Study Title “IVIg for Small Fiber Neuropathy With Autoantibodies to TS-HDS and FGFR3”

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## Protocol:

***Study Design:*** This was a prospective 6-month double-blind placebo-controlled pilot study of IVIG for treatment of suspected immune mediated SFN associated with TS-HDS or FGFR-3. The trial was registered with ClinicalTrials.gov (NCT03401073).

***Subjects***: Potential research subjects were recruited from the panel of patients seen by neuromuscular/peripheral nerve groups at Beth Israel Deaconess Medical Center (BIDMC) (Boston, MA) and HonorHealth Neurology (Phoenix, AZ) and by patients seen at neighboring institutions in the Boston and Phoenix areas. Additional referrals were obtained through ClinicalTrials.gov. All recruited subjects provided signed informed consent as approved by the Institutional Review Boards of BIDMC and HonorHealth.

***Inclusion Criteria:*** Patients were potentially eligible if they had clinically evident SFN in a length dependent or non-length dependent pattern as confirmed by history of symptoms that included burning, shooting, or prickling pain or dysesthesia for at least 6 months and examination findings demonstrating loss of pain sensation and/or loss of temperature detection in the distribution of pain. Subjects also required evidence of reduced intra-epidermal nerve fiber density (IENFD) seen on skin biopsy in the region corresponding to examination abnormalities, based on age and sex-matched normative values, using PGP 9.5 as the immunostain .[15] Subjects required a baseline daily pain score on a visual analogue scale (VAS) of greater than or equal to 4/10. Patients also required elevated titers of autoantibodies to TS-HDS or FGFR-3 as measured by the Neuromuscular Clinical Laboratory at Washington University in St Louis using standard enzyme linked immunoassay (ELISA) assays as previously published. [14, 16] Antibody titers were measured at the time of screening and abnormal values measured at the Washington University laboratory within the 6 months prior to screening were accepted. Normative values re <10,000 for TS-HDS and <3,000 for FGFR3 .

***Exclusion Criteria:*** Individuals were excluded if they had evidence of large fiber neuropathy such as abnormal nerve conduction studies, motor weakness, absent vibration, proprioception or deep tendon reflexes. Individuals were also excluded if they were found to have other known causes for small fiber neuropathy: diabetes, prediabetes, HIV, Sjogren's syndrome, vitamin deficiency, toxin exposure, or monoclonal gammopathy. Patients were also excluded for generalized, severe musculoskeletal conditions other than SFN that would have prevented accurate assessment by the physician, or for an underlying condition that would put them at risk of complication from a biopsy such as anticoagulant use

or high risk of infection. Other exclusions were a contraindication to IVIG because of renal dysfunction with creatinine >1.5 mg/dl, prior thrombotic event or a hypersensitivity reaction to IVIG in the past.

***Screening:*** Study subjects underwent a detailed history, physical examination, laboratory studies, pain VAS scores, the Short-From 36 question health survey (SF-36), antibody measurement of TS-HDS and FGFR-3 and skin biopsy evaluation of nerve fiber density at the distal leg and proximal thigh using standard methodology.

***Randomization and Blinding:*** Subjects who were eligible for study inclusion were block randomized into 2 groups (IVIG or placebo) by a blinded statistician. All study personnel, study subjects and support staff were blinded to treatment assignment. Only the single research pharmacist who mixed the IVIG/placebo was aware of the treatment assignment and was not in communication with any study staff. IVIG and placebo were placed in opaque infusion bags that covered the entire treatment product and infusion line to prevent unblinding.

***Study Medication and Treatment Allocation***: Subjects received either IVIG or placebo. IVIG treatment involved an initial dose of 1 gm/kg (Gamunex, Grifols, Los Angeles, CA) daily for 2 days followed by 1 gm/kg in a single dose every 3 weeks for 21 weeks (for a total of 8 doses) with a maximum of 80 gm IVIG infusion. Normal saline was used as placebo and included in a volume equivalent to that of IVIG.

***Study Procedures:*** The outline of the study protocol is seen in Supplemental Figure 1. At all study visits participants underwent review of inclusion and exclusion criteria, medical history, medication review, adverse effect review, and pain VAS scores (0-10 scale – reported as pain on the day of visit). At the screening and final study visits all subjects also completed skin biopsies at the distal leg,[17] the Utah Early Neuropathy Scale (UENS),[18] and the SF-36.[19]

***Physical Examination****:* Detailed neurologic examinations were performed at baseline and at the final visit. The physical examination was quantified via the UENS.[18] In brief, the UENS is a 42-point system that grades neuropathy from 0 (no neuropathy) to 42 (absent ankle reflexes, weakness at the toes, absent sensation to multiple modalities in the legs).

***Pain Scores***: Subjects rated their pain on an 11-point visual analogue Likert scale at every visit (where 0 = no pain and 10 = maximal pain). The location, exacerbating factors and character of pain were recorded at each visit. Subjects were treated with medications to reduce neuropathic pain at the discretion of the treating physician, including anti-convulsants, anti-depressants and opioids, typically in combination. Pain scores were measured while on medication. Subjects

were asked not to change doses of daily neuropathy pain medications while participating in the clinical trial. Subject use of pain medications was monitored at each study visit.

***Skin Biopsy Evaluation of Intra-Epidermal Nerve Fiber Density (IENFD)****:* All study participants underwent 3-mm punch skin biopsies 10 cm above the lateral malleolus at their baseline visit. Follow-up biopsies were taken 21-25 weeks later on the same leg adjacent to the original biopsy sites using standard techniques. Specimens were fixed and stained with PGP 9.5 (ubiquitin hydrolase, Chemicon).[20] All patients underwent IENFD counting by a blinded physician and results were expressed as a linear density for IENFD (number of fibers per millimeter).[21]

## Statistical Analysis Plan:

***Primary Outcome*****:** The primary efficacy endpoint for this study was the change in IENFD between visits 1 and 8. We defined a clinically relevant change in disease endpoint as a >2.0 fiber/millimeter increase in IENFD between screening and final visit. We anticipated no change in IENFD between visits 1 and 8 in the placebo treatment arm. The change in IENFD was based on prior studies of lifestyle modification in diabetic neuropathy where increases in IENFD of >2 fibers/mm over 1 year were seen in 25% of patients associated with an improvement in neuropathic pain.[22] Pain was not selected as a primary outcome measure because a separate ongoing clinical trial using IVIG in small fiber neuropathy was already investigating pain as an endpoint,[2] and also because of our specific interest was in evaluating IVIG for potential impact on disease modification.

***Secondary Outcomes*****:** Two secondary outcome measures are included in this study:

1) The change in neuropathic pain severity between visits 1 and 8.

   We expected that individuals with immune mediated small fiber neuropathy treated with IVIG would have a >2-point reduction in neuropathic pain VAS score between screening and final visit. We anticipated a <1.5-point reduction in neuropathic pain VAS score between screening and final visit in the placebo group.

2) The change in quantified UENS scores, between screening and final visit.

We expected that individuals with immune mediated SFN treated with IVIG would have a have a >1 point reduction in the UENS score between screening and final visit. We expected that the placebo treated group would have no change in UENS score between screening and final visits.

***Exploratory Outcomes*:** Safety in response to IVIG infusion and changes in distribution or character of pain associated with SFN in patients treated with IVIG.

This pilot study was being conducted in order to obtain adequate preliminary data for a larger clinical trial but was powered to answer the primary endpoint analysis. Secondary endpoints and exploratory endpoints are not included in the power analysis. The sample size of 10 participants per group was determined with an assumed change in IENFD rate of 0 fibers/millimeter in the placebo group and >2.0 fibers/millimeter in the IVIG group, a 1-sided $\alpha$ of 5%, and 80% power between the 2 groups, including the assumption of a dropout rate of 10% (2 patients). Therefore, the aim was to include in total 20 patients in the study.

The results were analyzed according to the intention-to-treat (ITT) protocol. For the primary ITT analysis, missing values were imputed as no change from baseline. Participants were analyzed for efficacy according to randomized treatment. For the SF-36 Health Survey, biopsy results, and UENS examination findings, the difference between baseline (screening period) and the end of the final visit was calculated. For the primary outcome measure, the proportion of patients with a decrease of at least 2.0 fibers/millimeter was compared between the IVIG and placebo groups with a $\chi^2$ test, with mean and standard deviations reported. For secondary outcome measure, mean change in values between groups was compared using a student's T-test (2 tailed, 2 sample, homoscedastic variance). For pain VAS scores, ANOVA was used to measure differences in pain scores between IVIG and placebo groups.