designee), will review study data (provided by the PI/available in OnCore) and communicate with the PI at least biannually. In addition, Dr. Vaena of the University of Tennessee will visit the University of Iowa and Wake Forest Baptist Health to review safety data on a quarterly basis and communicate his findings to Drs. Hammond and Thomas. A copy of this communication will be forwarded to the DSMC and PRMC Chairs.

Additional Reporting Requirements:

- A scanned copy of the completed eligibility checklist, with screening information and enrolling physician's signature, will be attached in OnCore for ongoing review by DSMC staff.
- Serious adverse events will be entered directly into an OnCore SAE report by the research team. OnCore will send an automatic notification to the DSMC Chair/acting Chair and staff for review.
- The DSMC utilizes a risk-based monitoring approach. The trial's research records will be monitored at minimum twice per year. Monitoring may be done more frequently depending on the protocol, risks to subjects, reported serious/adverse events, patient population and accrual rate. Records for a minimum of 25% of subjects will be monitored for the entire study.

Routine Adverse Event Reporting

For non-serious Adverse Events, documentation must begin from the first day of study treatment and typically continue through the 30 day follow-up period beginning one week after treatment is discontinued.

Collected information should be recorded in the electronic/Case Report Forms (eCRF/CRF) for that subject. A description of the event, its severity or toxicity grade (according to NCI's Common Toxicity Criteria (CTCAE), onset and resolved dates (if applicable), and the relationship to the study drug should be included. Documentation should occur in real time. The principal investigator has final responsibility for determining the attribution of the event as it is related to the study drug.

Serious Adverse Event Reporting

For any experience or condition that meets the definition of a serious adverse event (SAE), recording of the event must begin after signing of the informed consent and continue through the 30 day follow-up period after treatment is discontinued.

Investigators must report to the DSMC any serious adverse events (SAE), whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64).

An adverse event is considered **serious** if it results in ANY of the following outcomes:

1. Death
2. A life-threatening adverse event
3. An adverse event that results in inpatient hospitalization OR prolongation of existing hospitalization for ≥ 24 hours
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. A congenital anomaly/birth defect.
6. Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

SAEs must be reported via an OnCore SAE Report within 24 hours of learning of the event.

ChromaDex requires safety reporting to follow the requirements specified under 21 CFR § 312.32 IND safety reporting. ChromaDex will also be notified of all SAEs. Notifications should be sent to:

Sarah Garthe, M.S.
Regulatory Affairs Manager
10005 Muirlands Blvd., Suite G
Irvine, CA 92618 USA
SarahG@chromadex.com
www.chromadex.com
Tel: +1.612-512-8251.

Data Monitoring and Management

All studies that undergo PRMC review and/or utilize HCCC Clinical Research Services (CRS) resources are required to register subjects in OnCore. Subject registration includes the following:

- Consent date and the IRB approved consent used
- Date of eligibility and eligibility status (eligible, not eligible)
- On study date and subject's disease site (and histology if applicable)
- On treatment date (if applicable)

Subject Data

In addition to the subject registration and subject status data entered in OnCore for all HCCC trials, research staff also enters the subject study data into electronic case report forms (eCRFs) for HCCC investigator initiated studies. eCRFs are approved by the PI and statistician prior to study activation to ensure the most effective data acquisition. All information on eCRFs will be traceable to the source documents which are generally maintained in the subject's file. eCRF data are expected to be entered into OnCore within 30 calendar days after a subject's study visit.

Forms Monitoring

OnCore eCRF data are monitored on a routine basis (dependent on accrual) to ensure all mandatory fields are entered completely, accurately and within time requirements. The assigned DSMC monitor manages the logistics associated with the data monitoring review. Once the clinical trial is identified for monitoring, the monitor arranges for a selection of cases to review from among the subjects registered in OnCore. As part of the forms monitoring process, the assigned monitor will issue queries within the eCRF to resolve missing, incomplete and/or incorrect information. A member of the research team is expected to respond to monitoring queries within 14 business days.

This process can often identify a misunderstanding or deficiency in protocol requirements early in the study and can improve data quality.

Final Reports

A summary of each subject's data record is continually available to the PI, research staff, and DSMC from OnCore's Biostat Console. The availability of this information is a valuable tool for the preparation of final reports and manuscripts as well as ongoing deficiency reports.

APPENDIX C – Lab Manual

Keystone Bioanalytical, a GLP-compliant contract research organization will be used for measurement of the NAD^+ in red blood cells and N-methyl-nicotinamide in blood (Biomarker samples). Measurement of paclitaxel levels in blood will be conducted by the Department of Pathology at University of Iowa (Pharmacokinetic samples).

Refer to Section 6.2.4 Biomarkers

Refer to Study Calendar – Pre-Treatment NAD+ levels.

At UIHC, contact Dr. Hammond (office: 319-335-9595 or cell: 319-621-6110) as soon as you are aware that a sample for biomarkers will be collected/scheduled. At Wake Forest, contact Dr. Christopher Peters (336-716-3452) as soon as you are aware that a sample for biomarkers will be collected/scheduled.

Collection:

- Draw two 3-ml samples of blood into light blue top citrate tubes, which contain 3.2% citrate.
- Immediately invert the tubes four times, and place on wet ice.
- At UIHC, give to Dr. Hammond or her lab staff (who should be present). At Wake Forest, samples are to be messengered to Dr. Peters' laboratory in Hanes 2049.

Samples must be kept on wet ice and

processed within two hours of collection with no hemolysis

Processing:

For **each** sample:

- Centrifuge tubes at 2,000 *g* at 2-8˚C for 5 min to separate plasma and red blood cells.
- Aliquot 0.5 ml of plasma into a labeled cold cryovial, snap freeze, and store at -80°C. Decant remaining plasma and discard.
- Using a positive displacement pipette, aliquot 100 µL of the sedimented RBCs into a labelled chilled cryovial tube that contains 1 ml of cold 0.5 M perchloric acid. Invert tube 4 times, then snap freeze, and store at -80°C until analysis.

Shipping:

Biomarker samples collected at the University of Iowa will be processed by Dr. Hammond or her laboratory staff, stored in a -80 degree Celsius freezer at the University of Iowa, and shipped in batches of 200 or more by Dr. Hammond to Keystone Bioanalytical Laboratory for analysis.

Biomarker samples collected at Wake Forest will be processed by Dr. Peters' research staff, stored in a -80 degree Celsius freezer at Wake Forest, and shipped as a batch to Keystone Bioanalytics as directed by Dr. Hammond.

One plasma sample and one RBC sample will be shipped on dry ice to Keystone Bioanalytical as directed by Dr. Hammond. The other samples will be retained on site should the first samples be inadvertently destroyed.

Allan Xu, Ph.D.

Lab Director

Keystone Bioanalytical,Inc.

501 Dickerson Rd.

North Wales, PA 19454

215-699-8899

axu@keystonebioanalytical.com

Refer to Section 6.2.3 Pharmacokinetics

Refer to Study Calendar - Paclitaxel levels.

Collection:

- Two 5-ml blood samples will be collected, 30 ± 10 minutes AFTER the infusion of paclitaxel or between 15 and 30 minutes after the infusion of nab-paclitaxel, from the arm or port.
- Collect the blood samples in a purple (EDTA) top tube and place on wet ice.
- At UIHC, contact Dr. Hammond (319-335-9595 or 319-621-6110) once the sample is obtained.
- At Wake Forest, contact Dr.Christopher Peters (336-716-3452) once the sample is obtained.

Processing:

- At UIHC, a person from Dr. Hammond's lab will retrieve the samples on wet ice, centrifuge at 1,000 *g* at 2 to 8 degrees Celsius, and aspirate two 1-ml aliquots of plasma from each tube into two 1.8-ml polypropylene cryo tubes with inner threads.
- All four samples will be labeled with the study ID and stored in a -80 degree Celsius freezer. Avoid repeated freeze-thaw cycles.
- At Wake-Forest, the samples will be messengered to Dr. Peters' laboratory in Hanes 2049. He will process the samples as described immediately above.

Shipping:

- One aliquot of each sample (total of two) collected at Wake Forest, will be shipped on dry ice to the Department of Pathology at University of Iowa to the attention of David Frederick. An email will be sent to donna-hammond@uiowa.edu and to matthew-krasowski@uiowa.edu with tracking information. The other samples will be retained on site should the first samples be inadvertently destroyed.

Address Label:

Attn: David Frederick

Department of Pathology

University of Iowa Hospitals and Clinics

200 Hawkins Dr 6240 RCP

Iowa City, IA 52242

david-frederick@uiowa.edu

319-353-7018

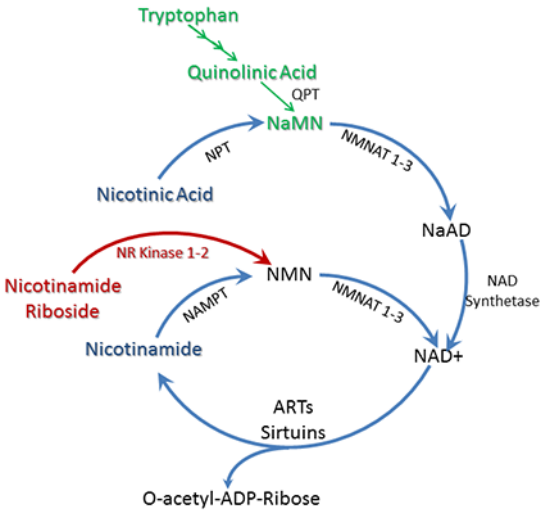
On day of shipment, please send e-mail notification along with sample tracking information to:

Donna L. Hammond, Ph.D.
Anesthesia Research 3000 ML
University of Iowa
200 Hawkins Drive
Iowa City, IA 52242

Phone: 319-335-9595
Mobile: 319-621-6110 (contact 24/7)
E-mail: donna-hammond@uiowa.edu

APPENDIX D - List of Tables

Title
Schematic of NAD+ biosynthetic pathways



APPENDIX E – FACT/GOG – NTX subscale

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
NTX 1	I have numbness or tingling in my hands.....	0	1	2	3	4
NTX 2	I have numbness or tingling in my feet.....	0	1	2	3	4
NTX 3	I feel discomfort in my hands.....	0	1	2	3	4
NTX 4	I feel discomfort in my feet.....	0	1	2	3	4
NTX 5	I have joint pain or muscle cramps	0	1	2	3	4
HI12	I feel weak all over.....	0	1	2	3	4
NTX 6	I have trouble hearing.....	0	1	2	3	4
NTX 7	I get a ringing or buzzing in my ears.....	0	1	2	3	4
NTX 8	I have trouble buttoning buttons	0	1	2	3	4
NTX 9	I have trouble feeling the shape of small objects when they are in my hand.....	0	1	2	3	4
An6	I have trouble walking.....	0	1	2	3	4

APPENDIX F – Total Neuropathy Score – clinical version

	Score				
Scale	**0**	**1**	**2**	**3**	**4**
Sensory symptoms	None	Symptoms limited to fingers or toes	Symptoms extend to ankle or wrist	Symptoms extend to knee or elbow	Symptoms above knee or elbow or functionally disabling
Motor symptoms	None	Slight difficulty	Moderate difficulty	Require help/assistance	Paralysis
Autonomic symptoms, n	0	1	2	3	4 or 5
Pin sensibility	Normal	Reduced in fingers/toes	Reduced up to wrist/ankle	Reduced up to elbow/knee	Reduced to above elbow/knee
Vibration sensibility	Normal	Reduced in fingers/toes	Reduced up to wrist/ankle	Reduced up to elbow/knee	Reduced to above elbow/knee
Strength	Normal	Mild weakness (MRC 4)	Moderate weakness (MRC 3)	Severe weakness (MRC 2)	Paralysis (MRC 0–1)
Deep tendon reflex	Normal	Ankle reflex reduced	Ankle reflex absent	Ankle reflex absent, others reduced	All reflexes absent

The Total Neuropathy Score – Clinical is a reduced version of the TNS that assesses only the signs and symptoms that are apparent during a clinical examination. The questionnaire is copyrighted, and has to be purchased. The table above is a summary of the key elements.