Validation of Cutaneous Nerve Demyelination in the Diagnosis and Treatment of CIDP

Amanda C. Peltier, MD MS, Associate Professor
Department of Neurology and Medicine
A-0118 Medical Center North
Vanderbilt University Medical Center

Jun Li, MD PhD, Professor and Chair
Department of Neurology
Wayne State University

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1.0 Background

CIDP is a chronic inflammatory polyneuropathy. The pathogenesis of CIDP is thought to be cell- and humoral-mediated autoimmune mechanisms acting on peripheral nerves. Many patients with CIDP have antibodies against Schwann cell or myelin antigens. In addition, treatments, such as plasma exchange and intravenous immunoglobulin are particularly efficacious in removing or interacting with antibodies, providing additional evidence of an antibody-mediated autoimmune disorder. Pathologic studies of nerves demonstrate segmental demyelination in teased fibers, demyelination and remyelination with onion-bulb formation. Inflammatory infiltrates are also common (1, 2).

The prevalence of CIDP is estimated to be between 1-9 cases per 100,000 (3, 4). However, diagnosis can be difficult and these numbers may be an underestimate of the true prevalence. Nerve conduction studies are specific but over 15 diagnostic criteria have been published in an effort to improve sensitivity (5). One of the possible sources of insensitivity can be low amplitudes which could reflect conduction block rather than axonal loss but cannot be discriminated on nerve conduction studies (6). CSF protein elevation is present in 90% of patients, but can be present in 50% of patients misdiagnosed as CIDP (7) Nerve biopsy is specific, but shows demyelination in only 50-66% of biopsies (8, 9). Teased fiber preparation is technically challenging and not performed routinely at all labs. In addition, none of these methods have proved useful for determining efficacy of treatments. Motor strength and disability scores are nonspecific but have been the primary endpoint measures used in CIDP studies. No tool currently exists for identifying demyelination in CIDP as either a diagnostic test or to assess treatment effect. This hypothesis-generating study will assess whether skin biopsy can discriminately identify demyelination in CIDP patients from healthy controls and other neuropathies such as diabetes mellitus. In addition, because skin biopsies can be repeated over time, this study will assess whether this biomarker is responsive to treatment, which has not been shown with any previous biomarker.

As mentioned, glabrous skin biopsy affords a method of pathological assessment of distal nerves which is minimally invasive and repeatable. Saporta et al published evidence of segmental demyelination in CIDP patients in skin biopsy using them as controls for an inherited neuropathy study (10). Segmental demyelination was observed in all four CIDP patients studied. We have used this method to demonstrate abnormalities in myelin and the node of Ranvier in diabetic subjects and healthy controls with average age of 53 (35-75). We observed 5% segmental demyelination in healthy controls and 7.3% in type 1 diabetic patients, 7.7% in type 2 diabetic patients (11). We propose to use this technique to study patients diagnosed with CIDP to evaluate its usefulness as a potential diagnostic tool and as a biomarker of treatment efficacy. We expect to observe a significantly higher percentage in CIDP than our previous diabetic

patients which will allow us to differentiate CIDP from diabetes and healthy aged controls. In addition, we expect to see demyelination improve after IVIg treatment with Privigen. In addition, we suggest that nodal architecture will be abnormal in CIDP compared to our data in healthy control patients.

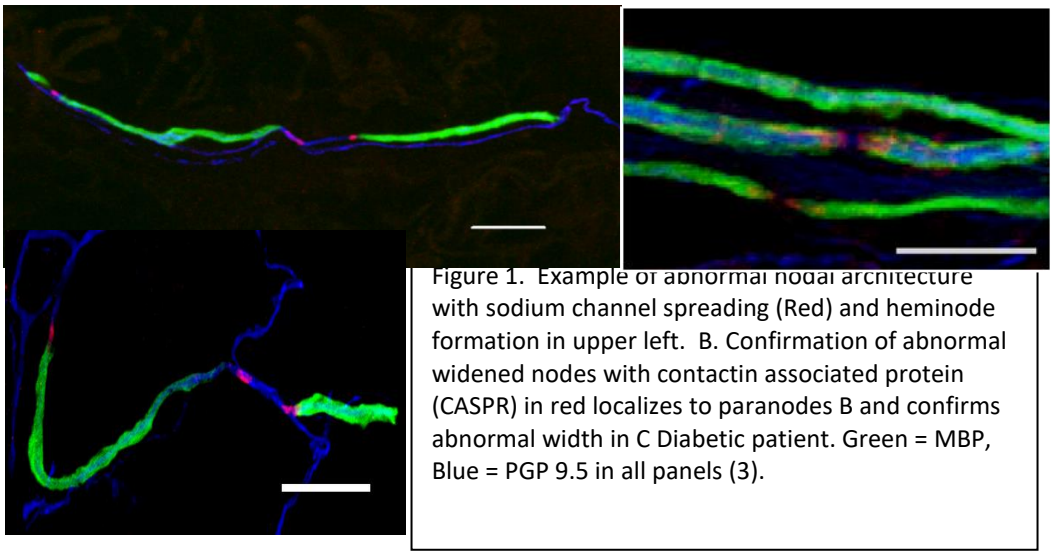

Figure 1. Example of abnormal nodal architecture with sodium channel spreading (Red) and heminode formation in upper left. B. Confirmation of abnormal widened nodes with contactin associated protein (CASPR) in red localizes to paranodes B and confirms abnormal width in C Diabetic patient. Green = MBP, Blue = PGP 9.5 in all panels (3).

2.0 Rationale and Specific Aims

Study Hypothesis and Objectives:

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a chronic, disabling illness where there is immune-modulated demyelination of motor and sensory myelinated axons. Unlike most polyneuropathies, CIDP is treatable, and responds to multiple immunotherapies, the most effective being intravenous immunoglobulin (12). Newer approaches include subcutaneous immunoglobulin (SCIg) which has been used successfully for other patients and has shown promise in the treatment of CIDP (13).

The main treatment of CIDP has been intravenous immunoglobulin (IVIg), typically given in pulses or repeated treatments every 3 to 6 weeks. About 50-70% of patients respond to IVIg treatment (14) as opposed to 40% of patients on prednisolone or 30-88% of patient to plasma exchange, but is difficult to deliver repeatedly (15-17). The goal of immunoglobulins or immunosuppressive therapy is to prevent demyelination which is secondary to autoimmune –mediated damage to peripheral sensory and motor nerves.

The major barrier to treating patients effectively has been identifying correctly patients with CIDP who will respond to immunomodulatory treatment. Nerve conduction studies and nerve biopsy are insensitive at identifying patients with acquired demyelination. Skin biopsy has been used as a diagnostic tool for neuropathies. Biopsy of glabrous skin can be repeated over time and using antibodies to myelin antigens tagged with florescent proteins, can demonstrate demyelination, and abnormalities at the node of Ranvier (gaps in myelin where

salutatory conduction takes place, important for axon potential propagation in myelinated nerves), in addition to quantifying density of myelinated fibers present, (18, 19). Segmental demyelination has been shown to be present in glabrous skin biopsies of CIDP patients (10) in a previous small study.

We hypothesize that dermal myelinated fibers in glabrous skin biopsies show segmental demyelination in CIDP patients, which will improve over time with treatment.

Aim 1. We will obtain glabrous skin biopsies in 10 CIDP patients to demonstrate increased percentage of myelinated fibers with segmental demyelination using immune-histochemical labeling of myelin and axonal proteins compared to those of historical healthy control and diabetic subjects. We will demonstrate that this method will be able to discriminate CIDP patients from healthy controls and diabetic neuropathy patients.
Aim 2. We will perform follow up biopsies 3 months and 6 months after treatment to demonstrate whether there is decreased segmental demyelination after treatment.
Aim 3. We will compare measurement of segmental demyelination in vivo to medical research council (MRC) scores for muscle strength for each patient.

The overall goal is to provide preliminary data for a future study to demonstrate using SCIg and IVIg is efficacious in preventing in vivo segmental demyelination and that this correlates with improvement in patient outcomes.

3.0 Animal Studies and Previous Human Studies

Dr. Jun Li and colleagues demonstrated demyelination in their CIDP samples used as comparison to Charcot-Marie-Tooth Neuropathy patients (Saporta et al.). Quantification of demyelination in these samples was not done as quantitatively as we performed in our diabetes population (Peltier et al.). The work described has never previously been done in the CIDP population. This pilot study will provide data for future larger studies to explore the effect of treatment on demyelination in CIDP.

4.0 Inclusion/Exclusion Criteria

Inclusion Criteria:

1. Adults (age ≥ 18 years) with definite or probable CIDP according to the EFNS/PNS criteria may enter the trial within 6 months of treatment onset or treatment naïve patients. Written informed consent is obtained by the local investigator before entry into the study.
2. IVIg treatment of 0.8 up to 1.2 g/kg per 30 days will be allowed up to 6 months. It is expected some patients may be on variable treatment regimens, and as along as they are not significantly declining, efforts will be made to continue treatment regimens already instituted.
3. Prednisone in doses up to 20 mg /day will be allowed as long as dose has been stable for 90 days and is not being escalated or tapered during study.

4. Previous plasma exchange will be allowed as long as it is not continued during the study.

Exclusion Criteria:

1. Other causes of polyneuropathy, multifocal motor neuropathy, diabetes mellitus, alcohol, family history of neuropathy, monoclonal gammopathy or malignancy, history of drug or toxin exposure, which could reasonably cause neuropathy. Coexistent monoclonal gammopathy will also be used as an exclusion as these patients may not have the same response to IVIg.
2. Any other disease that may cause neurological symptoms and signs or that may interfere with treatment or outcome assessments.
3. Severe conditions that may interfere with an evaluation of the study product or satisfactory conduct of the study such as current malignancy or history of allogeneic bone marrow/stem cell transplant, cardiac insufficiency (New York Heart Association Classes III/IV), cardiomyopathy, cardiac arrhythmia requiring treatment, unstable or advanced ischemic heart disease, congestive heart failure or severe hypertension, chronic kidney disease deemed too severe to safely use IVIg, known hyperprolinemia, known bleeding disorders, severe skin disease at the planned injection sites or biopsy site, alcohol, drug or medication abuse.
4. History of keloids or other reactions to local anesthetic making skin biopsies unsafe.
5. Patients with the following laboratory results:
   a. Positive result at screening on any of the following viral markers: human immunodeficiency virus-1 or 2, or hepatitis B or C virus.
   b. Abnormal laboratory parameters: creatinine greater than 1.5 times the upper limit of normal (ULN), blood urea nitrogen greater than three times the ULN if the increase is related to potential kidney disease, or hemoglobin less than 10 g/dL
6. Fulfilling the following general criteria: inability to comply with study procedures and treatment regimen; mental condition rendering the patient unable to understand the nature, scope, and possible consequences of the study; pregnancy or nursing mother; intention to become pregnant during the course of the study; female patients of childbearing potential either not using or not willing to use a medically reliable method of contraception for the entire duration of the study or not sexually abstinent for the entire duration of the study or not surgically sterile; participation in another clinical study or use of another investigational medicinal product within the same time period of the study.
7. Additional medications and treatments other than prednisone and IVIg for treatment of CIDP such as azathioprine, mycophenolate, rituximab and ongoing plasma exchange as this will either confound or interfere with effects of IVIg.

5.0 Enrollment/Randomization

The study will be posted on Clinicaltrials.gov in concordance with guidelines. 20 patients will be recruited by advertisement with a goal of 10 patients completing the study. Patients will be recruited within Vanderbilt University Medical Center and affiliated Neurologists, as well as local neurologists in Alabama, Kentucky, Tennessee and Georgia. Letters will also be sent to academic medical centers close by such as University of Kentucky, University of Tennessee, Emory University, and University of

Alabama. Advertisements will list the contact information of the PI, as well as ,the research study nurse.

All patients will be contacted by the study nurse after response or in the Vanderbilt Neurology Neuromuscular Clinic. The patients will be given information about the study and a copy of the informed consent to look over. Patients interested in the study will be scheduled for screening visits within the Vanderbilt Clinical Research Center (CRC). At the time of their screening visit they will be consented according to the principles of the Declaration of Helsinki and 2018 Common Rule Requirements.

Within 3 weeks, if a patient is found to be eligible at the time of screening an enrollment visit will be scheduled. All patients will be prospectively entered into the study. No randomization or blinding will take place as this is an observational prospective study utilizing new procedures for a biomarker without change in therapy.
All efforts will be made to recruit patients prior to therapy initiation without significantly delaying therapy.

Address for contact information will be Department of Neurology, Dr. Peltier Research, 1161 21st Avenue South, A-0118 MCN, Nashville, TN, 37232. Phone number is 615-322-8957.
List the process for randomization and/or registration; list the address, phone number or website where the registration will take place.

6.0 Study Procedures

Day -30 -0, Visit 1: Screening visit:

Procedures:
Consent: Patients will provide written consent according to the principles of the 2018 Common Rule and Declaration of Helsinki

H&P: Detailed history and physical with neurological examination will be performed.

MRC score: Medical Research Council (MRC) score for muscle strength will be performed.

Nerve Conduction Studies: Limbs will be warmed to at least 31 C minimum temperature. Antidromic sensory nerve conduction studies of the sural, median and ulnar nerve will be performed. Motor conduction studies of the peroneal, tibial, ulnar and median with proximal studies and F-waves will be performed. Unless patients have significant asymmetry, all studies will be done on the left. Conduction block and temporal dispersion will be noted.

Labs: Blood will be drawn for CBCPD, IgA, CMP. If previously performed will record CSF protein and cell count.

If patients are found to have history and exam consistent with CIDP, met ENFS criteria (cite), inclusion, exclusion criteria including less than 6 months of treatment for CIDP

they will be accepted into the study and baseline visit will be scheduled 24 hours to 30 days apart. No randomization will take place as all patients will be treated.

Day 1, Visit 2: Baseline visit:

Patients qualifying for the study will be invited back for baseline visit. If patients have not been on IVIg treatment this visit will be scheduled as soon as possible to avoid delays in treatment. If patients have been started on IVIg treatment, patients will be scheduled the day before their next infusion to approximate a nadir in treatment for best baseline measurement.
Procedures:
Physical exam: Interval history will be taken (if appropriate) and physical exam with neurological examination repeated.

MRC score: MRC score will be documented.

INCAT score: The INCAT score will be collected as a secondary endpoint as it has been used in both ICE and PATH studies (refs). The INCAT score for upper and lower extremities will be documented.

Skin biopsies:
Skin biopsies will be performed at the second study visit. The procedure below is similar to that of Li et al. 2005 (16). The biopsy is performed on the lateral aspect of the finger between the first and second interphalangeal joint 2-3 mm from the border of the hairy skin of the finger. The area is anesthetized with 1% lidocaine with epinephrine. A 2 mm punch is used with tip length of 8 mm (Miltex Instrument Co.). The skin punch is pushed perpendicularly to the surface of the skin and advanced to a depth of 5 mm (a little over half way to the punch tip). The biopsy wound will be compressed for at least 15 minutes as the fingers are rich in small vessels to stop bleeding. A bandage will be applied over the wound. Patients will be instructed to change the bandage daily until the wound is healed and to avoid immersing their fingers in water for prolonged periods until a scab has formed. The digit nerves and arteries are deeper than 5 mm and should not be harmed by the procedure.

Biopsies are preserved by 4% paraformaldehyde fixative for 30 minutes, followed by cryoprotection in phosphate-buffered sucrose solution. Sectioning will be done in a -20˚C temperature-regulated cryostat (Leica CM1950) after embedding in optimal cutting temperature (OCT) medium. Relatively thick sections of 60 µm will allow for capture and potentially reconstruction of large structures like internodes, Meissner corpuscles, etc. (8). This yields approximately 20-24 sections per biopsy.

Immunohistochemistry: Aim 1 will be achieved by immunostaining multiple neuronal structures conjugated with fluorophores of various wavelengths (e.g. Dy488, Cy3, Dy649; Jackson Immunoresearch) in order to identify the pan axonal marker, protein gene product 9.5 (PGP 9.5; AbD serotec), compact myelin basic protein (MBP; Chemicon) and voltage-gated sodium channels (Pan NaV; Sigma-Aldrich). Four sections of each biopsy will be stained with MBP, PGP 9.5 and PanNaV markers. Additional sections will be reserved for future exploratory measures.

Epifluorescence microscopy (Leica DM4000) will be sufficient for identifying and measuring Pan NaV-immunoreactive nodes of Ranvier, length of sections for density calculations, as well as other cutaneous structures. Internodal length will be measured three dimensionally (Metamorph) in confocal-acquired images (Leica LSM510). Quantitative morphometric parameters of dermal myelinated fibers include Meissner corpuscle density (receptors per mm2), the density of their myelinated afferents (fibers per mm2, axon diameter (μm), and further characterization of myelin structure in regards to internodal, nodal and paranodal length (μm) will also be calculated. Based on morphologic analyses, additional measures unique to dermal myelinated fibers may be generated and ROC curves developed to determine sensitivity and specificity. Confocal microscopy will be utilized in 3 patients at each site to confirm nodal measurements, myelinated fiber density, and confirm presence of segmental demyelination. Correlation of confocal measurements with florescence measurements will be evaluated to determine whether florescence is sufficient for accurate measurements. The PI and Dr. Jun Li will be analyzing the samples in a blinded fashion, and will train lab technicians in this technique which Dr. Li and Dr. Peltier have published.

Analysis of segmental demyelination: Measurement of myelinated fiber density from glabrous skin biopsies will be calculated according to methods of previous publication (7, 23, 24). Meissner corpuscle density will also be performed for comparison. Number of papillary, dermal internodes will be calculated. Analysis of nodes of Ranvier will include quantitation of nodes with greater width (> 6 um; (25)); branching at nodes, localization of ion channels within nodes will be quantified from 25 nodes from each biopsy, using at least 3 sections to ensure random distribution. Given the previous normal healthy control data demonstrating a 5% rate for healthy controls and 7.7% in diabetic subjects, 20% will be used as the cutoff for CIDP. 15.7% was the 95th percentile observed in both diabetic and healthy controls, with one diabetic subject having a 15% demyelination rate. Given that Dr. Li observe demyelination in multiple fibers from previous CIDP patients sampled, we believe this will ensure diagnostic efficiency with sufficient sensitivity (9). It is unknown what treatment effects we will find, but given the standard deviation was 4.1% in the previous study, we would expect a change greater than 5% to be meaningful.

Further characterization of nodal and internodal integrity for aim 1 will be investigated with nodal staining using pan-sodium channels (7), the paranodal staining of Contactin-associated protein (Caspr; Neuromab) and internodes will be labeled by staining for myelin basic protein (MBP). These will be helpful to further measure nodal width and alsoand to evaluate for decompartmentalization of ion channels which channels, which could contribute to conduction block. An internode will be defined as a segment with intact MBP, Pan-Na and PGP9.5 staining demonstrating a node on each end. A total number of nodes per biopsy (in 4 sections) will be calculated. We will estimate segmental demyelination percentage by calculating numbers of fibers with demyelination (identified as gaps of MBP stain within a fiber where MBP stain is visible on both sides of gap and PGP 9.5 stain is present confirming axonal integrity)/total number of fibers counted (25). Repeated measures for each patient will be calculated to determine if there is decrease in segmental demyelination over time after treatment.

IVIg infusions:

Treatment naïve patients will be initiated on IVIg treatment after baseline visit procedures are completed. This can occur later that day or within the next 14 days. Patients who are treatment naïve will be given a loading dose of 2 g/kg IV over 3-5 days at standard infusion rates which will take 4-6 hours daily, depending on comorbidities and age. Patients will receive acetaminophen and diphenhydramine as standard pre-infusion prophylactic treatment. Patients will receive Privigen which is FDA approved for CIDP treatment either at Vanderbilt University Medical Center hospital infusion center or outpatient infusion center depending on insurance. Patients will then have 1 g/kg IVIg infusions scheduled every 3 weeks for 6 months. Every attempt will be made to ensure Privigen will be used for IVIg product as it is FDA approved for CIDP, is temperature stable, and has no sucrose (26). No placebo will be used given this would be unethical. Patients will serve as their own controls, and the hypothesis is that percentage of segmental demyelination found should decrease as INCAT score and strength increases.

For patients previously treated:
Baseline visit will be arranged closest to the day prior to infusion to ensure the best possible baseline measurement. They will resume according to the patient's previous schedule. Patients will have up to 1.2 g/kg monthly to account for different patient management schedules and variation and patients will be on Privigen ideally.

Patients with escalating weakness not responding to IVIg will be allowed to try additional treatments but will be released from study. Additional treatment with prednisone up to 20 mg/day will be allowed but must be stable dosing and not escalating to avoid additional variability.

Patients will receive IVIg infusions q3 weeks. A monthly phone call will occur so the coordinator can confirm that patients are doing well, having no new treatment related side effects, and are receiving IVIg infusions either at home or in infusion centers.

Day 90, Visit 3:

Procedures (previously described):
History & Physical Exam: Note interval history.
MRC Score
INCAT Score
Skin Biopsy
Labs: BMP to check renal function

Day 180, Visit 4:

Procedures (previously described):
History & Physical Exam: Note interval history.
MRC Score
INCAT Score
Skin Biopsy
Labs: BMP to check renal function
Nerve conduction studies will be repeated from Baseline visit 1

Data Entry Study data will be collected on paper forms for each study procedure and entered into Redcap database. All Data entry will be reviewed by PI and team for accuracy. Use of Redcap will also provide tracking of data entry and security. Protocol compliance will be monitored by PI and investigator team.

7.0 Risks

1. *Risks of the study procedures include:*

a. Phlebotomy: bleeding, infection, possible syncope.

b. Nerve conduction testing: Possible risk of electrical injury (burn) from stimulation of nerves. The procedure can be uncomfortable, and many individuals find it painful.

c. Skin biopsy: bleeding, infection from skin biopsy site. Usually a stitch is not needed for hemostasis. Patients are warned not to immerse sites in water for 5-7 days (no baths or swimming). Theoretically numbness from injury to the digital nerve of the finger biopsied is possible but has not been observed in previous studies and was evaluated in cadaver studies to determine that depth used would not approach digital nerves or vessels.

2. *Risks of IVIg treatment include:*
   a) Rare, serious: venous and arterial occlusion, anaphylactic reactions, kidney damage (which could be irreversible), chemical meningitis, congestive heart failure.
   b) Common, moderate: headache, nausea, myalgias.
   c) Risk of IV access: bleeding, infection, superficial venous thrombus

3. *Risks of premedication with acetaminophen and diphenhydramine:*
   a) Sleepiness
   b) dry mouth
   c) dry eyes
   d) difficulty with urination
   e) constipation
   f) allergic reactions

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Adverse events will be recorded by either the study coordinator or investigators. The investigator, her collaborator Dr. Jun Li, and the study coordinator will monitor adverse events. Adverse events will be discussed at committee meetings so that any unanticipated events are evaluated and necessary protocol changes made. Serious adverse events are not anticipated, given the minimal invasiveness of the testing proposed. Because this is not a clinical trial, a formal data safety monitoring board will not be created, but the mentoring committee will act as ad hoc reviewers to evaluate

appropriateness of recruitment, and review any adverse events, which occur. We will report significant adverse events to CSL Behring, to monitor for any significant risks. The study procedures have been performed in previous studies by the PI with no adverse events.

A physician (either the primary investigator or a member of the GCRC) will perform all testing. The primary investigator will be paged if an adverse event occurs. All subjects will give the coordinator/PI the name and contact information of their primary physician in order to communicate any important medical information which is discovered as part of the study (for example, newly diagnosed diabetes mellitus) to ensure appropriate follow up care.

Any adverse events, such as wound infection will be reported to the IRB and to CSL Behring, either at time of continuing review, or if adverse events are serious and require hospitalization or emergency room visit, they will be reported within 24 hours of when the PI is notified. All patients are encouraged to report any signs of infection or uncontrolled bleeding to the PI and will be given contact numbers to reach the PI. Any minor, adverse events anticipated will be reported in the continuing review 12 months from initial IRB approval.

9.0 Study Withdrawal/Discontinuation

Patients can withdraw from the study at any time. Patients who choose to withdraw from the study will be referred back to their primary physician to resume care. Patients wishing to stop therapy may be discharged from the study. Patients wishing to initiate therapy outside of the study medications allowed will be discharged from the study. Patients can contact the PI, or the study coordinator to let them know of their intent to withdraw. Patients who withdraw after biopsies are performed will be given the option to destroy their tissue samples.

10.0 Statistical Considerations

As this is a pilot study and has never been performed in this population, a formal power calculation has not been performed. A sample of 10 CIDP patients was deemed appropriate to determine if abnormal dermal myelinated pathology exists which can be evaluated in further studies. It is possible patients may have insufficient pathology. This study will provide data for future studies and will enable power calculations. Morphometric analysis of IENF, myelin thickness, intermodal length, etc will be compared using nonparametric Wilcoxan test of means of CIDP patients and healthy controls collected from previous study (18).

The data collected from Aim 1 will also be used for both Aims 2 and 3. The primary outcome measures for segmental demyelination obtained from biopsy will include the number of fibers with segmental demyelination (out of a random sample of 25 fibers), the average length of demyelinated segments, and the number of fibers with sodium channel spreading over denuded axons. As a secondary outcome, clinical outcomes such as MRC scores from the routine standard of care will be also collected.

For Aim 2, signed rank tests will be used to test whether there would be any improvement after treatment in the primary outcomes measured at 3 months and 6 months separately, compared to those measured at the baseline. Also, the outcome measures obtained from confocal vs. florescence alone will be compared to examine whether the measurements are similar using a random-effects model along with application of bioequivalence testing methodology.

For Aim 3, to examine whether the primary outcomes and MRC scores are correlated, their correlations will be compared using Spearman rank correlations at 3 months and 6 months separately.

11.0 Privacy/Confidentiality Issues

Consents will be obtained in the Clinical Research Center at time of screening in a private room. Patients will be provided with a copy of the consent form prior to the screening visit, and the patient will be given adequate time to review with the study coordinator prior to consent. Paper copies of nerve conduction waveforms, autonomic test results and survey results will be kept in binders, locked in the PI's or study coordinator's office in a locked filing cabinet. No PHI will be kept on paper study forms. Data will be kept in a password protected REDCAP database. Electronic files of photos from skin biopsies will be kept on a password-protected shared drive without identifying information (PHI) on them.

All patients will be given a unique code identifying them for the study which will be used instead of names to protect confidentiality. Keys to the codes will be kept by the PI and study coordinator. The PI will maintain study information confidentially for a minimum of seven years as stipulated by the NIH and FDA. The codes linking study number with names, PHI information will be stored in a secure database, password protected in a redcap database and a paper form with the code will be stored in a binder in a locked cabinet in the PI's office. The codes will be generated by the order in which the patient is screened for the study (001, 002, for diabetics, 101, 102 for healthy controls). The PI will maintain the information obtained through the study for seven years and will be kept by the PI in a secure location in the PI's office in addition to the data stored in redcap database. Paper copies of waveforms, survey instruments will only have code numbers and the date of the survey, study on them, and will be kept in binders in the PI's office. Digital images of biopsies will be coded by the patient code number and will be kept on a Vanderbilt password protected secure server. Only the PI and the co-investigators, study coordinator will have access to PHI/demographic information.

Tissue will only be labeled with the date of specimen, side of body and site (ie finger, distal leg) and code number of patient to avoid any identifying information. Tissue will be kept indefinitely for further studies unless the patient specifies they wish their specimens to be destroyed. All data will be recorded on paper forms and electronically in a Vanderbilt Redcap database which is password protected and backed up continuously to a server. Paper forms will be kept in a patient specific notebook and a unique ID number given to each patient and recorded on each form. No identifying information will

be on forms other than study ID and patient initials. HIPAA information will be kept confidential in a locked cabinet in a locked office of the study coordinator. The Redcap database is password protected and authenticated and will be built to flag erroneous values.

12.0 Follow-up and Record Retention

The study is expected to last an additional two years maximum to recruit 20 patients to have 10 patients total complete the study. The study binders and documents will be kept for a minimum of seven years past the last publication from the study and will be kept by the PI in a locked cabinet in her office which is also locked. Study photos of skin biopsy specimens will be archived on a password protected shared server and will not have any PHI linked to them, but will be archived indefinitely.

Appendix A
Study Schedule

Study Procedures	**Visit 1** (Screening) Day -30-0	**Visit 2** (Baseline) Day 1-30	**Visit 3** Day 90	**Visit 4** Day 180
Informed Consent	X			
Incl/Excl Criteria	X			
Demographics	X			
Medical History	X			
Interval History			X	X
Concomitant Meds	X	X	X	X
Physical Exam[a]	X	X	X	X
Neurological Exam	X	X	X	X
Height	X			
Weight	X	X	X	X
Vital Signs	X	X	X	X
Blood Work : CBC with diff BMP IgA	ALL ~15mL	BMP only 5mL	BMP only	BMP only
Nerve Conduction Studies	X			X
AE		X	X	X
IVIg Infusion (up to 14 days after visit 2)		X		
INCAT score		X	X	X
MRC Score	X	X	X	X
Phone Follow- Up calls monthly following visit 2[b]				
Glabrous Skin Biopsy		X	X	X

[a] Complete physical examination at Screening Visit, abbreviated physical examination at other visits

[b]Phone calls will be made monthly to verify patients are receiving and tolerating IVIg treatment.

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