CLINICAL RESEARCH PROTOCOL

BIOMARKERS IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROTOXICITY: BETTER TOOLS AND UNDERSTANDING

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Version	DATE	DESCRIPTION OF CHANGE AND APPROVAL DATES
1.0	13FEB2017	02/14/2017: Submitted to CCRC. 03/10/2017: Conditional Approval from CCRC.
1.1	18MAR2017	03/27/2017: Conditional Approval from CCRC.
1.1	31MAR2017	03/31/2017: Primary and secondary objectives clarified in formatting of the text. 05/04/2017: Conditional Approval from CCRC.
1.2	08MAY2017	05/08/2017: Protocol updated with OCR monitoring plan and housekeeping changes. 05/11/2017: Submitted to CPHS. 07/17/2017: Approved
1.3	15AUG2017	08/15/2017: Protocol modified with risk of PET/CT false positive and minor housekeeping changes. 8/17/2017: Submitted to CPHS
2.0	20OCT2017	10/20/2017: Protocol modified such that injections of india ink will be placed in the distal fourth dorsal interosseous toe web, rather than the first interosseous toe web as stated in all prior versions of the protocol. The option for a research-only blood draw will be given to patients when a standard blood draw is not already scheduled. India ink changed from Printex to Carlo Erba Devin Prior, M.D. added to protocol.
	18DEC2017	Active and open to enrollment.
2.1	17JAN2018	SUDOSCAN added to protocol. 01/17/2018: Submitted to the CCRC for review. 02/05/2018: Approved by the CCRC. 02/08/2018: Submitted to CPHS. 02/27/2018: Approved by CPHS.
2.2	18MAY2018	AE language and reporting adjusted to align with institutional standards and expectations. Visit windows have been modified for ease of scheduling, and additional EPR readings have been added to accommodate for fluctuation of CIPN symptoms. 05/18/2018: Submitted to the CCRC for review. 05/22/2018 Approved by the CCRC. 06/13/2018:Submitted to CPHS. 06/21/2018: Approved by CPHS
2.3	19OCT2018	Neurofilament light chain biomarker was added to protocol. Protocol was also revised to address findings in monitoring report.

TABLE OF CONTENTS

List of Abbreviations

CCRC Clinical Cancer Review Committee

CE Carlo Erba (India ink made by suspending Carlo Erba manufactured carbon particulates)

CIPN Chemotherapy Induced Peripheral Neurotoxicity

CPHS Committee for the Protection of Human Subjects (Dartmouth's IRB used by DHMC)

D-H Dartmouth-Hitchcock (refers to D-H system of patient treatment clinics or inpatient units)

DHMC Dartmouth-Hitchcock Medical Center (refers to the physical location at One Medical Center Drive, Lebanon or the organizational complex of Geisel Medical School, WRJ-VA, and D-H)

DNA Deoxyribonucleic acid

DSMAC Data Safety Monitoring and Accrual Committee

EMG Electromyography

EPR Electron paramagnetic resonance

FDA Food and Drug Administration (US Dept of Health and Human Services)

HIPAA Health Insurance Portability and Accountability Act of 1996

IDE Investigational device exemption

L-band Frequency of microwaves used for the clinical EPR spectrometer at Dartmouth (~1-2 GHz)

MRDD Magnetic Resonance Diagnostic Devices

MTG-B Murine breast adenocarcinoma (tumor model)

NCCC Norris Cotton Cancer Center (at Geisel and D-H)

NCI National Cancer Institute, one of the National Institutes of Health

NO Nitrous Oxide

NSR Non significant risk (device)

PHI Patient health information

SNP Single nucleotide polymorphisms

Study Summary

Title	***BIOMARKERS IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROTOXICITY: BETTER TOOLS AND UNDERSTANDING***
Short Title	*Biomarkers in CIPN*
Protocol Number	*CPHS: #30310; Velos/CTO: D17062*
Phase	*Phase 1 -- Pilot*
Methodology	*Non-randomized, non-controlled*
Study Duration	*One year*
Study Center(s)	*Single-center: Dartmouth-Hitchcock Medical Center*
Objectives	*Obtain longitudinal pilot data to include clinical metrics, electrophysiologic metrics, and tissue oxygen as potential biomarkers for CIPN.* *Establish the relationship between tissue oxygen measurements and existing clinical and electrophysiological biomarkers as CIPN develops.* *The primary objective of the study is to prospectively compare peripheral tissue oxygenation levels between patients who later develop CIPN, and those who do not.* *The secondary objectives of the study are to: (i) establish the feasibility of the approach, (ii) to evaluate the utility of longitudinal clinical and electrophysiologic assessments and (iii) to describe changes in biomarkers of axonal degeneration including neurofilament light chain over the course of chemotherapy.*
Number of Subjects	*30*
Diagnosis and Main Inclusion Criteria	*Adult patients with a diagnosis of breast cancer and who are scheduled to receive chemotherapy with a life expectancy greater than or equal to 12 months.*
Study Product, Dose, Route, Regimen	*India ink (Carlo Erba) and electron paramagnetic resonance oximetry measurements.* *Standard, clinical electrophysiologic tests: nerve conduction study and needle electromyography.* *SUDOSCAN measure of sudomotor function.*
Duration of administration	*Ink administered once, EPR oximetry measurements and standard electrophysiologic studies at three distinct time points over the course of chemotherapy.As many as five measurements will be done for each time point.*
Statistical Methodology	*Correlations between peripheral tissue oximetry and clinical & electrophysiologic measures of CIPN and EPR assessments of hypoxia and response to oxygen. Descriptive statistics of NFL changes over time. Correlations between NFL and peripheral neuropathy assessments.*

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (International Conference on Harmonization ICHE6), the Code of Federal Regulations Title 21 especially device related parts 803 and 812, and other applicable government regulations and Institutional Research Board (i.e., Dartmouth's Committee for the Protection of Human Subjects, CPHS) research policies and procedures.

1.1 Background

As medical surveillance and effective therapies improve the survivability of cancer, the number of people living with a history of cancer will increase drastically. "Quality of survival" is increasingly being recognized as an important mission of cancer care. Chemotherapy induced peripheral neurotoxicity (CIPN) is a common, potentially severe and dose-limiting adverse effect of cancer chemotherapeutic agents. A major shortfall in management of subjects with CIPN is the absence of (i) early predictors for those at greatest risk of CIPN, and (ii) sensitive biomarkers to identify disease onset at an earlier stage.

The pathophysiology of CIPN is not completely elucidated; however, an important set of data implicates oxidative stress, mitochondrial dysfunction and microvascular injury (Carozzi et al., 2006). Alterations in antioxidant activity (glutathione reductase, catalase and superoxide dismutase) after exposure to chemotherapeutic agents increase the susceptibility of neurons to injury from reactive oxygen species (ROS). Some chemotherapeutic regimens have been associated with ROS accumulation in dorsal root ganglion neurons. TRPA1, a neuronal transient receptor potential channel A1 gene implicated in neuronal nociception, is activated by nitrous oxide (NO). Mitochondrial damage resulting from oxidative stress has been suggested as a mechanism for chemotherapy associated neurotoxicity. A specific single nucleotide polymorphism in a glutathione-S-transferase gene (GSTP1) has been extensively investigated in relation to peripheral neurotoxicity. These studies have revealed an association in approximately half of the studies completed. This association represents one of the strongest among candidate genes evaluated in polymorphism association studies. In an experimental model of neuropathy induced by Schwann cell mitotoxicity, preferential and early loss of small unmyelinated (Aδ and C) fibers was observed. This early effect on small, unmyelinated fibers was seen even in the setting of preserved Schwann cell proliferation and survival. This surprising finding indicates that Schwann cell mitochondrial function is vital for normal function and survival of, not only large myelinated fibers, but small unmyelinated fibers; further, those fibers most affected in CIPN (Aδ and C) are selectively vulnerable (Viader et al., 2011). Axons of C-fibers in rats exposed to paclitaxel demonstrate swollen and vacuolated mitochondria. These mitochondrial changes mirror pain behavior in the animals and further support pathophysiology related to mitotoxicity (Flatters and Bennett, 2006). Clinical trials with antioxidant therapies have been met with mixed success in terms of efficacy. A recent, well-designed clinical trial testing α–lipoic acid revealed no significant decrease in incidence and severity of peripheral neurotoxicity. However, smaller trials using other antioxidants, including α-tocopherol, reduced glutathione and oral glutamine, have been shown to reduce the incidence and severity of neuropathy. In addition to direct neuronal effects, oxidative stress has been implicated in endothelial cell apoptosis of the vasa nervorum. This is thought to result in peripheral nerve ischemia and is supported by the finding that antiangiogenic adjuvant therapy increases CIPN incidence. These data support the use of metrics of oxidative stress and microvascular injury as biomarkers of CIPN. Another important pathophysiologic mechanism of

CIPN is thought to be related to the production of proinflammatory cytokines and chemokines. The effect of inflammatory cytokines on nociceptive sensors or nerve fibers has been proposed as the etiology for the immediate painful paresthesias experienced after administration of chemotherapeutic agents (Wang et al., 2012). Oxidative stress promotes the production of proinflammatory cytokines; there is evidence that hypoxia can induce a pro-inflammatory phenotype in endothelial and tumor cells (Tellier et al., 2015). Therefore the ability to measure changes in tissue oxygen could provide a very effective tool to provide quantitative measurements of the key component of oxidative stress. We propose the use of a technique uniquely available at Dartmouth-Hitchcock (clinical oximetry based on electron paramagnetic resonance [EPR] spectroscopy) as a novel biomarker to predict the occurrence, severity and duration of CIPN in individual participants. EPR oximetry is a clinically applicable tool that has the unique capability of repeatedly measuring tissue oxygen. It is non-invasive and rapid, requiring less than 10 minutes to obtain a stable measurement of oxygen in tissues after the initial placement of the paramagnetic material, India ink, at the sites of interest. This allows for the longitudinal surveillance of tissue oxygen that can be correlated with clinical and electrophysiological markers of neuropathy as neurotoxicity develops, progresses and resolves. Correlation of this metric with established indices of neuropathy will not only provide critical insights into the underlying pathophysiology of CIPN, but may also provide the opportunity of a more sensitive biomarker for clinical trials.

Neurofilament light chain (NF-L) is emerging as a sensitive blood-based biomarker of axonal degeneration. NF-L is a component of the axonal cytoskeleton that leaks out of degenerating axons. NF-L has been reported to be elevated in plasma or serum in a wide range of neurodegenerative disorders, including CNS disorders such as multiple sclerosis (Siller et al., 2018) and ALS (Lu et al., 2015) as well as PNS disorders such as Charcot Marie Tooth (Sandelius et al., 2018) and Guillain-Barre syndrome (Mariotto et al., 2018). To date, there are no published reports of elevated blood NF-L levels in patients with CIPN, although it has been reported to increase in rat model of vincristine-induced neuropathy (Meregalli et al., 2018). We propose measuring blood NF-L levels at baseline and at each chemotherapy treatment session in order to determine if blood NF-L increases and how longitudinal changes in NF-L correlate with clinical and electrophysiological measures of neuropathy.

There are data linking single nucleotide polymorphisms (SNP's) to neurotoxicity (Baldwin et al., 2012). Polymorphisms in genes to include CYP 2C8, CYP 3A5, ABCB1 and FANCD2 have all been associated with CIPN, including differences in interindividual toxicity and response (Abraham et al., 2014; Boora et al., 2016). Data remain inconclusive however. In this proposal, we will provide study subjects with the option to donate blood for placement in a CIPN-biorepository. Although not in the realm of the current pilot study, this will allow for future studies of relevant polymorphisms in a cohort of very well phenotyped individuals.

1.2 Investigational Agents

1.2.1 – Electrophysiological Assessment

1.2.1a – **Nerve Conduction Studies:** Electrophysiological assessments, used as part of the assessment for neuropathy, will be conducted under the supervision of the PI (VL) using a certified Teca Synergy T5EP NCS EMP EP machine. The nerve conduction studies (NCS) will be measured using standardized techniques for surface recording and stimulation developed in our Clinical Neurophysiology laboratory at Dartmouth-Hitchcock for clinical care. The widely

accepted criteria for identification of abnormalities will be used. Sensory conductions may include sural, medial plantar and dorsal sural antidromic testing with assessment of peak to peak amplitudes of sensory nerve action potentials and sensory conductions. Motor conductions may also be tested in tibial and peroneal nerves with measurement of baseline to peak amplitude of the compound muscle action potential, motor conduction velocities and distal motor latencies. Recording will be performed from the extensor digitorum brevis and tibialis anterior muscles. Additional or different nerves may be tested at the discretion of the examiner. Temperature will be controlled and data compared with normative age-matched reference data. Testing will be performed by electromyography (EMG) technicians trained in performing electrodiagnostic studies. Testing will be repeated to ensure verifiable amplitudes. All devices and tests are considered part of routine, standard care; no investigational devices or tests will be used.

1.2.1b – **Sudoscan:** One limitation of NCS is that most toxic neuropathies involve primarily small, unmyelinated (C-) and small myelinated nerve fibers (Aδ-); these small fibers are less sensitively assessed with NCS than large fiber function. Changes in peripheral autonomic nervous system function are an early manifestation of distal small fiber neuropathy. Sudomotor dysfunction is one of the earliest detectable neurophysiologic abnormalities in distal small fiber neuropathies, including toxic neuropathies. SUDOSCAN (Impeto Medical, Paris, France) is a simple, noninvasive, easy-to-perform sudomotor test that measures sweat gland function that also compares favorably to other metrics of small fiber function, including quantitative sensory testing (QST), skin biopsy for epidermal nerve fiber density and sympathetic skin response. SUDOSCAN has been approved by the FDA as a sudomotor function test; the device uses a low DC voltage (≤ 4 volts) to activate sweat glands in the soles of the feet and the palms of the hand and measures the current of chloride ions that flow out of the sweat glands in response to the electric impulse. The test takes between two-three minutes.

1.2.2 – India ink and EPR[1]

A non-significant risk (NSR) investigational device and an exempt device will be used in this study: (1) EPR spectroscopy designed to assess oxygen in vivo in human tissues and has been previously recognized by the CPHS as a NSR device, and (2) the use of paramagnetic carbon particulates that are sensitive to the amount of oxygen that is present, suspended in a type of India ink and injected into superficial tissues; India ink is an exempt device. (See Section 5A for details about the Food and Drug Administration's (FDA) designation of India ink for medical useas a device, for the purpose of approving the marketing of sterile forms on India ink for medical use only. As detailed in Section 5A, the FDA considers tattooing inks to be cosmetic for regulatory purposes and does not require oversight of their manufacture or labeling or packaging).

(1) EPR oximetry: The clinical EPR instrument, located in 2K -- the Radiation Oncology clinical suite of DHMC, operates at ~430 G (0.043 T) magnetic field strength and ~1.2 GHz microwave energy as the radiofrequency (RF) source. The field is very low compared to whole-body clinical MRI scanners that operate typically at 1.5T or 3T magnetic field strength. The 1.2 GHz RF source in our clinical EPR scanner will use <20-mW power continuously. The maximum specific absorption rate (SAR) in the tissue at the position that should have the greatest density of RF, directly under the loop of the surface-loop resonator, has been estimated to be 3.7±1.2 W/kg at

[1] Note that this protocol will use the same ink and the same procedures for injecting it and for measuring oxygen in tissues with EPR oximetry as in Velos D17008/CPHS 12459 (*Measurement of the Partial Pressure of Oxygen in Cancers Using Electron Paramagnetic Resonance Oximetry with Injected India Ink); PI Harold Swartz). An overview of oximetry using India ink is presented here with emphasis on its application to this proposal.*

100 -mW incident 1.2 GHz RF power using a surface-loop resonator with an efficiency of about 0.1 $mT/W^{1/2}$ (Salikhov and Swartz 2005). This estimated value is substantially below the recommended limit (12 W/kg) in the relevant regulation for extremities. Thus the SAR for our measurements, even at 100-mW power, is well within the acceptable range for human applications. The protocol for our clinical EPR scanner will not use rapidly changing magnetic field gradients such as those used in MRI. However, the EPR measurements use three types of magnetic fields that change in time during the measurement:

1. Main magnetic field sweep: The sweeping of the main magnetic field is done at slow rates, typically a linear sweep of 8 G over a 30-sec period, which translates to a rate of ~$3x10^{-5}$ T/sec.

2. Modulation field: We typically use a modulation field of 1 G or less at 20 kHz, which will amount to 2T/sec.

3. Multisite oximetry: We apply a static gradient magnetic field, which will be applied during the entire 3-sec duration of data acquisition.

In all three scenarios, the rate of change of magnetic field is well below the limit of 20 T/sec set by the 1988 or 1995 Magnetic Resonance Diagnostic Devices (MRDD) Guidance. Hence the time-rate of change in magnetic field is not expected to have any significant effect on the subject.

L-band EPR spectroscopy for in vivo oximetry in human subjects has been used at Dartmouth since about 2002, in two protocols previously approved by CPHS (#16578 and #12459). This device is essentially identical to the L-band EPR spectrometer used to detect radiation in vivo in teeth, in protocol #15492). This device has been previously evaluated by CPHS and found not to require an Investigation device exemption (IDE), i.e., it has been classified as a nonsignificant risk device (NSR); see definition below).

Under 21 CFR 812.3(m), a non significant risk (NSR) device means an investigational device that does NOT meet any of the following criteria for being a significant risk, i.e., it should NOT fit these criteria:

- _Is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject;
- _Is purported or represented to be for use supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject;
- _Is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject; or
- _Otherwise presents a potential for serious risk to the health, safety, or welfare of a subject.

The EPR oximetry device does not fit any of these criteria and should continue to be considered a NSR device.

(2) The India ink we propose to use in this study consists of 20-50µL of Carlo Erba ink injected in subcutaneous tissue at the distal fourth interosseous toe web. This ink (referred to herein as Carlo Erba ink, CE ink, or India ink) is based on a paramagnetic black pigment: purified and depyrogenated charcoal manufactured by Carlo Erba that is prepared as a sterile ink using the protocol developed at the lab of the NIH-Funded Principal Investigator of Project 1, Professor Bernard Gallez, using the Standard Operating Procedure (SOP): 63.2 EPR Center Standard Method: Preparation of a Carlo Erba charcoal suspension for EPR Oximetry. A summary of the components of CE follows:

Materials in Carlo Erba ink	Prior clinical use of carbon in India ink as a device
1. Purified charcoal from Carlo Erba (Code 332658), depyrogenated CAS# 7440-44-0	Pigment: Carbon particulates including charcoal have been used safely in numerous studies for the localization of lesions in humans and animal models in the U.S. and abroad; see details in Section 5A.
2. Gum arabic (USP quality) The FDA's Select Committee on Generally Recognized as Safe (GRAS) Substances recognizes Gum Arabic as a GRAS and says, "There is no evidence in the available information on gum Arabic that demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced." http://www.fda.gov/ Food/IngredientsPackagingLabeling/GRAS/SCOGS/ucm260422.htm	Suspension materials: The FDA classifies inks used for permanent cosmetic tattoos and for medical uses in marking as a cosmetic, which does not require registering or oversight of the manufacture and labeling of the products. It also has approved the *voluntary* 510(k) premarket notification request to market Endomark, a sterile India ink, and for Spot ink as *devices* to be used for medical injections subcutaneously. Hence we conclude that our use of India ink is a device; Section 5A has details.
3. Saline (NaCl at 0.9% concentration in water) (medical grade)	

Section 5A and Appendix C present additional details about why India ink is a device and about its risks. To summarize: Carlo Erba does not fit any of the criteria for being a significant risk device; more importantly, Carlo Erba ink has been recognized by the FDA as not being required to apply for IDE, i.e., India ink fits the criteria in 21 C.F.R. 812.2(c) as being *exempt from the IDE requirements.* This section 1.2 (and the required form for devices) present details about why EPR oximetry is a nonsignificant risk device. Therefore, we request that CPHS designate India ink as being exempt from IDE requirements and designate EPR oximetry as being a NSR device when approving this protocol.

1.3 Preclinical Data

As detailed in Section 1.1, preclinical studies have produced a wealth of evidence suggesting that oxidative stress, mitochondrial dysfunction, and microvascular injury mediate the relationship between chemotherapeutic agents and peripheral neuropathy. Changes in tissue oxygen levels can provide a measure of oxidative stress and, in effect, microvascular injury – potential biomarkers of CIPN.

The development of India ink as an oxygen probe for pre-clinical and clinical applications has been carried out by the laboratories of Dr. Swartz at the EPR Center at Dartmouth and Dr. Gallez at Catholic University of Louvain (CUL) in Brussels, Belgium.

The preparation and paramagnetic properties and suitability for *in vivo* oximetry of 36 commercially available charcoals, before and after suspending as an ink, were carried out by

Jordan et al. (1998). While they found that several charcoals had sufficient paramagnetic properties to be suitable for EPR spectroscopy, only three—including two independent batches of Carlo Erba—demonstrated appropriate stability over time, as assessed by long term continuation of observing responsiveness of mice to breathing enriched oxygen and compression-induced hypoxia. For these reasons, and because charcoal based inks have been preferred in Europe for medical use as injections in humans, Gallez' laboratory (in Brussels) continued to conduct a series of *in vitro* and *in vivo* studies of oximetry using Carlo Erba as the basis of the India ink sensor.

For both charcoal-based India inks (e.g., Carlo Erba) and carbon black-based India ink (e.g., Printex U), the suspending agents and any additives for the inks (added either to obtain a viscosity suitable for injection or to enhance the physical stability of the suspension) needed to be based on biocompatible and safe materials that also had properties suitable for stable paramagnetic signals and demonstrable responsiveness to the presence of oxygen in tissues, particularly at low (hypoxic) levels of greatest clinical interest. An additional consideration was to identify pigments with the properties needed for oximetry and which could be reliably reproduced from different batches of manufactured carbon particles and whose manufacturing process could be certified as to its purity. Therefore, a series of tests were conducted on commercially available carbon particulates to identify the most suitable pigments as well as to identify other ingredients for an ink to be used as an in vivo paramagnetic marker/sensor for oximetry.

The preparation and biocompatibility testing of oxygen sensitive inks for EPR oximetry using carbon black (the basis of Printex U India ink) has been described in detail by Charlier et al. (2004). The biocompatibility studies were performed for a range of ink preparations based on carbon black, including the Printex U carbon black (originally manufactured by Degussa-Hüls). The coatings were a biocompatible polymer carboxy methyl cellulose (CMC), hydroxypropyl methyl cellulose (HPMC), and polyvinyl pyrrolidone (PVP). Tests included cytotoxicity via tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Aldrich) assay, hemolysis, and histology using hematoxylin-eosin (H-E) staining of mouse muscle tissue. No significant cytotoxicity was observed with the MTT assay for any ink preparations, irrespective of biocompatible polymer coating. A slight hemolytic effect was observed for the various ink preparations relative to the negative control (Polyethylene USP88), though effects for all preparations were small when compared to the positive control (copper plate) and coating with CMC served to reduce the effect. Using the Printex U ink preparations, histology showed no sign of toxicity or necrosis surrounding the carbon black at the site of injection. This biocompatible ink has been successfully applied to volunteers and patients at Dartmouth without any serious toxicity.

Additional details about the preclinical data for EPR oximetry using India ink are contained in protocol 29880.

1.4 Clinical Data to Date

Section 1.1 describes recent clinical trials testing antioxidant therapies to reduce peripheral neurotoxicity. Such studies have provided mixed results, but two trials in particular (Cavaletti et al., 2004 and Velasco et al., 2014) support precise clinical and electrophysiological evaluation of the PNS to predict the incidence and severity of CIPN. No clinical trials to date have identified and measured biomarkers for assessing the extent of CIPN or predicting outcomes.

Researchers at Dartmouth have conducted a series of *in vivo* EPR oximetry measurements in human subjects, using India ink as the paramagnetic material. India ink is highly sensitive to tissue oxygen levels, is very stable in tissue, and already has been approved for use in human subjects (Swartz et al., 1994). Some existing oximetry measurements in human subjects have been made in subcutaneous tissues in the feet of normal volunteers, performed under a CPHS approved protocol to study healthy volunteers. The primary clinical goal of these studies has been to develop and validate EPR oximetry as a technique to monitor tissue oxygen levels in the feet of diabetic patients to guide therapeutic intervention for peripheral vascular disease. These experiments confirm that the method can be used effectively and repeatedly in human subjects, as detailed further in protocol 29880.

1.5 Risk/Benefits

The most important potential risk is that participation in a study of neurotoxic sequelae to a chemotherapeutic regimen may increase anxiety about the therapy; this is especially true given the subjects will likely have recently learned of their cancer diagnosis and will have just been introduced to all the possible short-term and long-term risk of chemotherapy. Discussion of chemotherapy risk is part of a dedicated pre-therapy assessment and education visit ("pre-chemo visit") to place the risk of neurotoxicity within its appropriate context. This will also be discussed during recruitment and as part of the consent process. A potential benefit is the enhancement of opportunities to educate subjects regarding CIPN. Other risks include the discomfort of electrophysiologic evaluation, the time needed to complete questionnaires/ undergo neurologic examination, and loss of privacy relating to collection of study data.

Electrophysiologic evaluation of large fiber neuropathy is performed using nerve conduction studies (NCS) ; this is a test used routinely in the clinic for diagnosis of nerve disorders. Nerve conduction studies involve delivery of a mild electrical stimulation to the skin while recording the response from a superficial disc electrode placed over top of the skin. There is a risk of discomfort with the test but discomfort is generally mild and will be mitigated with careful study procedures. Evaluation of sudomotor function and small-fiber neuropathy is performed using SUDOSCAN, which may cause a slight tingling, but painless, sensation in the feet or the hands when the electrical impulse is being generated.

There is a risk of mild discomfort with placement of the paramagnetic material for EPR oximetry. The material is placed using a needle and syringe. In addition to the discomfort of the needle, there is a risk of minor swelling and discomfort after the injection (can persist for up to 3 days) and a chance of the India ink spreading, resulting in a larger dark spot than is expected. It should be noted that IRB approval has already been granted for the use of this technique in other applications (#16578 and #12459) and is in process of being approved for #29880 where these risks are detailed.

If a patient receives a PET/CT scan for restaging a tumor in the area of the injection, there may be a false positive at the site of the injection. Because this study does not involve ink injections into a tumor, this is suspected to be a very minimal risk.

Subjects will be protected against unnecessary risk with the use of careful study procedures. Records containing personally identifying information of subjects participating in the study will be coded and kept in a locked office in a secure environment. Access to code key will be limited to the PI and study coordinator. The risk of discomfort with the placement of the paramagnetic ink is minimal. Patients will be fully informed that the ink will make a permanent mark similar to a

small tattoo. The risk of breathing 100% oxygen through a nonrebreather mask for 10 minutes is minimal. The risk of discomfort with electrodiagnostic study will be minimized by performing a focused evaluation with the minimal number of electrical stimuli/needle electrode penetrations as possible and as per standard clinical practice.

Patients will be informed of any risk associated with blood draws which includes discomfort during the procedure, bruising, bleeding, swelling at the puncture site, infection, and feeling lightheaded or passing out during the procedure. Since blood will be generally drawn in conjunction with the patient's routine laboratory assessments for their chemotherapy, the additional risk should be minimal.

Patients will be informed of the risks of participating in research involving the study of their genetic information. The informed consent form will have an opt-in box ensuring that each patient is aware of the risks and research purposes of the blood draw and genetic analysis being conducted.

Patients will be informed that they may not personally benefit from participation in this study; however, the study aims to help patients in the future. The identification of a biomarker that not only provides information regarding the underlying pathophysiology, but also allows for early prediction of those patients at greatest risk of developing chemotherapy-induced peripheral neurotoxicity, is of significant benefit to patients undergoing chemotherapy. Early identification/prediction can help oncologists tailor and advise patients about side effects they may expect to develop and help them make informed choices about their therapy. Advancements in the understanding of the pathogenesis of this neurotoxicity will lead to better therapies, both in the prevention and the management of CIPN.

2 Study Objectives

The overall objective is to extend the pathophysiologic understanding of CIPN and identify better biomarkers for patients at greatest risk of developing early and/or severe or refractory CIPN.

Primary Objectives:
The primary objective of the study is: ***to prospectively compare peripheral tissue oxygenation response between patients who later develop CIPN, and those who do not.***

Secondary Objectives:
The secondary objectives of the study are to: (i) establish the feasibility of the approach, (ii) to evaluate the utility of longitudinal clinical and electrophysiologic assessments and (iii) to describe changes in biomarkers of axonal degeneration including neurofilament light chain over the course of chemotherapy
Specific Aim #1: *Adapt the existing clinically established techniques for EPR oximetry to measurements of oxygen to be relevant to the pathophysiology of CIPN*. In the proposed research, we will use an India ink injection and obtain an initial oximetry reading assessing local oxygen metabolism. The objective of this aim will be to establish feasibility of the approach and obtain preliminary data for a more definitive and statistically robust cohort. Five measurements will be done within each study visit window to improve the strength of the readings.

Specific Aim #2: *Establish a pre-exposure neurologic phenotype and baseline tissue oximetry in cancer patients scheduled to receive neurotoxic chemotherapy*. In order to assess a potential biomarker for CIPN, it must be correlated to metrics of CIPN that can be reliably followed and quantitated as a function of progression and severity.

Specific Aim #3: *Re-evaluate neurologic phenotype and tissue oximetry in symptomatic patients after exposure to a standard regimen of neurotoxic chemotherapy.* Neurologic phenotype and EPR oximetry will be re-assessed at mid-chemotherapy treatment regimen or at the onset of symptoms. The assessments will be repeated at the completion of chemotherapy and will provide longitudinal data for comparison against baseline characteristics.

Specific Aim #4: *In order to develop a proposal for a statistically robust cohort, use pilot data to (i) inform regarding the feasibility and potential utility of EPR oximetry as a CIPN biomarker, (ii) focus on the most sensitive metrics to follow, and (iii) to suggest trends of correlation in sub-metrics.* This study is designed for the collection of preliminary data and to establish the feasibility and optimization of the EPR oximetric technique in CIPN. It is anticipated that this data will guide a larger study for which additional, external funding will be sought.

Specific Aim #5: Describe changes in serum NFL levels over the course of chemotherapy to determine if NFL can be used as a biomarker for axonal damage in patients who develop CIPN. NFL will be measured at baseline, before each round of chemotherapy and at the completion of chemotherapy. Changes in NFL will be compared between patients who develop CIPN and those that do not.

3 Study Design

3.1 General Design

This protocol is for a pilot study designed primarily to a) strengthen the evidence for using detailed clinical and electrophysiological characterization to predict the outcome and incidence of CIPN; b) obtain initial data evaluating the role of tissue oxygen as a potential biomarker for CIPN using EPR oximetry; and c) establish the feasibility of using EPR oximetry to correlate peripheral tissue oxygenation metrics with clinical and electrophysiological indices of CIPN. As a secondary objective, this pilot study will seek to demonstrate effective recruitment, screening, assessment, and retention in order to justify a larger trial in the future. The investigators will aim to enroll thirty (30) patients into the study over a one-year enrollment period. Subjects will have three study visits: Visit 1, before beginning chemotherapy treatment; Visit 2, mid-treatment; Visit 3, after completing chemotherapy. All subjects will undergo a neurologic evaluation and electrophysiological assessment in the form of nerve conduction studies and motor conductions at each study visit. Patients will have EPR tests at all study visits.

3.2 Primary Study Endpoints

The primary endpoint identified for the purposes of driving the sample size and primary analysis is: **the between-groups difference in longitudinal EPR oximetry measures by CIPN status.**

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Scheduled to receive chemotherapy with taxane compounds for the treatment of breast cancer.
2. No prior taxane or platinum chemotherapy prior to enrollment.
3. Life expectancy greater than or equal to 12 months.
4. Able to provide independent informed consent for the study.
5. Able to undergo EPR oximetry
6. Age 18 years or older

4.2 Exclusion Criteria

1. Central nervous system or other impairments that interfere with clinical and electrophysiological assessment.
2. Unable to provide independent informed consent.
3. Pacemaker or other metallic objects that would be contraindicated for MRI.
4. A requirement for supplemental oxygen at baseline, or known, severe COPD.
5. Previous exposure to neurotoxic chemotherapeutic agents.

4.3 Subject Recruitment and Screening

Subjects will be recruited for the study by referral from their oncologist as soon as possible after the decision is made to proceed with taxane monotherapy for breast cancer, but before therapy is started. The oncologist will obtain the patient's consent to be contacted by our study team to explain the study. Informed consent will be obtained by the PI or designee. Potential subjects will be informed of the objectives and procedures of the study as well as adequately informed of the potential risks. Consent will be documented with the use of an IRB-approved consent document, and saved in the patient's electronic medical record.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects will be withdrawn from the study if they are no longer able or willing to participate. If a participant is unable to continue with the study procedures or is deemed lost to follow up, the PI or designee will mail a signed letter to the patient to inform them of their withdrawal from the study. Subjects will be deemed lost to follow up if they fail to present for ongoing cycles of chemotherapy. If a subject declines additional cycles of chemotherapy or cannot complete the planned number of cycles, they will not be withdrawn, but a post chemo-therapy assessment will be performed. Subjects will be withdrawn if they develop an impairment that would interfere with clinical and electrophysiologic assessment.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Once a patient has been withdrawn from the study s/he will not be contacted by the research staff to conduct further assessments; however, survival data will be collected until one year after enrollment.

5 Study Device

5.1 A. Description of India ink

India ink (also called 'Chinese ink') is composed of *carbon particles* (black pigments traditionally produced from charring organic materials such as wood or bone and more recently from petroleum products) combined with a suspending agent (usually water) to form a liquid. The carbon particles used in India inks are usually in the form of a powder mainly comprised of amorphous particles of carbon (Bäumler 2015). The India ink used for this study, Carlo Erba ink consists of carbon particles from a well-defined charcoal. The SOP with specifics of this ink and its preparation, also developed by Gallez' lab and the same pharmacy in Brussels, is attached (SOP 63.2). The company that makes the charcoal preparation (Carlo Erba, headquartered in Milan, Italy; manufacturer =Carlo Erba Reagents, Chaussée du Vexin, Parc d'Affaires des Portes - BP616) maintains a website with details of the contents of its charcoal products and its safety data sheets, https://www.carloerbareagents.com/en/catalog/product/view/id/420433/category/28637.

To be used in EPR oximetry, the carbon particles in ink need to have a detectable EPR signal that is affected by the presence of molecular oxygen in a quantitative and sensitive manner. Due to the amorphous 3-dimensional structure of carbon, not all inks made with suspended carbon particles have a sufficiently narrow EPR line-width to be usually used for EPR detection of hypoxia. Gallez' laboratory (cf., Charlier et al. 2004, Jordan et al. 1998, and Gallez 2003) has conducted a series of experiments to test the EPR signal of various formulations of India ink that they manufactured from known samples and following pharmaceutical standards appropriate for in vivo human use. They determined that inks made from Printex-U and Carlo Erba have the necessary characteristics to be useful for EPR.

The FDA and its counterparts worldwide have opted not to regulate the manufacture of India inks used in cosmetic tattoos or for medical uses in marking, in large part because it has a very long established history as a safe product to use in humans. Instead they have agreed to brand name marketing of inks used for marking, based on the 'predicate' of India ink being used in humans well before May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act that do not require approval of a premarket approval application.

India ink (Carlo Erba ink) is not a treatment per se. It is a paramagnetic material whose properties when queried by EPR provide an assessment of the oxygen level in tissues. It is inert and stable and permanent and so, after the initial injection and response to a foreign object in the body (i.e., akin to tattoo response described above) it permits noninvasive in vivo measurements using EPR spectroscopy. Approximately 50micL of sterile India ink, will be injected into the distal fourth dorsal interosseous toe web.

Dartmouth's inks to be used for EPR oximetry (prepared with certifications and under SOPs meeting both US and EU/Belgian Pharmacopia Standards)			
Printex-U India ink	Carbon black (Channel Type Black Printex U Degussa AG - D 60287 Frankfurt Germany)	Carboxymethylcellu-lose sodium salt (CMC-Na), ultra-low viscosity Saline water (water plus .9 NaCl), medical grade	SOP used at Dartmouth was developed and tested by Gallez's CUL Laboratory and the associated hospital pharmacy Brussels, Belgium
Carlo Erba India ink	Charcoal, vegetable powder	Saline water (water plus .9NaCl), medical grade	SOP used at Dartmouth was developed and tested

	(certified as pure); Carlo Erba Reagents in France CAS 7440-44-0 product code 332658; pyrogenated	Gum Arabic, medical grade	by Gallez's CUL Laboratory and the associated hospital pharmacy Brussels, Belgium

5.1 B. Description of Nerve Conduction Study and Regimen

Electrophysiologic assessment is performed by completing a nerve conduction study (NCS). Nerve conductions are obtained using surface electrodes placed on the skin and provide a measurement of the speed of conduction of an electrical impulse through a nerve. Changes in NCS reflect axonal loss and/or damage to myelin. To perform the test, surface electrodes are placed on the skin of the distal extremity. The underlying nerve is stimulated with a very mild electrical impulse. This results in an evoked response that is recorded from a distal surface electrode. This is repeated for each nerve being tested. The response evoked by stimulation of the peripheral nerve is analyzed for morphology, speed of response and amplitude.

5.1 C. Description of SUDOSCAN

1 Image of SUDOSCAN device.

The Impeto Medical SUDOSCAN is a galvanic skin response measurement device that measures the concentration of chloride ions produced by sweat gland activity. Patients place their hands and feet on the SUDOSCAN's stainless steel plates. A low-voltage current is applied to the feet through the plate's sensor electrodes, which stimulates sweat glands to produce chloride ions. The entire scan takes between two-three minutes to complete. It is non-invasive and painless. SUDOSCAN has been cleared by the FDA since 2010 and is routinely used in clinical settings to evaluate neuropathies.

5.2 Method for Assigning Subjects to Treatment Groups

Subjects will not be randomized into treatment groups. All participants will receive EPR oximetry, clinical evaluation, and electrophysiologic testing.

5.3 Subject Compliance Monitoring

Patients must complete their baseline assessment prior to starting chemotherapy. The study staff will strive to complete EPR oximetry, electrophysiologic testing, and clinical evaluation during a single visit after the patient enrolls in the study. Testing may be separated into distinct visits if scheduling conflicts arise or per patient preference. Patients' chemotherapy progress will be monitored in the electronic medical record so that follow up visits can be scheduled during and after completion of chemotherapy. Aside from attending and completing the scheduled study visits, patients will have no further compliance requirements.

5.4 Protocol for Preparation, Administration and Maintenance of Carlo Erba ink

5.4.1 Storage (Carlo Erba India Ink)

The India ink capsules will be stored at room temperature, in sterile packaging. As available, the ink capsules are stored on a continuously rolling platform located in the study treatment room for oximetry measurements. A complete sterile kit containing all necessary items for the injection is stored in the cabinet space in the same room and clearly marked. The vortexer that is to be used immediately prior to injecting a subject is located in the same area as is a camera top record injection sites.

5.4.2 Preparation and Administration of Study Device (Carlo Erba Ink)

This study will follow the EPR Center's standard protocol for the preparation and dispending of India ink. For detailed instructions on the preparation of Carlo Erba ink, please see *EPR Center's Standard Method 63.2, "SOP for Preparation of Carlo Erba Ink: For injection in Humans"*. Briefly, the materials used are purified charcoal Carlo Erba (Cod 332658) (Depyrogenated for human use) as the pigment and saline water (NaCl 0.9%) and gum Arabic [2](USP quality) for the aqueous suspension. Briefly, the methods can be described as: First create a solution of gum Arabic and saline water. Then add the charcoal pigment, then distribute into vials, place in an ultrasonic bath and sonicate for 30 sec. Add stopper and cap. Seal and crimp the vials and sterilize the vials at 121°C for 20 min using the "liquids" program of the autoclave. Add labels to each individual vial with pertinent information about the date, use, batch, expiration date, etc.

The methods and materials for making Carlo Erba ink were developed and tested in Brussels under the supervision of the Project 1 PI for the NCI grant, Dr. Bernard Gallez. These methods and tests have been published (see Jordan et al 1998) and the materials used have already been used for more than 20 years in St- Luc Hospital for preoperative marking (about 200 patients/year). Charcoal particles (Carlo Erba, Codex, diameter 3 to 10 micrometer) will be suspended (agitation and sonication) using a suspending agent (3% Arabic gum in saline) approved for clinical use of parenteral injection. Endotoxins will be removed by dry heating of the charcoals (2 hours at 220° C). Sterilization procedure will be carried out by using moist heat in an autoclave at 121°C for 20 min. The batches will be prepared in single use 1mL serum bottles with rubber stoppers to facilitate the injection procedure. Materials will be released for human use after regular quality control tests (sterility) are carried out.

The administration (injection) of the ink is detailed in SOP 71.2 (attached). Only designated clinicians who have completed standard in-person training by an experienced physician injector will be allowed to perform the ink injections. Briefly, these are the steps: in advance of injecting the ink, the study team should have in place two vials of India ink, the kit for injection, a camera, data collection forms, and the vortexer. Then, for a consented subject, the study physician doing the injection should first prepare the site (written here for skin) using 70% alcohol or other appropriate skin preparation such as betadine or other preparative skin cleanser. To inject the ink, first vigorously shake/mix the ink vial, preferably with a vortex mixer, for at least 5 minutes prior to drawing up ink into syringe. Continue to shake and warm bottle of ink by hand until ready to ready to inject. Draw up about 50μl of ink into a 0.5 ml insulin or tuberculin syringe with an attached 29g ½ in needle immediately before injection. Wipe the needle with an alcohol wipe to

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1.1 [2] From the e-CRF code, current as of August 11, 2016: Listing of ingredients generally recognized as safe in Title 21 of the FDA: §582.7330 Gum Arabic. (a) Product. Acacia (gum arabic). (b) Conditions of use. This substance is generally recognized as safe when used in accordance with good manufacturing or feeding practice.

remove excess ink before injecting into patient. Place needle at 20-30 degrees to the skin and inject between 2 to 5 units using the syringe units, which corresponds to ~20-50μl at depth of ~5mm from skin surface. Allow the pressure to stabilize for a few seconds and then, while withdrawing the needle, pull back on plunger to minimize ink release in needle track. Then remove needle and dispose safely. Clean the injection site of ink using fresh skin cleaner. Mark sites with non-permanent ink and photograph site. Offer a band-aid. Record pertinent information on the data recording form.

5.4.3 Dispensing of Study Device (Carlo Erba ink)

Regular device reconciliation checks will be performed. Information tracked will include which device was injected in a given subject; which device container(s) was opened; whether the subject actually received the assigned device; and which device(s) was inadvertently damaged in handling/dispensing and therefore not to be used for human subjects. This reconciliation will be logged on the device reconciliation form, and signed and dated by the study team members who are designated to perform this task.

5.4.4 Return or Destruction of Study Device (ink)

At the completion of the study, there will be a final reconciliation of the vials of ink that were shipped, vials consumed, and vials remaining. This reconciliation will be logged on the device reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study device. Vials destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Schedule of Assessments

	Visit 1 (prior to beginning chemotherapy)	Visit 2 (mid-point of chemotherapy treatment or at onset of CIPN symptoms)	Visit 3 (one week or more after completion of chemotherapy)	Unscheduled EPR oximetry readings	Blood Draw Visits
Informed Consent	X				
Inclusion/Exclusion Criteria	X				
Neurological Exam	X	X	X		
Neurologic History	X				
Ink Injection	X				
Nerve Conduction Study	X	X	X		
Sudoscan	X	X	X		
EPR Oximetry Reading	X[1]	X[2]	X[2]	X	
Total Neuropathy Score	X	X	X		

Toronto Clinical Neuropathy Scoring System	X	X	X		
National Common Institute – Common Toxicity Criteria V.3	X	X	X		
Questionnaires- - McGill pain - Survey of Autonomic Symptoms - Subset of NQol-DN -NTSS-6	X	X	X		
Blood Draw for Genetic Testing					X[3]
Blood Draws for NF-L Assay					X[4]

1. As many as 5 EPR measurements may be performed prior to beginning chemotherapy.
2. As many as 5 EPR measurements may be performed in the ±18 days surrounding the electrophysiologic assessments.
3. Blood for genetic testing can be drawn at any time during study participation.
4. Blood for NF-L testing will be drawn prior to the beginning of chemotherapy, prior to each scheduled chemotherapy treatment, and after the completion of chemotherapy.

6.2 Visit 1

Before beginning chemotherapy, and after providing informed consent, patients will receive an injection of India ink. Patients will then be scheduled to meet with a designated investigator for clinical assessments, which will occur prior to the start of chemotherapy. The neurologic assessment will include a standard neurologic history, neuropathy symptom questionnaire (NTSS-6 [Bastyr et al., 2005]), portions of the Total Neuropathy Score (TNS [Cornblath et al., 1999]), the National Common Institute – Common Toxicity Criteria V.3 (NCI-CTCv3), Toronto Clinical Neuropathy Scoring System, McGill pain questionnaire (Melzack, 1975), Survey of Autonomic Symptoms questionnaire, and a subset of a neuropathy-specific quality of life instrument (NQol-DN, [Vinik et al., 2005]). As part of the neurologic examination, strength, sensation, and reflexes will be tested. Sensation testing may include pin, temperature, and vibration assessments using a Rydel-Seiffert tuning fork. Specific tests conducted will be at the discretion of the study neurologist. Assessments will be performed by one of the study neurologists.

Subjects will also receive their baseline electrophysiological assessment at this visit. See section 1.2.1 for device information. Sensory conductions may include sural, medial plantar and dorsal sural antidromic testing with assessment of peak to peak amplitudes of sensory nerve action potentials and sensory conductions. Motor conductions may also be tested in tibial and peroneal nerves with measurement of baseline to peak amplitude of the compound muscle action potential, motor conduction velocities, and distal motor latencies. Recording will be performed from the extensor digitorum brevis and tibialis anterior muscles. Additional or different nerves may be tested at the discretion of the examiner. Temperature will be controlled and data compared with normative age-matched reference data. Testing will be performed by EMG technicians trained in

performing electrodiagnostic studies or the study neurologist. Testing will be repeated for each nerve to ensure verifiable amplitudes. The SUDOSCAN recordings will be made with both the patients' hands and feet.

Subjects will report to the Department of Radiation Oncology at Dartmouth-Hitchcock Medical Center for EPR oximetry. The EPR Center's established procedures for the injection of India ink will be followed. Subjects will receive an injection of ink in the foot over the distal fourth dorsal interosseous toe web. Subjects may require 2 or more days after the injection in order for the ink to settle before the first EPR oximetry measurement can be performed. As many as five measurements will be performed prior to beginning chemotherapy.

The standard oximetry measurement procedure consists of acquiring continuous EPR scans to characterize 1) baseline tissue oxygen levels 2) tissue oxygen levels in response to inhalation of enriched oxygen mixture and 3) response to oxygen levels in tissue to discontinued breathing of enriched oxygen. The total time for one measurement is approximately 30 minutes: approximately 10 minutes while breathing room air, then ~10 minutes while breathing enriched oxygen by non rebreather face mask, followed by ~10 minutes while resuming breathing room air.

6.3 Visit 2

Visit 2 will be scheduled at the onset of CIPN symptoms or at the mid-point of chemotherapy treatment. If a patient reports symptoms of CIPN to their nurse or physician, or if the patient's provider suspects CIPN is present prior to midpoint of therapy, the subject will be scheduled for Visit 2, in which the electrophysiologic and clinical assessments are standard of care. For patients who do not show signs of CIPN by the mid-point of their treatment, the study staff will coordinate a visit for clinical and electrophysiological assessment. These assessments will be identical to the tests conducted at Visit 1.

Patients will also return to the Department of Radiation Oncology for their second round of EPR measurements. The continuous EPR will take approximately 30 minutes (~10 minutes breathing room air, ~10 minutes of enriched oxygen, ~10 minutes breathing room air again). As many as five measurements will be made in the ±18 days surrounding the electrophysiologic assessments at Visit 2.

6.4 Visit 3

Visit 3 will take place no sooner than one week after the completion of chemotherapy. The procedures for this visit will be the same as Visits 1 and 2: patients will receive clinical and electrophysiological assessments, and EPR oximetry measurement. As many as five measurements will be made in the ±18 days surrounding the electrophysiologic assessments at Visit 3.

6.5 Unscheduled EPR Oximetry Readings

Subjects who develop symptoms of CIPN may experience fluctuations of these symptoms. Unscheduled EPR oximetry measurements may be conducted if a subject experiences change in these symptoms as the PI sees as appropriate.

6.6 Blood Draws for Serum Biomarker Assessment

Prior to the start of chemotherapy, prior to each scheduled chemotherapy treatment, and after the completion of chemotherapy, patients will provide a blood sample (2 tablespoons) for

measurement of NFL. All attempts will be made by the study team to obtain the blood sample at the time of the patient's clinical blood draw prior to chemotherapy. In the event that this is not feasible, the patient will have the option of having a separate blood draw done for study purposes. A tube will be filled and labelled with the subject ID #, the study name, and date of draw. The tube will be processed and stored in a -80 degree freezer until they are shipped for analysis. Samples will be batched for shipment. A lab requisition form will be included in the shipment. Stored samples will not have patient information on the label.

6.7 Blood Draw for Genetic Research (Optional)

Patients will be given the option to provide whole blood to a biobank for CIPN. Willing participants will give an additional tube of blood (1-2 tablespoons) to be stored in the CIPN biobank. All attempts will be made by the study team to obtain the blood sample at the patient's next standard blood draw. In the event that this is not feasible, the patient will have the option of having a separate blood draw done for study purposes. A tube will be filled and labelled with the patient ID # and the study #. Samples will be stored in the Department of Neurology -80 degree freezer for storage and eventual Deoxyribonucleic acid (DNA) extraction. The samples will remain in the bank until they are used for another CPHS approved research study. An electronic document will hold the patient Id – study ID code. Stored samples will not have patient information on the label. The electronic code will be stored on an encrypted DHMC drive and not removed from Dartmouth-Hitchcock servers.

7 Statistical Plan

7.1 Sample Size Determination

This pilot study is designed for proof of concept and to establish the bedrock for a future trial with a more robust sample. The secondary objective of this pilot is to demonstrate effective recruitment, screening, assessment processes, and high participant retention. We will assess the proportion of potential participants that: meet inclusion criteria, consent to participate, and complete all assessments.

Thirty (30) patients will be enrolled in the study, and it has been estimated that ten (10) patients will develop CIPN during their taxane treatment. Accurate incidence of CIPN varies significantly; the average incidence is 70% (Seretny et al., 2014), with ranges from 30% to 90%. A conservative estimate is that 30%-50% of patients in this trial will develop symptoms of neuropathy during their chemotherapy treatment. Development of CIPN will be determined based on the results of their neurologic assessments. For data analysis, the patients will be divided by this CIPN status into two groups. Primary endpoints are continuous metrics: Endpoint 1) neurological phenotyping involves electrophysiological assessment (e.g. nerve conduction, EMG readings, SUDOSCAN results), and Endpoint 2) tissue oximetry involves the EPR measures). The mean levels of each electrophysiological or oximetry metric will be assessed between the two groups of patients (with CIPN vs. without) over time (at timepoints: before, mid-, and post-chemotherapy) using repeated measures ANOVA. This method will also assess the within-subject changes in the electrophysiological and EPR oximetry metrics over time, from baseline through post-chemotherapy, as well as the interaction between CIPN status and time. With 15 patients per group, we anticipate that we will have 80% power to detect a between-groups difference in longitudinal EPR oximetry measures by CIPN status of 28%, assuming a correlation of 0.5 among repeated measures, a standard deviation of 25%, alpha=0.05. The within-subjects and interaction tests are estimated to be able to detect an 8.5% change over time, with these same parameters (calculated using G*Power 3.1.9.2).

We are particularly interested in identifying the metrics that allow the early identification of patients who go on to have a clinical diagnosis of CIPN. Within the pre- or mid-chemotherapy timepoints, the metrics that show a combination of the largest magnitude difference and lowest t-test p-value by CIPN status will be prioritized for future study. For primary endpoint 3), we will also use Pearson correlation to assess the relationship between metrics, for example, EPR oximetry will be assessed vs. a continuous CIPN metric (e.g. nerve conduction, temperature sensation, vibratory sensation) within a timepoint, e.g. post-chemotherapy). With n=30 patients, we anticipate having 80% power to detect at least a 33% difference in the means of a metric using an independent samples t-test comparing patients with CIPN vs. without, or a regression slope of 0.66 among continuous metrics, with a standard deviation of 0.25, and alpha of 0.05. This data will guide a larger study for which additional, external funding will be sought.

The potential utility of the EPR measures will be assessed in these CIPN patients using either a minimum within-subjects increase over time in the tissue oximetry measure of (e.g. 5%) compared to baseline, or a Pearson r threshold (e.g. -0.3) for the correlation between the tissue oximetry and electrophysiological measures of sensory or motor nerve conduction.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:
- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event
An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:
- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A serious adverse event is any AE that is:
- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect

- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as non-serious adverse events.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up.

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

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

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

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

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

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

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

8.2 Recording of Adverse Events

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

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

Summaries of study enrollments, clinical status, and data will be submitted for regular review. In the case of any unexpected adverse events, the patient's medical record will be reviewed by a study physician, and if necessary, the patient will be examined by a study physician and appropriate medical action taken. Any unexpected or serious adverse events, or reactions that are determined possibly to be related to the study procedures, will be reported to the Dartmouth IRB and the FDA as required.

8.3 Investigator reporting: notifying the Dartmouth IRB

This section describes the requirements for safety reporting by investigators who are Dartmouth faculty, affiliated with a Dartmouth research site, or otherwise responsible for safety reporting to the Dartmouth IRB. The Dartmouth IRB requires reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Dartmouth IRB requires researchers to submit reports of any incident, experience, or outcome that meets each of the following criteria:
• Unanticipated in terms of nature, severity, or frequency given: (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and consent document; and (b) the characteristics of the subject population being studied; and
• Possibly related to participation in the research means there is a reasonable possibility that the incident, experience, or outcome may have been associated with research participation; and

• The problem suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, emotional, economic, legal, or social harms) than was previously known or recognized.

Reporting Process
Unanticipated problems posing risks to subjects or others as noted above will be reported to the Dartmouth IRB using the form: “Unanticipated Problem Involving Risks to Subjects or Others (UPR).”

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator’s study file.

Other Reportable events:
For clinical trials, the following events are also reportable to the Dartmouth IRB:
• Any adverse experience, defined as an untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject’s participation in research, whether or not considered related to the subject’s participation in the research), that is considered:
– Serious: Death; a life-threatening adverse drug experience; inpatient hospitalization or prolongation of existing hospitalization; a persistent or significant disability or incapacity; or a congenital anomaly or birth defect; and
– Unexpected: Any adverse experience, the specificity or severity of which is not consistent with the current investigator brochure or consent form; and
– Possibly related: There is a reasonable possibility that the incident, experience, or outcome may have been associated with the procedures involved in the research; and
– Is experienced by a participant in a trial open at a site subject to Dartmouth IRB review
• Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
– An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
– Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
– A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
• Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
• Breach of confidentiality
• Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
• Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
• Protocol deviation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

9 Data Handling and Record Keeping

9.1 Confidentiality

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

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

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

9.2 Source Documents

Source data and original records will be maintained by the research coordinator at Dartmouth-Hitchcock Medical Center. All source data will be stored in a locked cabinet or office, or will be part of subjects' electronic medical records. Demographics and outcome data of participants in the study will be manipulated and stored on a secure, web-based application called REDcap and/or shared drive used for the Department of Neurology's research purposes and managed by the Information Technology Department at Dartmouth-Hitchcock Medical Center. The department follows industry standard procedures for securing the computers, servers, and networks physically and electronically. REDcap will be used as an electronic case report form. Participants will be assigned a random generated number and any identifying information or PHI will be removed prior to analysis.

9.3 Records Retention

Electronic data will be stored indefinitely. The physical forms and paper data will be kept for three years after completion of the trial.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored by the Data Safety Monitoring and Accrual Committee (DSMAC) of the Norris Cotton Cancer Center. The Committee meets quarterly to review accrual rates and information for studies that have accrued participants. The Clinical Cancer Review Committee (CCRC) determines the frequency of DSMAC review. The DSMAC has the authority to suspend or to recommend termination to the CCRC of all research activities that fall within its jurisdiction. In the event that a study is suspended or terminated, that information will be forwarded to the CPHS (Dartmouth IRB) office.

10.2 *Data and Safety Monitoring Plan*

Internal monitoring is conducted by appropriately trained staff of the NCCC Office of Clinical Research and Dartmouth-Hitchcock Medical Center Clinical Trials Office who are not involved in the study. This monitoring will include periodic assessment of the regulatory compliance, data quality, and study integrity. Study records will be reviewed and directly compared to source documents and the conduct of the study will be discussed with the investigator. Monitors may

request access to all regulatory documents, source documents, CRFs, and other study documentation for on-site inspection. Direct access to these documents is guaranteed by the investigator, who must provide support at all times for these activities.

11 Ethical Considerations

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

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

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

12 Study Finances

12.1 Funding Source

This study is being funded by the Reeves Fund of the Dartmouth-Hitchcock Department of Neurology.

12.2 Conflict of Interest

All Dartmouth investigators will follow the Dartmouth conflict of interest policy.

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