CARBIDOPA IN FAMILIAL DYSAUTONOMIA
PHASE II STUDY, IND 117435

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Study Product: Carbidopa (Lodosyn®)

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Study Summary

Title	**CARBIDOPA IN FAMILIAL DYSAUTONOMIA PHASE II STUDY, IND 117435, Date: 01/07/13**
Short Title	*CARBiFD*
Protocol Number	*13-00065*
Phase	*Phase 2*
Methodology	*Double-blind, Randomized, placebo control; cross-over design*
Study Duration	*3 years*
Study Center(s)	*Single-center*
Objectives	The overall study objectives are to determine whether carbidopa (Lodosyn®) is safe and well tolerated and to assess whether it can inhibit catecholamine-induced paroxysmal hypertension and normalize or reduce the exaggerated blood pressure variability in patients with familial dysautonomia
Number of Subjects	*30*
Diagnosis and Main Inclusion Criteria	Diagnosis: Familial Dysautonomia ▪ Male or female patients with FD age 10 or older ▪ Unstable blood pressure: ▪ Systolic BP standard deviation >15 mmHg ▪ Or coefficient of variation >15% ▪ Or documented episodic hypertensive peaks (≥140mmHg) ▪ Confirmed diagnosis of FD (historical confirmation of subjects' genetic diagnosis will be obtained for this study) ▪ Providing written informed consent (or ascent) to participate in the trial Ability to comply with the requirements of the study procedures.
Study Product, Dose, Route, Regimen	*Carbidopa* (Lodosyn®), Dose: 100mg, 200mg, placebo, oral, three time a day
Duration of administration	*1 month on each dose.*
Reference therapy	*Placebo*
Statistical Methodology	We will examine the sample distribution of SD of daytime SBP, and if it deviates from the normal distribution, we will employ the nonparametric Wilcoxon test for robustness. In addition, to account for potential covariates, such as age, gender, and disease severity, the analysis of (co)variance (ANOVA and ANCOVA) methods will be used to model the repeated measures data.

1 Introduction

Patients with the rare genetic disorder familial dysautonomia (FD) suffer from frequent surges in blood pressure that irreversibly damage their organs, for which there are no adequate treatments. This clinical trial will test whether carbidopa could effectively and safely treat the problematic high blood pressure. If successful, this will have a major impact in the quality of life of these patients, and could potentially be a treatment for other disorders of uncontrolled sympathetic activation.

This document is a protocol for a human research study. This study is to be conducted in accordance with US government research regulations, and applicable international standards of Good Clinical Practice, and institutional research policies and procedures.

1.1 Background

The study objective is to determine whether carbidopa (Lodosyn®) is safe and tolerable in patients with familial dysautonomia (FD), and to learn whether it can inhibit catecholamine-induced paroxysmal hypertension and reduce their exaggerated blood pressure (BP) variability. FD is a brutal genetic disease caused by a developmental defect in primary sensory neurons. The nerves that relay information from arterial baroreceptors are particularly affected resulting in unstable BP. Even mild anxiety can trigger a pronounced release of catecholamines causing paroxysmal hypertension and tachycardia. The subsequent exaggerated BP variability correlates closely with target organ damage in FD. Current drug treatments have little efficacy or intolerable side effects, and none specifically targets BP variability. Carbidopa is a reversible competitive inhibitor of aromatic L-amino acid decarboxylase (DOPA-decarboxylase). It cannot cross the blood brain barrier, and only prevents the formation of catecholamines in the periphery. We recently showed that carbidopa reduces the spillover of dopamine into the circulation and decreases the frequency of nausea in FD patients. Preliminary observations suggest that carbidopa may also lessen the exaggerated BP variability by reducing the formation of norepinephrine outside the brain. To follow up on this finding, we propose to conduct a well-powered study to test of the hypothesis that carbidopa might dampen norepinephrine-driven periods of paroxysmal hypertension in FD patients and thus lessen BP variability. We will use a randomized, double blind, 14-week cross over study comparing two doses of carbidopa and placebo. The sample size will be 30 patients with FD, who will act as their own controls across the two active doses and placebo. In random order, patients will receive high dose carbidopa (600 mg/day), low dose carbidopa (300 mg/day) or matching placebo in three separate 4-week treatment periods. We will monitor adverse events and safety/tolerability parameters throughout. The primary efficacy end-point will be the standard deviation of systolic BP variability. To understand the physiological effects of carbidopa we will measure 24-h catecholamine excretion and diurnal and short-term BP variability. If successful, this would be a major therapeutic breakthrough for FD patients, and could serve as the basis for the use of carbidopa in other more common BP disorders with similar pathophysiology.

The overall study objectives are to determine whether carbidopa (Lodosyn®) is safe and well tolerated and to assess whether it can inhibit catecholamine-induced paroxysmal hypertension and normalize or reduce the exaggerated blood pressure variability in patients with familial dysautonomia (FD, also called hereditary sensory and autonomic neuropathy type III or Riley-Day syndrome).[1]

FD is a rare hereditary disorder caused by splice mutations that lead to a deficiency of elongator-1 protein (ELP-1 or IKAP).[2,3] The defect prevents normal development of sensory (afferent) neurons,[4] including those that relay information from arterial baroreceptors.[5] Although reduced in number,[6] when activated, the remaining efferent sympathetic nerves can dramatically raise blood pressure.[1,5] Even mild arousals trigger surges in circulating catecholamines levels and characteristic paroxysmal hypertensive episodes.[1,5,7] Patients also have orthostatic hypotension.

Renal failure is a major problem for patients with FD.[8] Paroxysmal hypertension and excessive blood pressure fluctuations contribute to the progression of renal damage.[9,10] Treatment of hypertension in patients with FD is fraught with difficulties. Clonidine and benzodiazepines are currently used to decrease emotionally-induced hypertension [11] but they produce sedation, respiratory depression and dramatically worsen orthostatic hypotension.

We've recently shown that carbidopa, a reversible competitive inhibitor of DOPA-decarboxylase that does not cross the blood brain barrier,[12] successfully blocks the peripheral formation of dopamine and effectively reduced the frequency and severity of the retching/vomiting attacks, a disabling recurrent feature of these patients.[13] In

the small study, carbidopa was well tolerated and safe. New preliminary data indicates that carbidopa also reduces the synthesis of norepinephrine outside the brain in patients with FD. Further exploratory findings suggested that carbidopa might lessen the hypertensive peaks and reduce the exaggerate blood pressure variability over 24-hours, but further evidence is needed.

We now propose to perform a double-blind randomized trial with a cross over design to compare high dose (600 mg/day) and low dose (300 mg per day) carbidopa blockade with placebo. Patients will be randomly assigned to a high-dose/low-dose/placebo sequence, low-dose/placebo/high-dose sequence or placebo/high-dose/low-dose sequence. Participants will remain on each treatment period for 28-days.

Aim 1: To evaluate the safety and tolerability of carbidopa in FD patients with particular emphasis on the orthostatic fall in blood pressure.

Aim 2: As proof of concept, examine the hemodynamic effects of carbidopa and determine its effects on norepinephrine production, BP variability and paroxysmal hypertension.

Aim 3: In a dose finding study, compare the effects of low (300 mg/day) and high (600 mg/day) dose carbidopa blockade vs. placebo on BP variability and paroxysmal hypertension.

The study is well powered to answer whether carbidopa can successfully dampen norepinephrine-driven periods of paroxysmal hypertension, and compare the effectiveness of high-dose and low-dose blockade. As far as we are aware, this is the first time carbidopa has been used clinically to suppress norepinephrine synthesis in peripheral sympathetic neurons. If successful, effectively reducing catecholamine-driven paroxysmal hypertension without central side effects would be a major therapeutic breakthrough for these patients.

1.2 Investigational Agent

Carbidopa (DL-α-methyl-α-hydrazino-3, 4-dihydroxyphenyl-propionic acid, HMD, MK-486, Lodosyn ®) is a reversible competitive inhibitor of DOPA-decarboxylase that prevents dopamine formation outside the brain.[33] It is structurally similar to DOPA except for one extra methyl (CH_3) and a hydrazine (N_2H_4) rather than an amino group.[34] By forming a series of strong but ultimately reversible bonds with the enzyme's co-factor pyridoxine, carbidopa prevents the binding of DOPA and inhibits dopamine production.[34] It has been used for many years to block the peripheral synthesis of dopamine in patients with Parkinson disease treated with L-DOPA.[35]

1.3 Preclinical Data

N/A

1.4 Clinical Data to Date

Familial Dysautonomia (FD):

FD is a rare autosomal recessive disease characterized by profound sensory[14] and autonomic dysfunction.[1,5] The disease was first described by Conrad Riley and Richard Day in 1949.[1] The original report featured 5-children who had "*an undue reaction to mild anxiety characterized by* [...] *transient but marked arterial hypertension*" (Fig.1).[1] These episodes of paroxysmal hypertension are due to the release of norepinephrine into the circulation.[5]

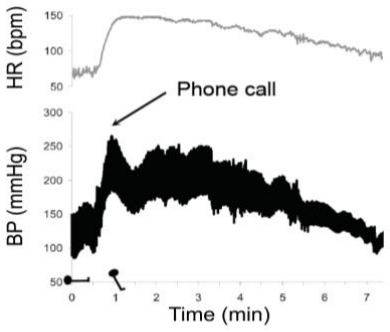


Fig. 1: *Shows a typical episode of paroxysmal hypertension in a patient with FD triggered by receiving an unexpected phone call from a friend. Note, rise in systolic BP to 250 mmHg.*

In the last 40-years, 666 patients with FD have registered at the Dysautonomia Center of NYU. Three hundred and forty of those patients are alive, being actively followed by the Center and have molecular confirmation of the FD gene mutations.[2] This is the largest cohort of FD patients in the world. The Center is dedicated to research and clinical care of these rare patients.

Genetics of FD:

Over 99% of FD cases are homozygous for a point mutation in the gene that encodes for the protein elongator-

1 (ELP-1, also known as IKAP).[2] ELP-1 is expressed most strongly in the nervous system during development.[15] Neural crest cells express particularly low levels of ELP-1 and fail to migrate and differentiate normally.[16] The mutation occurs in a non-encoding intron and some tissues mysteriously retain the ability to express normal levels of ELP-1. For unknown reasons, the nervous system is particularly "hard-hit", producing almost no normal protein.[2]

Blood Pressure Abnormalities in FD:

The deficiency of ELP-1 during embryogenesis affects the development of afferent neurons, including fibers sensing pain and temperature,[17] proprioception [14] as well as the cranial nerves relaying information from the chemoreceptors[18] and baroreceptors.[5] However, there is a relative sparing of efferent sympathetic neurons [19] which remain functional and release catecholamines.[5,7] FD patients have a unique autonomic phenotype with periods of paroxysmal hypertension driven by surges in circulating catecholamines. Due to lack of normal baroreflex restrain, arousals result in periods of paroxysmal hypertension and tachycardia throughout the day (Fig.1).[5] Many patients frequently suffer attacks of nausea and vomiting, as dopamine [7] and norepinephrine [5] spillover into the circulation. Blood pressure is dramatically unstable in patients with FD. This instability contributes to an overall increase in variability, which appears to be directly related to 24-h norepinephrine excretion (Fig. 2). Although patients with FD also have orthostatic hypotension,[5] the most disabling features of the disease are related to heightened sympathetic activity.[7,10]

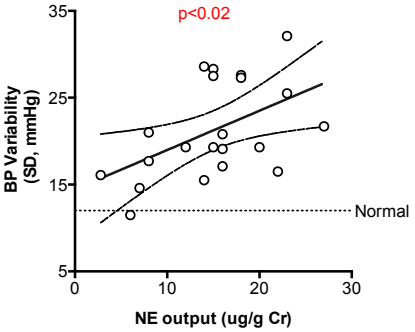


Fig. 2. Scatterplot shows relationship between NE excretion and BP variability in patients with FD

End-Organ Target Damage:

The main sites of end-organ target damage in patients with FD are the heart [20] and kidneys.[8,9] Patients have an increased incidence of renal failure that frequently begins at an early age.[8] Twenty-percent of patients that survive to age 25 have end-stage renal disease, requiring dialysis or transplantation.[21] Renal biopsies are consistent with chronic hypertensive nephrosclerosis and periods of accelerated hypertension.[9] While the underlying cause of the renal failure is unknown, several factors appear to influence its progression. Fludrocortisone, a drug until recently used in the treatment of orthostatic hypotension in patients with FD,[11] dangerously hastens the decline in glomerular filtration rate and is now used sparingly.[10] As was shown in hypertensive patients,[22,23] FD patients that have higher blood pressure variability (as expressed by the standard deviation [SD] of 24-h ambulatory blood pressure readings) have a greater degree of renal damage.[10] The same association has been shown in animal models of baroreflex deafferentation.[24] Our recent retrospective analysis (Fig. 3) shows that patients who had the most pronounced blood pressure variability (i.e., the highest SD in 24-h or during the day) had a faster rate of renal decline over the subsequent 13-years of follow-up.

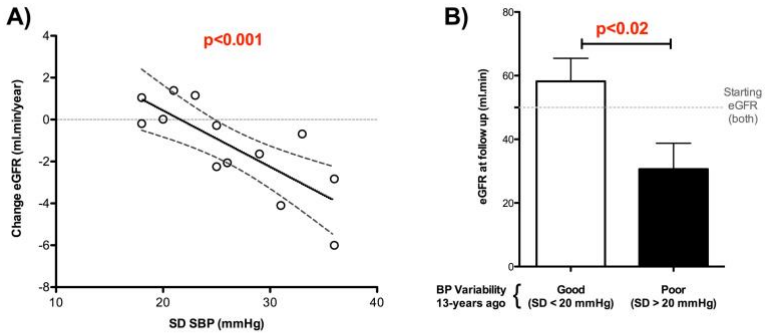


Fig. 3: *Scatter plot (A) shows faster decline in renal function in patients with more exaggerated variability. Bar chart (B) shows patients with variability >20 mmHg had a significantly lower est. glomular filtration rate (eGFR) when followed-up 13-years later. Groups had similar starting eGFR and same age/gender distribution.*

While mean blood pressure is a powerful risk factor for vascular events, recent findings emphasize the importance of paroxysmal hypertension and increased blood pressure variability as the cause of residual risk.[25]

Catecholamine Pathways in FD:

The clinical phenotype featuring flushing, hypertension, tachycardia and agitation is caused by sympathetic over

activity and the release of norepinephrine into the circulation. However, pathological samples show that only a third of peripheral sympathetic neurons survive development.[6] Infusion of norepinephrine produces an exaggerated pressor response, characteristic of denervation supersensitivity.[26]

Several enzyme abnormalities along the catecholamine pathway have been described in patients with FD. Plasma levels of dihydroxyphenylalanine (DOPA) [27,28] and dopamine are elevated,[7,27,28] while plasma levels of norepinephrine are normal.[5,7,27] Intraneuronal plasma norepinephrine metabolites are reduced.[27,28] In urine, dopamine metabolites are high (twice the normal value),[26] while free-norepinephrine [26] and normetanephrine excretion are both normal,[29] and vanillylmandelic acid (VMA) levels are sometimes decreased.[26] Reports on enzyme expression are conflicting; over expression of tyrosine-hydroxylase,[19] under-expression of dopamine-β-hydroxylase [30,31] and down-regulation of mono-amine oxidase [32] have all been suggested. The overall profile suggests increased catecholamine biosynthesis in sympathetic nerves and a decrease in neuronal re-uptake, leading to increased excretion rates.[27] Because of absent baroreceptor afference there is no feedback inhibition and arousal evokes the unrestrained release or norepinephrine and paroxysmal hypertension (Fig. 1).[5]

Current Treatments for Managing Paroxysmal Hypertension in FD:

Benzodiazepines (like diazepam and clonazepam [11]) are effective anxiolytics and frequently lower blood pressure during a prolonged anxiety-induced paroxysmal hypertensive attack. Many patients, however, quickly become tolerant and require increasing doses,[7,13] which can cause severe respiratory depression as patient also lack inputs from chemoreceptors.[18] The central sympatholytic agent clonidine has been used with some success,[11] but its use is limited by the unwanted side effects of sedation, hypotension and rebound hypertension. Beta-blockers and ACE-inhibitors frequently produce hypokalemia. Vasodilators like calcium channel blockers, hydralazine and nitrates frequently worsen orthostatic hypotension.

Dopa-Decarboxylase-Inhibition and Blood Pressure Variability:

At rest, carbidopa does not have profound effects on tissue concentrations of norepinephrine[33] or on blood pressure.[36] In patients with classic (efferent) autonomic failure, carbidopa does not worsen orthostatic hypotension.[37] Most relevant to this proposal, experimental data shows that at times of emotional arousal, when rates of catecholamines synthesis increase, carbidopa effectively blocks the additional production of dopamine, norepinephrine and epinephrine.[33] Animal data shows that the suppression of norepinephrine synthesis in sympathetic nerves is even more effective at larger doses.[38] If carbidopa had the same effect in patients with FD, inhibiting catecholamine surges at times of arousal would help dampen paroxysmal hypertensive peaks and should subsequently reduce overall variability. Since carbidopa itself cannot cross the blood brain barrier,[12] even at high doses,[36,39] it has no effects on the CNS, thus making it a viable alternative to benzodiazepines and clonidine for the treatment of catecholamine-induced paroxysmal hypertension in FD. Its effects on the severity of orthostatic hypotension in patients with FD are not clear and warrant further investigation to ensure the drug is safe.[13]

C: Preliminary Data

Recently, in a double-bind, placebo-controlled trial sponsored by the FDA's Office of Orphan Products Development [13] we showed the short-term safety of carbidopa and its antiemetic efficacy in patients with FD. Carbidopa successfully lessened the nausea/retching/vomiting episodes and reduced the need for benzodiazepines.[13] Preliminary results show carbidopa also has a beneficial effect on stabilizing the blood pressure and reducing its variability.

1) Effect of Carbidopa on Blood Pressure Variability in FD:

Six of the 12 patients with FD that participated in the carbidopa nausea trial wore 24-hour ambulatory BP monitors while taking carbidopa (dose 450 to 600 mg/day, divided into three doses 6 hours apart). These patients were similar in age, gender and clinical features to the total population (mean age 18±2 years [range 15-28 years] 3 males: 3 females). In these 6-patients, we compared pre-treatment blood pressure recordings with those obtained while taking carbidopa. Overall average systolic (111±10 vs. 117±6 mmHg) and diastolic (73±3 vs. 70±8 mmHg) pressures were similar on and off carbidopa treatment, but heart rate was 13 beats lower while on carbidopa (86±3 to 76±2 bpm). While taking carbidopa, measures of blood pressure variability during the day were significantly lower (SD. $p<0.05$; coefficient of variation [CV] $p<0.05$, Fig. 4). Minimum captured BP was similar on and off carbidopa, but maximum captured BP was significantly lower on carbidopa (Fig. 4).

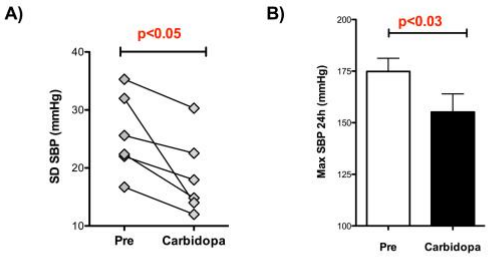


Fig. 4: *Graph A shows systolic blood pressure (SBP) variability (standard deviation SD) and graph B shows maximum SBP captured on an ambulatory recording before and after treatment with high-dose carbidopa.*

2) Effect of Carbidopa on Catecholamines in FD:

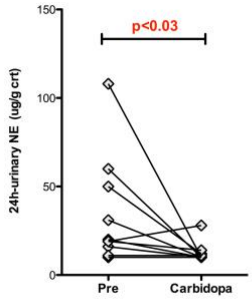


Fig. 5: *Twenty-four hour noreprinephrine (NE) excretion before and after treatment with high-dose carbidopa*

Twenty-four hour urinary catecholamine levels were assayed pre-treatment and after patients were titrated to their maximum tolerated dose. In addition to the expected decrease in dopamine excretion, norepinephrine excretion was also dramatically reduced (Fig. 5).

These results suggest that in patients with FD, inhibiting catecholamine synthesis upstream blunts the surges in norepinephrine as a knock on effect. We hypothesize, therefore, that by dampening the hypertensive peaks, carbidopa will reduces overall blood pressure variability in patients with FD.

We will carefully examine the hemodynamic effect of carbidopa and its effects on orthostatic hypotension. We will also determine whether a lower dose of carbidopa has similar beneficial effect.

Overview:

This clinical trial will enroll 30 patients with FD who have excessive blood pressure variability. We will define excessive blood pressure variability as a SD of systolic blood pressure >15 mmHg or a coefficient of variation > 15% (i.e., 2 SD above our own laboratory age-matched control data) or paroxysmal hypertensive peaks >140 mmHg captured in ambulatory blood pressure recordings.

The trial will use a double-blind crossover design to evaluate the safety and efficacy of high-dose (600 mg) and low-dose (300 mg) carbidopa compared with placebo. Each treatment period will last 28-days with a 2-day dose de-escalation (Day 1: 2/3 dosage and Day 2: 1/3 dosage) and 2-day washout period between dose changes. After baseline evaluation (visit 1), enrolled patients will be randomly assigned to one of three treatment sequences (high-dose/low-dose/placebo, low-dose/placebo/high-dose or placebo/high-dose/low-dose, see Fig. 6). Study visits (in the office or via 2-way televideo conferencing with voice capabilities) will occur on the last day of each 28-day treatment period. Evaluation will include: adverse event monitoring, vital signs, safety bloods, urinalysis, 12-lead ECG, blood pressure supine and standing, orthostatic hypotension symptom severity questionnaires, autonomic testing, urinary catecholamine assay, and ambulatory blood pressure monitoring. Fig. 7 describes the timing sequence of events during the office visit.

We will monitor safety and tolerability throughout and safety bloods will be obtained after 4-weeks on each treatment arm (**Aim 1**). As proof of concept, we will examine the hemodynamic effects of carbidopa by measuring ambulatory BP recordings BPs supine/sitting/standing (in the office or during a telemedicine visit), and continuous BP measurements during cognitive arousal (Stroop test). We will also assess 24-h urinary catecholamine excretion (**Aim 2**). To learn more about the appropriate dose, we will compare 600 mg/day with 300 mg/day **(Aim 3).**

1.5 Dose Rationale

Within 30 minutes of ingestion, carbidopa accumulates in the spleen, liver, lungs, kidneys and in plasma.[12] Only half of the oral dose is absorbed [12] and half-life in plasma is 1.3 hours.[34] DOPA-decarboxylase activity remains inhibited up to 6 hours after dosage.[12] In patients with FD, we are aiming for steady state of DOPA-decarboxylase

inhibition during waking hours when BP instability is at its most pronounced. In our previous trial,[13] we achieved this with a dosing schedule q6h (i.e., 7AM, 1PM and 7PM). In patients that were predictably hypertensive and at a certain time (most commonly when awakening from sleep when catecholamine levels surge), administration was tailored to 1.5 hours before awakening (via gastrostomy). In animal models, the mean effective dose of carbidopa needed to prevent L-DOPA-induced vomiting is 16 mg/kg (range 10 – 28 mg/kg).[40] In adult patients with chronic autonomic failure, a dose of 200mg of carbidopa completely blocked the conversion of dihidroxyphenylserine (DOPS or droxidopa) to norepinephrine.[37,41] The same 200mg dose q6h (total daily dose: 600 mg) was effective at reducing norepinephrine excretion and BP variability in patients with FD (see preliminary data and ref: 13). We do not know if lower doses are as effective.

Carbidopa is sold commercially as Lodosyn®, in pills of 25 mg strength. This requires the patient to take 8-pills 3 times a day, and has obvious compliance issues. Lower doses are effective in blocking nausea after L-DOPA administration in patients with Parkinson disease. Thus comparing 600 mg/day versus 300 mg/day doses (the lowest dose used in the trial) will allow us to collect preliminary data to understand which doses to use in a future phase III trial. While we saw no serious adverse events with carbidopa, we will nevertheless provide patients with an *individualized dosing schedule* so that they can titrate down prior to washout when switch between high-dose or low dose blockade and placebo. Dose de-escalation instructions will be provided at the end of each study visit (Appendix). To ensure investigators still remain blind to the treatment allocation, the Investigational Pharmacy will dispense all pills including those for the titration down schedule. To ascertain *compliance,* patients will be instructed to return unused medication. Since carbidopa is eliminated from the body after a short period of time,[12] we expect there to be no carryover between successive treatments 28-days apart. Nevertheless, between crossovers, we will have a washout period of 2 days (see Fig. 6 for details).

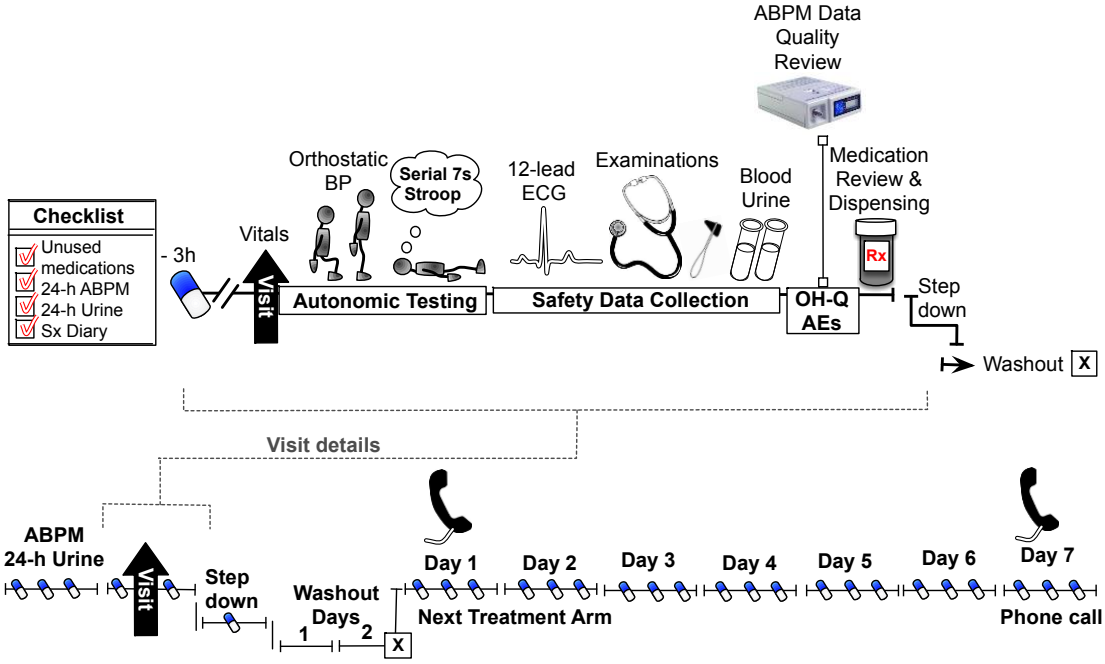


Fig. 6: Office visit details: *Prior to visits 2, 3 & 4, patients will be instructed to collect urine for 24-h to be assayed for catecholamines. Over the same period, patients will undergo ambulatory blood pressure monitoring (ABPM) and will be instructed to arrive at the laboratory wearing the ABPM and return all unused medications. Office visits and telemedicine visits will be scheduled in the morning and patients will be told to take their morning carbidopa dose 3-h before testing. On arrival at the clinic or at the start of the telemedicine conference, vital signs will be measured and autonomic function tests will be performed. Orthostatic blood pressures will be measured supine (5-min), seated (2-min) and standing (5 min). To re-check the calibration on the ABPM values, simultaneous measurement of blood pressure on the ABPM and on an automated sphygmomanometer will be performed during the active stand test. At all in office visits, patients will perform the Stroop test with continuous blood pressure and RR interval monitoring. During telemedicine visits, heart rate will be measured at home with a wireless activity wristband while performing*

serial seven subtractions. A 12-lead ECG and physical/neurological examination will be conducted. Blood and urine will be obtained for safety analyses. Patients will answer the OH-Questionnaire and be questioned about adverse events. ABPM recordings will be reviewed to determine data quality and will be repeated if not satisfactory. Medication compliance will be checked by asking patients to bring in or email a time stamped photograph of unused pills.. Medications will be dispensed by the Investigational Pharmacy (including those for the titration down and those for the next treatment arm). Prior to starting the next treatment arm, patients will be instructed (in writing [Appendix] and verbally) to titrate down their current dose, by taking one pill in the afternoon of testing and one pill the following day. They will then proceed to a 2-day washout period. After the washout, they will start their next batch of medications three-times per day (q6h). On the day they start their new medications, all patients will receive a phone call to check for adverse events, if concerned, we will bring the patients into the Center for an unscheduled visit. Seven-days after their office visit, patients will be contacted by phone to check for tolerability issues and adverse events. Thereafter, patients will be contacted on a weekly basis and questioned about adverse events. They will also fill a daily symptom diary, which prompts them to note any adverse events.

1.6 Research Risks & Benefits

1.6.1 Risk of Study Drug

All human studies will be done by trained personnel in a quiet room, within the Dysautonomia Center. The monitoring procedures, including 12-lead electrocardiogram and blood pressure with a sphygmomanometer are non-invasive and should produce only minor discomfort. Peripheral venous blood sampling dose not produce pain, but can leave bruising at the insertion site and will be explained on the consent form. Carbidopa is an FDA approved drug for the adjunct treatment of Parkinson's disease, but it is not approved for this purpose. Prominent side effects of carbidopa are related to combined treatment with levodopa. Carbidopa does not cross the blood brain barrier so central side effects will not occur. Doses similar to those used in this trial have not shown to be carcinogenic, mutagenetic or impair fertility. There have been no controlled trials of carbidopa in pregnant or lactating women or children. Carbidopa, at doses as high as 120 mg/kg/day were without teratogenic effects in the mouse or rabbit. In the event of over dosage, general supportive measures will be employed, along with immediate gastric lavage. Intravenous fluids will be administered judiciously, and an adequate airway maintained. Electrocardiographic monitoring will be instituted and the patient carefully observed for the development of arrhythmias; if required, appropriate anti-arrhythmic therapy will be given.

1.6.2 Other Risks of Study Participation

To minimize any potential risk, a physician and a research nurse will be present and monitor all procedures. Careful and continuous monitoring of vital signs, blood chemistries, 12-lead echocardiograms and adverse events will help minimize potential risks. Confidentiality will be maintained for the identity of participants in this study, except as necessary for oversight by the Secretary of the Department of Health and Human Services or his designated representative. To minimize the theoretical risk of rebound hypertension following abrupt carbidopa withdrawal, prior to wash-out, between crossovers, we will reduce the dose of study medication by 2/3 for one day (the day of the office visit) and then to 1/3 the following day. We will monitor adverse events with a phone call on the first day of adjusting dosages, and at weekly intervals thereafter. We will emphasize to patients and caregivers the importance of contacting the Center should they experience a change in their health status. We will also collect daily diaries prompting patients/caregivers to write down adverse events. To minimize the potential risks of orthostatic hypotension, we will provide patients with a practical advice sheet to quickly raise blood pressure including to lay supine (to aid venous return), ensure good hydration (consume 500 ml of water) and be cognitively aroused (doing something they enjoy, talking, homework, playing with a computer game console which raises blood pressure).

1.6.3 Potential benefits

No benefits can be guaranteed nor will they be implied when consent is obtained. Under the conditions of careful bedside monitoring outlined in this proposal, the risk to any subject will be minimized. We consider these risks smaller than the practical and theoretical benefits derived from the information obtained.

There is a potential benefit that carbidopa may reduce blood pressure variability and paroxysmal

hypertension.

2 Study Objectives

Aim 1: To evaluate the safety and tolerability of carbidopa in FD patients with particular emphasis on the orthostatic fall in blood pressure.

Aim 2: As proof of concept, examine the hemodynamic effects of carbidopa and determine its effects on norepinephrine production, BP variability and paroxysmal hypertension.

Aim 3: In a dose finding study, compare the effects of low (300 mg/day) and high (600 mg/day) dose carbidopa blockade vs. placebo on BP variability and paroxysmal hypertension.

3 Study Design

3.1 General Design

Double-blind, Randomized, placebo control; cross-over design

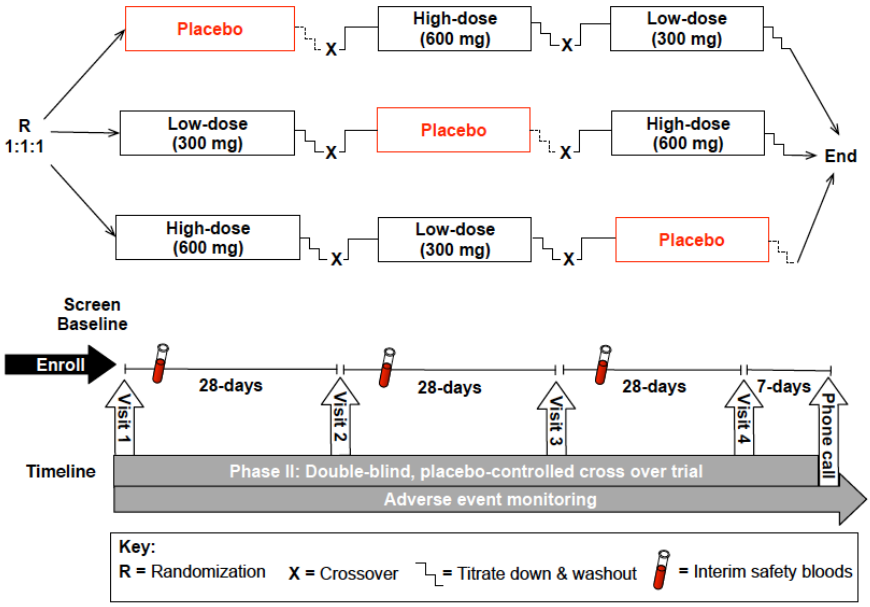


Fig. 7: Overall trial design: *This is a 14-week study. Patients will receive, in random order, high dose carbidopa (600mg/day), low dose carbidopa (300 mg/day) or placebo. Between each crossover, there will be a titration down over 2-days followed by a 2-day washout. An interim safety phone call will be made 7-days after crossover. Safety bloods will be collected each study visit. Patients participating in telemedicine visits will be given prescriptions to collect their safety bloods and urine locally. Adverse events will be monitored throughout by weekly phone calls and a daily symptom diary log. There will be 4 evaluation visits (in the office or via 2-way teleconferencing), 28 days apart and another follow-up phone call 7-days after completing the study. See text for details. A 14-day window either side of scheduled office visits will be allowed to accommodate school/work commitments. Because patients with FD are prone to frequent illnesses as part of their underlying medical condition, we will allow patients undergoing medical care for an unanticipated/unexpected adverse event to cease taking the study medication and resume again when the issue is resolved and/or the patient is recovered. In all instances, this will be documented in the case report forms.*

3.2 Primary Study Endpoints

Primary (hemodynamic) efficacy outcome variables

- SD of systolic BP variability (daytime)

- Highest systolic BP 24-h

3.3 Secondary Study Endpoints

Secondary efficacy outcome variables

- 24-h Urinary norepinephrine excretion
- CV of systolic BP variability (daytime)
- Weighted SD of BP variability
- Percent/number of systolic readings >140 mmHg in 24-h
- Maximum hypertensive peak produced by Stroop test (cognitive arousal) or hear rate during congnitive stimulation while performing serial sevens testing
- Short-term beat-to-beat spontaneous SD of BP or heart rate (5-minutes) breathing spontaneous
- Morning surge in systolic BP on awakening from sleep (24-h)

3.4 Primary Safety Endpoints

Safety measures

- Adverse events
- Laboratory values
  - CBC
  - Metabolic panel
- Urinalysis
- Vital signs
- Body temperature
- Weight
- Blood pressure (sitting and standing)
- ECG (at screening and termination visit)

Tolerability measures

- Number of subjects (%) who discontinue the study
- Number of subjects (%) who discontinue the study due to AEs.
- Severity of hypotension during an active stand test
- Lowest systolic BP (24-h)
- Worsening in OH-Q scores
- Frequency of symptoms noted in their diary

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

- Male or female patients with FD age 10 or older
- Unstable blood pressure:
  - Systolic BP standard deviation >15 mmHg
  - Or coefficient of variation >15%
  - Or documented episodic hypertensive peaks ($\geq$140mmHg)
- Confirmed diagnosis of FD (Historical confirmation of subjects' genetic diagnosis will be obtained for this study)
- Providing written informed consent (or ascent) to participate in the trial
- Ability to comply with the requirements of the study procedures.

4.2 Exclusion Criteria

- Patients taking MAO-inhibitors
- Patients taking metoclopramide, domperidone, risperidone or other dopamine blockers
- Patients taking tricyclic antidepressants
- Patients taking neuroleptic drugs (haloperidol and chlorpromazine)

- Patients with a known hypersensitivity to any component of this drug.
- Patients with atrial fibrillation, angina or significant ECG abnormality
- Patients with significant pulmonary, cardiac, liver, renal (creatinine >2.0 mg/ml)
- Patients who have a significant abnormality on clinical examination that may, in the investigator's opinion might jeopardize their healthy participating in this trial.
- Women who are pregnant or lactating.

4.3 Subject Recruitment and Screening

Since most patient with FD take medications by opening capsules or crushing pills and flushing them through their gastrostomy, it is necessary to use carbidopa powder (rather then re-encapsulate the commercially available pills) to maintain treatment allocation concealment. Subjects randomized to receive placebo will receive an identical small capsule containing a matching powder, as we've previously done. Thirty subjects will be randomized. Randomization will be performed by the Investigational Pharmacy of NYU, and participants, caregivers and assessors will be blinded at all times from the randomization sequence. Randomization sequences will be generated using computer-based system using a permuted block design and the Investigational Pharmacy of NYU will dispense all medications and provide dosing instructions for titration down and washout between treatment arms (Appendix). Investigators will not be involved in specifying the actual doses patients take and will remain blind to treatment allocation throughout. The PI will have the capacity to break the blind and remove any patient from the study at anytime for safety concerns. To monitor compliance, patients will be instructed to return all unused study medications at study visits.

4.3.1 Assessment of mental capacity to consent.

There are few formal assessments of cognitive function in patients with FD. Published studies likely underestimate the frequency of cognitive impairment owing to the bias of only "higher functioning" patients being able to complete the cognitive assessment tests. We estimate that around 40% of patients have impaired cognitive function. Given this we anticipate difficultly completing recruitment goals without enrolling patients with diminished capacity, as this is a rare genetic disease. We have included the following measures to ensure adequate assessment of cognitive impairment and capacity are in place.

In order to provide a consistent method to assess subject capacity at screening, we will use a modified version of the Montreal Cognitive Assessment (MoCA), that has been specifically adapted for patients with FD. This a cognitive screening test is validated to assist health care professionals in evaluating cognitive impairment. It is a widely used and well accepted screening tool. It is a 30-point test which can be administered in less than 5 minutes. It evaluates short-term memory recall, visuospatial abilities, multiple aspects of executive functions, attention, concentration, working memory, language, and orientation to time and place. Because all patients with familial dysautonomia have some degree of impaired vision and proprioceptive dysfunction, the MoCA test has been adapted for this population to omit the items that depend on vision and coordination as not to underestimate their cognitive ability. Therefore, the Modified MoCA version that we will be using will rate cognitive deficits in a 25-point scale. Scores of 13 or less will be considered as moderate or severe cognitive impairment. Subjects with a score of 13 or less will be assumed not to have capacity and consent from a guardian will be required. If the patient has legal guardianship, a copy will be obtained and filed in the study chart. If legal guardianship has not been officially sought, a note to file will be made to document the consent process in the study chart to document a process of confirming of a Legally Authorized Representative.

Rational for inclusion of subject with diminished mental capacity to conent. This is the population that have a genetic lesion that impairs their blood pressure control and lead to early onset renal failure. Preliminary results show carbidopa has a beneficial effect on stabilizing the blood pressure and reducing this variability.

The MoCA will be administered by a licensed clinical investigator (MD or NP) and will be interpreted by a neurologist who is familiar with the specific needs of patients with FD (Dr. Horacio Kaufmann or Dr. Jose Alberto Palma).

While it is not expected that subjects will lose the capacity to consent, should there be a change to the subject's mental status that the PI believes warrants reassessment of capacity, the modified MoCa test will be repeated.

4.3.2 Vulnerable Populations

Both children as well as adults without the capacity to consent for themselves will be enrolled in this study.

For Children:

a. *parental permission will be obtained from one parent*
b. *Assent from the child will be sought, if possible*

For cognitively impaired adults:

a. *Consent from the subject's legal authorized representative will be obtaind*
b. *Assent from the subject will be sought, if possible*

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

We have close connection to all FD families and do not anticipate that any patient will be lost to follow-up. To minimize the possibility of early withdrawals, we will make all patients fully aware of the study commitments prior to enrolling. We will make efforts to determine the reason for dropouts from the trial. In previous trials, patients with FD are occasionally unwilling to be randomized to placebo if they had experienced symptomatic improvement during an open label phase. In this trial, however, we do not anticipate dropouts due to unwillingness to be randomized to placebo, as the efficacy end-point is not primarily a symptom. Therefore, we do not anticipate problems retaining patients on the trial.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Every attempt will be made to collect as much follow-up data as possible on subject which withdraw from the study.

5 Study Drug

5.1 Description

Carbidopa (DL-α-methyl-α-hydrazino-3, 4-dihydroxyphenyl-propionic acid, HMD, MK-486, Lodosyn ®) is a reversible competitive inhibitor of DOPA-decarboxylase that prevents dopamine formation outside the brain.[33] It is structurally similar to DOPA except for one extra methyl (CH_3) and a hydrazine (N_2H_4) rather than an amino group.[34] By forming a series of strong but ultimately reversible bonds with the enzyme's co-factor pyridoxine, carbidopa prevents the binding of DOPA and inhibits dopamine production.[34] It has been used for many years to block the peripheral synthesis of dopamine in patients with Parkinson disease treated with L-DOPA.[35]

5.2 Treatment Regimen

Dose will be 100mg / 200 mg / placebo, oral administration, with a treatment duration of 1 month on each dose.

5.3 Method for Assigning Subjects to Treatment Groups

Double-blind, Randomized, cross-over design.

5.4 Preparation and Administration of Study Drug

The study drug will be stored and dispensed by the NYU investigational pharmacy. The NYU Investogational Pharmacy will maintain the subject randomization codes.

5.5 Subject Compliance Monitoring

At each study visit the study coordinator and reseach nurse will review compliance with each subject.

5.6 Prior and Concomitant Therapy

All concomitant medications will be collected

Concomitant medicines/therapies not permitted during the study are:

- MAO-inhibitors
- metoclopramide, domperidone, risperidone or other dopamine blockers
- tricyclic antidepressants
- neuroleptic drugs (haloperidol and chlorpromazine)

5.7 Packaging

Study drug will be packaged in 100 mg/ 200mg/ and matching placebo capsules and dispensed in a 30 day supply.

5.8 Blinding of Study Drug

Study is randomized Double-blind, the NYU Investigational Pharmacy will maintain the randomization codes for each subject.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

TownTotal Compounding Pharmacy will ship each subject's drug supply directly to the NYU Investogational pharmacy for storage and dispensing. Any unused medication will be returned to the Investigational pharmacy.

5.9.2 Storage

Storage of study drug will be maintained by the NYU-Investigational pharmacy, Study drug can be stored at room temperature. The investigational Phamacy has 24/7 temperature logs.

5.9.3 Dispensing of Study Drug

The NYU Investigational Pharmacy will dispense study drug. Study team personnel will check drug reconcillation at each study visit.

5.9.4 Return or Destruction of Study Drug

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

6 Study Procedures

Table 1 provides a complete list of the procedures at each study visit (in the office or via telemedicine 2-way conferencing) and figure 7 provides a timeline for the procedures at visits.

Table 1: Study procedures

	Visit 1	**Visit 2**	**Visit 3**	**Visit 4**
Procedure	Baseline/R	Crossover 1	Crossover 2	Final
Consent/ascent	☑			
Urine pregnancy test (reproductive females)	☑			☑
Inclusion/exclusion criteria	☑			
Concomitant medications/compliance check	☑	☑	☑	☑
Vital signs (temperature, weight)	☑	☑	☑	☑
Metabolic panel	☑	☑	☑	☑
Urinalysis	☑	☑	☑	☑
CBC	☑	☑	☑	☑
12-lead ECG*	☑	☑	☑	☑
Physical/neurological examination	☑	☑	☑	☑
Adverse event questioning	☑	☑	☑	☑
OH-Questionnaire	☑	☑	☑	☑
24-h ambulatory BP recording	☑	☑	☑	☑
24-h urine catecholamine excretion	☑	☑	☑	☑
Supine/seated/standing BP	☑	☑	☑	☑
Beat-to-beat BP or HR at rest	☑	☑	☑	☑
Beat-to-beat BP during Stroop (Office)	☑	☑	☑	☑
HR with serial 7s subtractions (Virtual Visit)	☑	☑	☑	☑
Symptom/AE diary collection		☑	☑	☑
Dose de-escalation		☑	☑	☑
Washout		☑	☑	
Dose adjustment day safety phone call		☑	☑	
Day 7 adverse event monitoring phone call	☑	☑	☑	☑

CBC; complete blood count, ECG; electrocardiogram, BP; blood pressure, AE, adverse event; R; randomization.

Visit 1. Baseline/Randomization:

Patients will be screened for eligibility and enrolled in the trial. Informed consent or ascent will be obtained. Patients will be given a tutorial and provided with written instructions on using the ambulatory blood pressure monitors and 24-h recordings will be obtained. Supine/sitting/standing office blood pressures will be measured. RR-interval and beat-to-beat blood pressure will be recorded for 5 minutes before and during a Stroop test (3-minutes) and during serial 7 subtractions (3 minutes). Patients will be asked to rate their symptoms of orthostatic hypotension on the validated orthostatic hypotension-questionnaire (OH-Q)[42]. Baseline safety bloods (CBC & metabolic panel), a 12-lead ECG and a physical and neurological exam will be obtained. After completing all baseline measures, patients will be randomized to the treatment order sequence (Fig. 6) in a 1:1:1 ratio. For patients that opt to continue follow-up via 2-way telemedicine conferencing, investigators will ensure that the patient has access to a validated blood pressure and heart rate monitor, weighing scale and thermometer at home. These will be provided, if needed. Patients will be given a continuous, automatic, wireless wrist-based heart rate tracker (Fitbit Charge HR, Fitbit, USA), which will be synchronized to allow remote monitoring at the Center. Patients and caregivers will be trained in how to perform a timed active stand test and to obtain blood pressure and heart rate supine (5-min), seated (2-min) and standing (5 min). Written instructions and a video guide will be provided.

Before leaving the office, patients will be instructed to keep a daily symptom diary (a modified version of the Rhodes Nausea/Retching/Vomiting Index[39] with additional space to note adverse events). Additionally, patients will be told to contact the Center to report any adverse event. They will be contacted by phone call on the day that they adjust their dose. Seven-days into their treatment arm patients will be contacted again to monitor tolerability and adverse events. Thereafter, we will contact patients on a weekly basis to monitor potential adverse events. They will return for the next study visit in 28-days.

Patients that have been seen at the Center within the last 3-months may have the option of completing the study screening and enrollment proceedures via virtual visits, at the discretion of the PI (a treating investigator).

Visit 2. Crossover #1:

For in office visits, patients will be instructed to take their study pill 3-h before attending the office visit. The timing of the medication and testing will be noted in the case report forms. Patients will bring with them to the office visit a 24-hour urine collection for urine catecholamine assay. They will be instructed to arrive wearing the ambulatory BP monitor. Upon arrival at the clinic, vital signs will be taken and blood pressure will be measured supine, seated and standing. Ambulatory monitor blood pressure values will be calibrated against simultaneous measurements of blood pressure in the opposite arm using an automated sphygmomanometer during the orthostatic challenge. Ambulatory recordings will be downloaded and the data reviewed for quality. If recordings are found to be inadequate, they will be repeated with the patient remaining on the same dose of medication for 1 additional day before titrating down and washout. Patients will be instructed to return their unused medications to monitor compliance Beat-to-beat BP and RR interval changes before/during stoop and serial 7 subtractions will be repeated. Patients will have blood drawn and urine collected for safety measures and a 12-lead ECG will be performed.. Neurological and physical exam will be performed. Patients will be questioned about adverse events and complete the OH-Q. Before leaving the office, all medications will be dispensed and dosing instructions will be provided (Appendix).

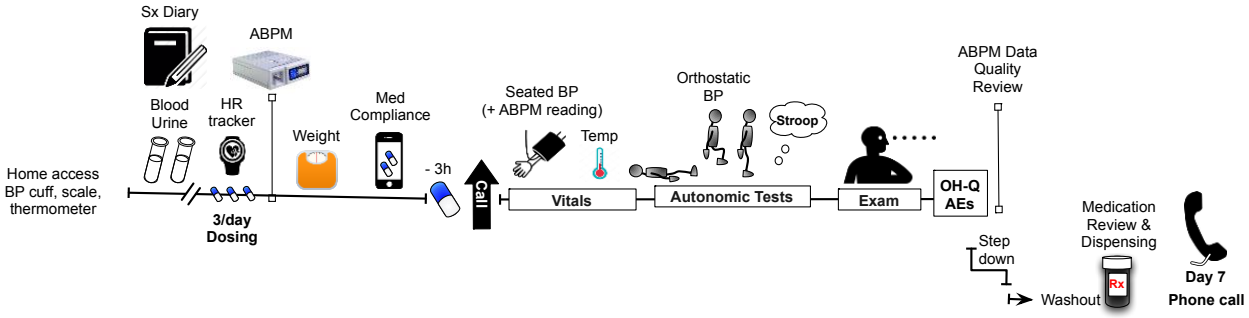


Fig 8: Details of the telemedine visits: *Study visits may be performed virtually, through 2-way audio visual conferencing. See text for details.*

As shown in Figure 8, for telemedicine visits, prior to the visit, investigators will ensure patients have access to 2-way video conferencing with audio and that continuous HR measurements can be acquired from the wireless wrist tracker. Symptoms diaries will be collected. Patients will be instructed to continue their three times daily dosage of carbidopa. Safety labs, ambulatory blood pressure monitoring and 24-h urine collections will be performed while at the steady state.

Before the call, patients will be instructed to (1) weigh themselves in the morning, (2) to take their study medication 3-h before the scheduled time of the teleconference, (3) email a photograph of their unused pills for medication compliance check, (4) be wearing their ambulatory monitor at the time of the video call, and (5) have their wrist-based heart rate tracker on.

When video conferencing begins, to monitor vital signs, patients will be instructed to take a blood pressure and heart rate reading (while seated), obtain their temperature, and display the results on screen. On the opposite arm, a simultaneous manual measurement on the ambulatory monitor will be performed, and both measurements noted in the case report forms for calibration of the ambulatory readings. Patients will be guided through a timed active stand test and acquire blood pressure and heart rate measurements supine, sitting and standing. After recovering, continuous HR will be obtained from the wireless wrist tracker for 5 minutes at rest while seated and over a 3-minute period while the patient is performing serial 7 subtractions, as described.[5]

The following is a summary of how a physical and neurological evaluation will be performed during a telemedicine visit:

All aspects of the physical and the currently performed neurologic exam can be performed via telemedicine except auscultation (which can be assessed in other ways). The following is an outline of how this will be performed with video monitoring:
- Vital signs including height, weight, temperature (the patient is asked to have a home thermometer available), and blood pressure and heart rate via the 24 hour ambulatory monitor.
- General appearance: The general appearance can be evaluated with video monitoring and conversing and watching the patient throughout the telemedicine visit.
- HEENT: The patient can perform ocular movements, the sclera can be evaluated, and the clinician can evaluate the eyes and throat via the video screen and a flashlight can be utilized when needed for additional visualization.
- CV: Heart rate and blood pressure can be evaluated as previously mentioned.
- Pulm: We can have the patient cough and deep breath to evaluate for presence of new cough, chest congestion, and wheezing. FD patients are familiar with performing these types of evaluations by phone as they are often calling from around the world with questions or concerns about their respiratory status.
- Abdomen: The abdomen can be evaluated visually including the integrity of the gastrostomy tube and looking for distention.
- GU: Information can be obtained via the review of systems and the safety urinalysis with microscopy.
- Lymphatics: Usually only evaluated if there is a problem or concern.
- Musculoskeletal: Upper extremity and lower extremity musculature can be visually evaluated, along with change in weight.
Skin: The skin be evaluated visually during the televisit.
- Neuro: Cognition, gait, facial expression, coordination, and evaluation for tremor can all easily be evaluated during a televisit.
- A full review of systems can be performed during the televisit and if there are any questions or concerns related to an organ system then subsequent follow up can occur either at the NYU Dysautonomia Center or by visiting a local physician who we can communicate with after his/her evaluation.
This protocol will be repeated for each telemedicine visit.

Before cross over onto the next allocated treatment and beginning their new batch of medications, patients will be titrated down (reducing the dose to 2/3 and then 1/3) and washed out for 2-days. Seven-days after being on their treatment, they will be contacted again. Thereafter, we will contact patients on a weekly basis to monitor potential adverse events. Patients will undergo another visit (virtual or in-office) after 28-days of treatment bringing with them a 24-hour ambulatory recording, a 24-h urine collection. They will be instructed to continue documenting their symptom in the custom diary.

Visit/Televisit 3. Crossover #2:

At this visit, supine/seated/standing BP and beat-to-beat changes during stoop will be measured 3-h post dose or heart rate during serial sevens testing. Ambulatory recordings will be calibrated against office blood pressure readings for in-office visits and will be downloaded and reviewed for data quality. Safety bloods and 12-lead ECG will be obtained and physical examination will be conducted. Patients will be questioned about adverse events and score the OH-Q. They will be crossed over to receive their final treatment allocation, depending on their randomization sequence. Dosing instructions will be provided to titrate down for 2-days and washout for 2-days. Patients will be asked to maintain their symptom diary and return for follow-up in 4-weeks with a 24-hour urine collection and ambulatory BP recording and the symptom diary. As described for visit 2, patients will be contacted on the day that they begin their new treatment and seven days after being on their treatment. Thereafter, we will contact patients on a weekly basis to monitor potential adverse events. Patients will return for follow-up after 28-days of treatment bringing with them a 24-hour ambulatory recording and a 24-h urine collection. These visits may be performed virtually, with the standard operating proceedures outlined in Figure 8. They will be instructed to continue documenting their symptom in the custom diary.

Visit/Televisit 4. Final:

At the final study visit or televisit (see Fig 8 for details), 3h post-dose we will conduct the orthostatic blood pressure measurements supine/seated/standing while obtaining concomitant measurements of blood pressure of the ambulatory monitor or using the ambulatory monitor for televisit patients. Vital signs and Stroop-induced changes in BP will be assessed in office or heart rate monitoring via the iPhone application while performing

serial sevens will be done during the televisit. We will obtain safety bloods, urinalysis, a 12-lead ECG and all patients will undergo a physical and neurological evaluation. Patients will fill the OH-questionnaire and be questioned about adverse events. Ambulatory recordings will be downloaded to check for data quality and 24-h urine collections will be assayed for catecholamines. Diaries and unused medications will be collected or returned to the Center. Dose de-escalation instructions will be provided for the end of the study.

Follow-up phone call:

One week after completion of the cross over periods, patients will receive another follow-up phone call to monitor any adverse events.

24-hour Ambulatory Blood Pressure Monitoring:

Patients with FD frequently undergo ambulatory BP monitoring while keeping a detailed log of their activities (sleep/meal-times/medications/posture/symptoms). Compliance is excellent and they tolerate the procedure well. The majority of patients have home nursing support that will aid with fitting the ambulatory monitors at home, and we will also provide a tutorial as well as written instructions on how to fit the monitor (Appendix), including instrumentation on the non-dominant arm and the distance (in cm) from the elbow. We will use video calls to aid fitting the ambulatory monitor when necessary.

To ensure consistency between measurements, cuff size will be recorded in the case report forms and the same cuff size will be used throughout. We will standardize the times of the recordings so that the data collection begins and ends at the same time (i.e., is over the same 24-h period: e.g., 9 AM to 9 AM) on each treatment period. Monitors will be calibrated prior to use following the manufacturers guidelines (SpaceLabs, Service Manual). For this study, patients will be fitted with a validated ambulatory blood pressure monitor (90207 monitor, SpaceLabs, Washington, USA) and instructed to go about their daily activities. For in office visits the patients will be instructed to arrive for the appointment still wearing the ambulatory monitor and simultaneous measurements of blood pressure will be obtained with an automated sphygmomanometer during the orthostatic challenge test to recheck the calibration (Colin Pilot, Texas, USA). For televisits the patients will be instructed to keep the ambulatory blood pressure monitor on during the televisit so that blood pressures and heart rates may be obtained under video supervision by the clinician. Recordings will be downloaded at study visits and repeated if the success rate is not adequate (<80%) or office and ambulatory readings differ by more than 20%. Due to afferent baroreceptor dysfunction, the episodes of paroxysmal hypertension in FD occur predictably in response to emotional or cognitive stimuli. Thus, it is the blood pressure instability during the day (when the patient is awake) that is the major contributor to the increased overall variability.[25] Therefore, our primary efficacy endpoint will focus on the change in blood pressure variability during waking hours (determined from the patient's activity log). Since nocturnal BP fall contributes significantly to 24-h variability, and a non-dipping pattern is associated with end-organ target damage,[43] we will look at changes in blood pressure variability during the entire 24-h period independently. We will also evaluate the weighted variability, to look at the influences episodes of paroxysmal hypertension during the nighttime sub-period. Variability in systolic, diastolic and mean blood pressures will be examined independently.

The following variables will be evaluated and each may have different primary determinants.[25]
Average blood pressures: The theoretical underlying level of blood pressure calculated by averaging several blood pressure values collected over the defined time periods (entire recording, sleep hours, wake hours).
Standard Deviation: The variability in blood pressure overtime determined by the standard deviation (entire recording sleep hours, wake hours).
Coefficient of Variation: The measurement of blood pressure variability based on the standard deviation that also takes into account the underlying level of BP. Calculated as follows: CV = [SD/mean BP] x 100.
Nighttime Dipping: Analysis of the circadian pattern based on the difference in average daytime and nighttime values and expressed as a percentage. With normal night-time "dipping" defined as a 10% fall in BP during sleeping hours.[43]
Weighted Standard Deviation: Using a "weighted" measurement of standard deviation of 24-h ambulatory BP that avoids the contribution of nocturnal dipping to the overall variability, but still includes the BP variability during the night has been suggested as an accurate measurement of cardiovascular risk.[23] Calculated as follows: wSD = [daytime SD x14 + nighttime SD x6]/20).
Morning Surge in Blood Pressure: The increase in blood pressure that occurs on awakening from sleep and is exaggerated in patients with FD. The morning surge will be calculated as the difference between the mean

systolic blood pressure during the hour that included the lowest blood pressure during sleep and maximum value detected within 2-h of awakening from sleep (as previously described[10])

Abbreviations: BP, blood pressure; SD, standard deviation; CV, coefficient of variation. wSD, weighted standard deviation.

Questionnaires:

Patients will complete 2-questionnaires to assess tolerability.

OH-Q: The orthostatic hypotension questionnaire (abbreviated to OH-Q) was recently validated and consists of 10-items graded on an 11-point scale (0 to 10).[42] Six items assess symptoms of neurogenic OH and 4 address the impact they have on activities of daily living (Appendix 1).

Symptom Diary: As we did in our previous trial,[13] we will use a tailored questionnaire to examine symptoms over the treatment period and the used of as needed medications. Each day will have a designated page. Since nausea/vomiting and hypertension occur together in FD we will use a diary consisting of a simplified version of the Rhodes Index[44] symptoms of nausea/retching, with items addressing vomiting/throwing up omitted, as most participants will have had anti-reflux surgery to prevent vomiting (fundoplication), graded on a 5-point scale (appendix 2). The diary will also include space to write down any adverse events on a daily basis.

Catecholamines:

Prior to arriving for a study visit or performing a televisit, patients will be asked to collect a 24-hour urine sample in a bottle shielded from light containing preservative. Patients will be instructed to refrigerate their sample and bring it on the morning of their visit in a cool bag or provided with a prescription to take it to a local laboratory. Catecholamines will be measured using high-pressure liquid chromatography as previously described.[45] Because urine catecholamine collections reflect periods of sympathetic nerve activation over an entire day, in this particular circumstance urinary excretion will more clinically useful information, than a plasma "snap-shot" obtained from a blood draw at the office, which has the emotional component of a having and IV inserted in the arm (which despite not feeling pain, is not well tolerated by these patients).

Autonomic Testing:

The effect of carbidopa on blood pressure will also be investigated 3-h post dose. All procedures will be carried in a temperature-controlled environment by investigators trained in performing autonomic function tests and experience in working with FD patients. Blood pressure will be measured using finger plethysmography (Finometer/PortaPres) and the arm supported at heart-level on a custom made rest for the in-office patients. RR intervals will be recorded from chest wall electrodes. All data will be synchronized, digitized and acquired using the PowerLab System (AD Instruments). Recordings will be made for 5-minutes while the patient is relaxed, calm and breathing spontaneously (pacing the breathing it impossible for most patients with FD to achieve). Baseline variability will be assessed using descriptive statistics (SD, CV see above for calculations). Blood pressure responses to cognitive stress will be evaluated during the Stroop Word Color Test Application administered on an iPad (First Generation, Apple Inc.) over 3 minutes as described.[46] The maximum increase in blood pressure while concentrating on the Stroop will be used to define cognitive-stress induced hypertension. We have successfully obtained autonomic data using these techniques before.[46] For the patients performing televisits the heart rate and unrestrained sympathetic activity will be measured using a smart phone application under visual supervision by the clinician.

7 Statistical Plan

7.1 Sample Size Determination

See section 7.2

7.2 Statistical Methods

We have a dedicated statistician for this clinical trial (M. Liu, PhD). Our inclusion criteria use a baseline SD of systolic blood pressure variability ≥15 mmHg (2 SD above our age-matched control data [8.7±3 mmHg]). The mean coefficient of variation in 24-h BP readings in patients with FD is 19.1±5 and 99% of FD patients meet the inclusion criteria. Preliminary data shows that treatment with high dose carbidopa (600 mg/day) reduces blood pressure variability (SD of daytime SBP) by 7.0±5.5 mmHg. Power analysis for the primary outcome (reduction in SD of daytime SBP variability) is detailed in table 3. A sample size of 20 achieves 80% power to detect a reduction of 2.5 (or 2.9) from the null hypothesis of no reduction with an estimated standard deviation of 4.4 for the difference in SD at a significance level of 0.05 using a one-sided (or two-sided) one-sample t-test. The minimum detectable differences are all well below the observed reduction of BP fluctuation of 5.1 from our preliminary data. To ensure that the study is well powered, we will choose a sample size of 30 patients. To account for a drop out rate of around 15%, we will enroll 36 patients in the trial. We will examine the sample distribution of SD of daytime SBP, and if it deviates from the normal distribution, we will employ the nonparametric Wilcoxon test for robustness. In addition, to account for potential covariates, such as age, gender, and disease severity, the analysis of (co)variance (ANOVA and ANCOVA) methods will be used to model the repeated measures data.

Table 3: Power Calculation and Sample Size

Minimal detectable difference: SD BP variability high vs. low dose		
Sample size	T-test	T-test
	One-sided	Two-sided
20	-2.5	-2.9
30	-2.0	-2.3
40	-1.8	-2.0

7.3 Subject Population(s) for Analysis

- *All-treated population: Any subject randomized into the study that received at least one dose of study drug*
- *Protocol-compliant population: Any subject who was randomized and received the protocol required study drug exposure and required protocol processing*

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. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

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. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality 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 and adverse event.

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

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required 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 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.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

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

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 8.1).

For Narrative Reports of Safety Events
If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the study sponsor

The following describes events that must be reported to the study sponsor in an expedited fashion.

Initial Report: within 24 hours:
The following events must be reported to the study sponsor by telephone within 24 hours of awareness of the event:

- Unanticipated problems related to study participation,
- Serious adverse events, regardless of whether they are unexpected.

Additionally, an FDA Form 3500A must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator shall maintain a copy of the MEDWATCH Form on file at the study site.

Follow-up report: within 48 hours:
As a follow-up to the initial report, within the following 48 hours of awareness of the event, the investigator shall provide further information, as applicable, on the unanticipated device event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Unanticipated Problem form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing unanticipated adverse device effects shall be provided promptly to the study sponsor.

Other Reportable events:

- **Deviations from the study protocol**
  Deviations from the protocol must receive both Sponsor and the investigator's IRB approval before they are initiated. Any protocol deviations initiated without Sponsor and the investigator's IRB approval that may affect the scientific soundness of the study, or affect the rights, safety, or welfare of study subjects, must be reported to the Sponsor and to the investigator's IRB as soon as a possible, but ***no later than 5 working days*** of the protocol deviation.

- ***Withdrawal of IRB approval***
  An investigator shall report to the sponsor a withdrawal of approval by the investigator's reviewing IRB as soon as a possible, but ***no later than 5 working days*** of the IRB notification of withdrawal of approval.

8.3.2 Investigator reporting: notifying the IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULMC IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

Report Promptly, but no later than 5 working days:
Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- ***Unanticipated problems including adverse events*** **that are unexpected and related**
  - *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
  - *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
  - *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:
The following events also require prompt reporting to the IRB, though ***no later than 5 working days***:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*
- ***Breach of confidentiality***
- ***Incarceration of a participant*** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- ***New Information indicating a change to the risks or potential benefits*** of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Reporting Process
The reportable events noted above will be reported to the IRB using the form: "Reportable Event Form" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

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

8.3.3 Sponsor reporting: *Notifying the FDA*

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

- ***Within 7 calendar days*** *(via telephone or facsimile report)*
  Any study event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening

- ***Within 15 calendar days*** *(via written report)*

Any study event that is:
- associated with the use of the study drug,
- unexpected, and
- serious, but not fatal or life-threatening

-or-

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

Any finding from tests in laboratory animals that:
- suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

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

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

8.3.4 Sponsor reporting: Notifying participating investigators

It is the responsibility of the study sponsor to notify all participating investigators of any adverse event that meets the FDA 15-day reporting requirement criteria as note above in section 8.3.4. The same materials and timeline used to report to the FDA are used for notifying participating investigators.

8.4 Unblinding Procedures

Unblinging will be at the PIs discretion on a case by case basis.

8.5 Stopping Rules

There are no specific stopping rules.
The principal investigator (H Kaufmann, MD) together with the treating investigators will be responsible for ensuring both data integrity and that patients who participate in the study will be properly cared for, and that all adverse events are noted, followed and reported to the IRB (when appropriate). In addition, a DSMB Chaired by Dr. Michael Brownstein will oversee the protocols outlined in this proposal. Any adverse event rated moderate or severe will be immediately reported to the IRB and the DSMB. All adverse events will be reported to the IRB on an annual basis. All necessary information will be reported to the FDA in accordance with the guidelines outlined for a treatment IND.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.6.1 Data Monitoring Committee

Safety data will be collected at each study visit (12 lead ECG, urinalysis, CBC, metabolic panel, vitals, neurological & physical examinations). In addition, to further monitor safety between study visits, we will contact

by phone patients on the day of and again 7-days after adjusting doses to question them about potential adverse events. We will continue to call patients on a weekly basis during the outpatient phase to monitor adverse events (table 2). All data will be collected on standardized case report forms. Data will be entered into a study database created on the REDCap platform and hosted by NYU's Clinical and Translational Science Institute (CTSI). All data will be de-identified, encrypted and stored behind a secure firewall accessible only to designated investigators. To check for potential data entry errors, data will be graphed and examined for outlying values prior to analysis.

A data and safety monitoring board (DSMB, Chaired by Michael Brownstein, MD) will review safety data on an ongoing basis. The study will continue until the target number of patients is enrolled or the DSMB recommends stopping the trial. To allow for a **flexible trial design,** there will be an **interim efficacy analysis** with access to the un-blinded after the first 15 patients have completed the trial. If carbidopa is found to significantly reduce blood pressure variability and the 300 mg/day (low-dose) is found to be equivalently effective as the 600mg/day (high-dose), we will decide, with the DSMB, whether to amend the protocol to add an additional treatment arm to test doses lower than 300 mg/day. Alternatively, if there is insufficient evidence to suggest that 600 mg/day of carbidopa reduces blood pressure variability, before terminating the trial for futility, we will drop the ineffective arms and collect exploratory data to explore the effectiveness of higher carbidopa doses up to 900 mg/day (for 28 days). We will modify the trial to include a titration from upwards of 600 mg/day in increments of 50 mg/day increases per week. To monitor safety closely, we will collect additional safety bloods (CBC and metabolic panel), 12 lead ECG and conduct comprehensive neurological examinations with each incremental titration step up and patients will be contacted by interim phone-calls to monitor adverse events. Only patients that live within 2-hours of the Dysautonomia Center will be included in the subsection to explore the efficacy of higher carbidopa dosages. We will conduct new power analyzes with the accumulated data and revise the sample size accordingly. Final analysis will be performed after the final study patient has completed the trial and the study is closed for enrollment. All end-points will be first examined using descriptive statistics. Each patient will serve as their own control allowing paired comparisons to be made between high-dose, low-dose and placebo data.

9 Data Handling and Record Keeping

9.1 Confidentiality

Study subjects will be given a unique ID code, data will be maintained on password protected computers and subject charts will be stored in locked offices in locked cabinets.

9.2 Confidentiality and HIPAA

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.

9.3 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists,

pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.4 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.5 Records Retention

Study documents will be maintained indifiently.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB) or independent Ethics Committee (EC) in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB/EC 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 IRB/EC 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. This consent form will be submitted with the protocol for review and approval by the IRB/EC for the study. The formal

consent of a subject, using the IRB/EC-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.

In addition, describe who will obtain consent and how the process of informed consent will be structured to be conducive to rational and thoughtful decision making by the subject/subject's legally authorized representative. If children and/ or cognitively impaired adults will be subjects, include a specific plan to assess comprehension during assent or the subject's agreement Individuals who are authorized to obtain consent must be listed on the protocol (or FDA form 1572) and consent form document. If necessary to use 'Auditor/Witness' and/or translator, these roles would be described in this section.
Include a plan for assessing subject capacity in cognitively impaired subjects. Describe the anticipated degree of impairment relative to their ability to consent and the anticipated direct benefits to the subjects.

12 Study Finances

12.1 Funding Source

This study is being funded by a grant from the Food and Drug Administration.

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable University conflict of interest policies.

12.3 Subject Stipends or Payments

There is no subject stipend/payment for participation in the study. Subject's may be reimbursed for travel cost to our center on a case by case basis.

13 Publication Plan

The PI holds the primary responsibility for publication of the results of the study.

14 References

1 Riley, C. M., Day, R. A., Greeley, D. M. & Landford, W. S. Central autonomic dysfunction with defective lacrimation: I. Report of five cases. *Pediatrics* **3**, 468-478 (1949).
2 Slaugenhaupt, S. A. *et al.* Tissue-specific expression of a splicing mutation in the IKBKAP gene causes familial dysautonomia. *Am J Hum Genet* **68**, 598-605, doi:S0002-9297(07)63100-0 [pii] (2001).
3 Anderson, S. L. *et al.* Familial dysautonomia is caused by mutations of the IKAP gene. *Am J Hum Genet* **68**, 753-758, doi:S0002-9297(07)63113-9 [pii] 10.1086/318808 (2001).
4 Pearson, J., Pytel, B. A., Grover-Johnson, N., Axelrod, F. & Dancis, J. Quantitative studies of dorsal root ganglia and neuropathologic observations on spinal cords in familial dysautonomia. *J Neurol Sci* **35**, 77-92 (1978).

5 Norcliffe-Kaufmann, L., Axelrod, F. & Kaufmann, H. Afferent baroreflex failure in familial dysautonomia. *Neurology* **75**, 1904-1911, doi:10.1212/WNL.0b013e3181feb283 (2010).
6 Pearson, J. & Pytel, B. A. Quantitative studies of sympathetic ganglia and spinal cord intermediolateral gray columns in familial dysautonomia. *J Neurol Sci* **39**, 47-59 (1978).
7 Norcliffe-Kaufmann, L. J., Axelrod, F. B. & Kaufmann, H. Cyclic Vomiting Associated With Excessive Dopamine in Riley-day Syndrome. *J Clin Gastroenterol*, doi:10.1097/MCG.0b013e3182582cbf (2012).
8 Pearson, J., Gallo, G., Gluck, M. & Axelrod, F. Renal disease in familial dysautonomia. *Kidney Int* **17**, 102-112 (1980).
9 Rekhtman, Y. *et al.* Renal Transplantation in Familial Dysautonomia: Report of Two Cases and Review of the Literature. *Clin J Am Soc Nephrol*, doi:CJN.01750210 [pii] 10.2215/CJN.01750210 (2010).
10 Norcliffe-Kaufmann, L., Axelrod, F. B. & Kaufmann, H. Developmental abnormalities, blood pressure variability and renal disease in Riley Day syndrome. *J Hum Hypertens* **27**, 51-55, doi:10.1038/jhh.2011.107 (2011).
11 Axelrod, F. B. Familial dysautonomia: a review of the current pharmacological treatments. *Expert Opin Pharmacother* **6**, 561-567, doi:10.1517/14656566.6.4.561 (2005).
12 Porter, C. C., Watson, L. S., Titus, D. C., Totaro, J. A. & Byer, S. S. Inhibition of DOPA decarboxylase by the hydrazino analog of alpha-methylDOPA. *Biochem Pharmacol* **11**, 1067-1077 (1962).
13 Norcliffe-Kaufmann, L., Martinez, J., Axelrod, F. & Kaufmann, H. Hyperdopaminergic crises in familial dysautonomia: A Trial of Carbidopa
. *Neurology* **In press.** (2013).
14 Macefield, V. G., Norcliffe-Kaufmann, L., Gutierrez, J., Axelrod, F. B. & Kaufmann, H. Can loss of muscle spindle afferents explain the ataxic gait in Riley-Day syndrome? *Brain* **134**, 3198-3208, doi:10.1093/brain/awr168 (2011).
15 Mezey, E. *et al.* Of splice and men: what does the distribution of IKAP mRNA in the rat tell us about the pathogenesis of familial dysautonomia? *Brain Res* **983**, 209-214, doi:S0006899303030907 [pii] (2003).
16 Lee, G. *et al.* Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature* **461**, 402-406, doi:nature08320 [pii] 10.1038/nature08320 (2009).
17 Hilz, M. J. *et al.* Assessing function and pathology in familial dysautonomia: assessment of temperature perception, sweating and cutaneous innervation. *Brain* **127**, 2090-2098, doi:10.1093/brain/awh235
awh235 [pii] (2004).
18 Filler, J., Smith, A. A., Stone, S. & Dancis, J. Respiratory Control in Familial Dysautonomia. *J Pediatr* **66**, 509-516 (1965).
19 Pearson, J., Brandeis, L. & Goldstein, M. Tyrosine hydroxylase immunoreactivity in familial dysautonomia. *Science* **206**, 71-72 (1979).
20 Nussinovitch, U., Katz, U., Nussinovitch, M., Blieden, L. & Nussinovitch, N. Echocardiographic abnormalities in familial dysautonomia. *Pediatr Cardiol* **30**, 1068-1074, doi:10.1007/s00246-009-9497-z (2009).
21 Elkayam, L., Matalon, A., Tseng, C. H. & Axelrod, F. Prevalence and severity of renal disease in familial dysautonomia. *Am J Kidney Dis* **48**, 780-786, doi:S0272-6386(06)01230-3 [pii] 10.1053/j.ajkd.2006.07.024 (2006).
22 Parati, G., Pomidossi, G., Albini, F., Malaspina, D. & Mancia, G. Relationship of 24-hour blood pressure mean and variability to severity of target-organ damage in hypertension. *J Hypertens* **5**, 93-98 (1987).
23 Bilo, G. *et al.* A new method for assessing 24-h blood pressure variability after excluding the contribution of nocturnal blood pressure fall. *J Hypertens* **25**, 2058-2066, doi:10.1097/HJH.0b013e32829c6a60 (2007).
24 Shan, Z. Z., Dai, S. M. & Su, D. F. Arterial baroreflex deficit induced organ damage in sinoaortic denervated rats. *J Cardiovasc Pharmacol* **38**, 427-437 (2001).
25 Rothwell, P. M. Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. *Lancet* **375**, 938-948, doi:S0140-6736(10)60309-1 [pii]

10.1016/S0140-6736(10)60309-1 (2010).

26 Smith, A. A., Taylor, T. & Wortis, S. B. Abnormal catechol amine metabolism in familial dysautonomia. *N Engl J Med* **268**, 705-707 (1963).

27 Goldstein, D. S., Holmes, C. & Axelrod, F. B. Plasma catechols in familial dysautonomia: a long-term follow-up study. *Neurochem Res* **33**, 1889-1893, doi:10.1007/s11064-008-9662-4 (2008).

28 Axelrod, F. B., Goldstein, D. S., Holmes, C., Berlin, D. & Kopin, I. J. Pattern of plasma levels of catecholamines in familial dysautonomia. *Clin Auton Res* **6**, 205-209 (1996).

29 Gitlow, S. E., Bertani, L. M., Wilk, E., Li, B. L. & Dziedzic, S. Excretion of catecholamine metabolites by children with familial dysautonomia. *Pediatrics* **46**, 513-522 (1970).

30 Weinshilboum, R. M. & Axelrod, J. Reduced plasma dopamine-beta-hydroxylase activity in familial dysautonomia. *N Engl J Med* **285**, 938-942 (1971).

31 Freedman, L. S., Ebstein, R. P., Goldstein, M., Axelrod, F. B. & Dancis, J. Serum dopamine-beta-hydroxylase in familial dysautonomia. *J Lab Clin Med* **85**, 1008-1012, doi:0022-2143(75)90391-1 [pii] (1975).

32 Rubin, B. Y. & Anderson, S. L. The molecular basis of familial dysautonomia: overview, new discoveries and implications for directed therapies. *Neuromolecular Med* **10**, 148-156, doi:10.1007/s12017-007-8019-5 (2008).

33 Porter, C. C. Aromatic amino acid decarboxylase inhibitors. *Fed Proc* **30**, 871-876 (1971).

34 Huebert, N. D., Palfreyman, M. G. & Haegele, K. D. A comparison of the effects of reversible and irreversible inhibitors of aromatic L-amino acid decarboxylase on the half-life and other pharmacokinetic parameters of oral L-3,4-dihydroxyphenylalanine. *Drug Metab Dispos* **11**, 195-200 (1983).

35 Markham, C., Diamond, S. G. & Treciokas, L. J. Carbidopa in Parkinson disease and in nausea and vomiting of levodopa. *Arch Neurol* **31**, 128-133 (1974).

36 Watanabe, A. M., Judy, W. V. & Cardon, P. V. Effect of L-DOPA on blood pressure and sympathetic nerve activity after decarboxylase inhibition in cats. *J Pharmacol Exp Ther* **188**, 107-113 (1974).

37 Kaufmann, H. *et al.* Norepinephrine precursor therapy in neurogenic orthostatic hypotension. *Circulation* **108**, 724-728 (2003).

38 Wurtman, R. J. & Watkins, C. J. Suppression of noradrenaline synthesis in sympathetic nerves by carbidopa, an inhibitor of peripheral dopa decarboxylase. *Nature* **265**, 79-80 (1977).

39 Brod, L. S., Aldred, J. L. & Nutt, J. G. Are high doses of carbidopa a concern? A randomized, clinical trial in Parkinson's disease. *Mov Disord* **27**, 750-753, doi:10.1002/mds.24998 (2012).

40 Lotti, V. J. & Porter, C. C. Potentiation and inhbition of some central actions of L(-)-dopa by decarboxylase inhibitors. *J Pharmacol Exp Ther* **172**, 406-415 (1970).

41 Goldstein, D. S., Holmes, C., Kaufmann, H. & Freeman, R. Clinical pharmacokinetics of the norepinephrine precursor L-threo-DOPS in primary chronic autonomic failure. *Clin Auton Res* **14**, 363-368 (2004).

42 Kaufmann, H., Malamut, R., Norcliffe-Kaufmann, L., Rosa, K. & Freeman, R. The Orthostatic Hypotension Questionnaire (OHQ): validation of a novel symptom assessment scale. *Clin Auton Res* **22**, 79-90, doi:10.1007/s10286-011-0146-2 (2012).

43 Vagaonescu, T. D., Saadia, D., Tuhrim, S., Phillips, R. A. & Kaufmann, H. Hypertensive cardiovascular damage in patients with primary autonomic failure. *Lancet* **355**, 725-726 (2000).

44 Rhodes, V. & McDaniel, R. The Index of Nausea, Vomiting, and Retching: a new format of the Index of Nausea and Vomiting. . *Oncol Nurs Forum* **26**, 889-894 (1999).

45 Holmes, C., Eisenhofer, G. & Goldstein, D. S. Improved assay for plasma dihydroxyphenylacetic acid and other catechols using high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Appl* **653**, 131-138 (1994).

46 Macefield, V. G., Norcliffe-Kaufmann, L. J., Axelrod, F. & Kaufmann, H. Cardiac-Locked Bursts of Muscle Sympathetic Nerve Activity Are Absent in Familial Dysautonomia. *J Physiol*, doi:10.1113/jphysiol.2012.246264 (2012).

15 Attachments

Appendix:

ORTHSTATIC HYPOTENSION QUESTIONNAIRE

We are interested in measuring the symptoms that occur because of your problem with low blood pressure (orthostatic hypotension) and the degree that those symptoms may interfere with your daily activity. It is important that we measure the symptoms that are due ONLY to your low blood pressure, and not something else (like diabetes or Parkinson's disease). Many people know which of their symptoms are due to low blood pressure. Some people who have recently developed problems with low blood pressure may not easily distinguish symptoms of low blood pressure from symptoms caused by other conditions. In general, symptoms of your low blood pressure problem will appear either upon standing or after you have been standing for some time, and will usually improve if you sit down or lie down. Some patients even have symptoms when they are sitting which might improve after lying down. Some people have symptoms that improve only after sitting or lying down for quite some time.

Please answer the next questions keeping in mind that we want to know only about those symptoms that are from your problem with low blood pressure.

OH SYMPTOM ASSESSMENT (OHSA)

Please tick the number on the scale that best rates how severe your symptoms from low blood pressure have been on the average over the past week. You should respond to every symptom. If you do not experience the symptom, circle zero (0). YOU SHOULD RATE ONLY THE SYMPTOMS THAT ARE DUE TO YOUR LOW BLOOD PRESSURE PROBLEM.

1. Dizziness, lightheadedness, feeling faint, or feeling like you might black out

None	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Worst possible

2. Problems with vision (blurring, seeing spots, tunnel vision, etc.)

None	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Worst possible

3. Weakness

None	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Worst possible

4. Fatigue

None	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Worst possible

5. Trouble concentrating

None	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Worst possible

6. Head and neck discomfort

None	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Worst possible

OH DAILY ACTIVITY SCALE (OHDAS)

We are interested in how the low blood pressure symptoms that you experiences affect daily life. Please rate each item by ticking the number that best represents how much on the average the activity has been interfered with over the past week by the low blood pressure symptoms you have experienced. If you cannot do the activity for reasons other than low blood pressure, please check the box at right.

1. Activities that require standing for a short time											☐ **Cannot do for other reasons**	
No interference	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐	**5** ☐	**6** ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☐	**Complete interference**
2. Activities that require standing for a long time											☐ **Cannot do for other reasons**	
No interference	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐	**5** ☐	**6** ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☐	**Complete interference**
\\\\\\\												
3. Activities that require walking for a short time											☐ **Cannot do for other reasons**	
No interference	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐	**5** ☐	**6** ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☐	**Complete interference**
4. Activities that require walking for a long time											☐ **Cannot do for other reasons**	
No interference	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐	**5** ☐	**6** ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☐	**Complete interference**

SYMPTOM DIARY

Name (last, first):						
Current date:		**Time:**				

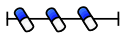
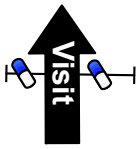
Retching is the effort your body makes to vomit

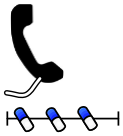
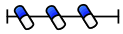
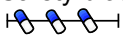
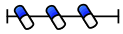
Please mark the box in each row that most clearly corresponds to your experience. Please make only one mark on each line.

In the last 24 hours, I have felt nauseated or sick to my stomach	*Not at all* ☐	*1-hour or less* ☐	*2-3 hours* ☐	*4-6 hours* ☐	*More than 6 hours* ☐	
In the last 24 hours, from nausea/sickness, I have felt "……" distress	*No*	*Mild*	*Moderate*	*Great*	*Severe*	
In the last 24 hours, I have had periods of retching.	*No*	*1 – 2 hours*	*3 – 4 hours*	*5 – 6 hours*	*7 or more*	
What time of day was the worst?						
Did you miss school or work?	☐ No ☐ Yes					
Did you take something for your nausea today?	☐ No ☐ Yes		☐ Clonidine: Dose _____ mg ☐ Valium: Dose _____ mg ☐ Other: Details_____			
Did you take something for your blood pressure today?	☐ No ☐ Yes		☐ Clonidine: Dose _____ mg ☐ Valium: Dose _____ mg ☐ Other: Details_____			
Did you have anything else that bothered you today (an Adverse Event)?						

Diary Day: 1 of 28

Sample Instructions for Testing/Dose administration:

Day	Instructions
Apr 1st The day before your visit 	At 9 AM fit the BP cuff to your <<LEFT>> arm (<<6>> cm above your elbow). Turn the monitor on, start the recording by pressing the blue button, go about your daily activities. Fill in the diary your meal times, times you took medications and the time you go to sleep and wake up. Starting this same morning, discard your first morning urine. For the next 24-h collect your urine in the container provided. Write here when you started the collection. **START TIME:** __ __ : __ __ AM You should continue to take your study medication every 6-hours.
Apr 2nd The day of your visit 	Your appointment or televisit at the Dysautonomia Clinic is scheduled for <<10:00>> AM. Finish your urine collection the same time you started it the previous day. Be sure this time to include your first early morning urine sample after waking up. Write here when you ended the collection: **END TIME:** __ __ : __ __ AM Take your first morning dose of study medication at <<8:00>> AM. Write down here the exact time you took your medication. **MEDICATION TIMING:** __ __ : __ __ AM Come into the clinic for your study visit. At 11 AM we will perform your autonomic function tests in the office of via telemedicine. DO NOT take off your BP monitor yet, will take it off when you arrive at the clinic. **Here is a check list with things to bring with you to the clinic or have prepared for the televisit:** ☐ **Your symptom diary** ☐ **Your 24-h urine collection** ☐ **The blood pressure monitor (which you should be still wearing)** ☐ **All your unused medication** **After your visit:** So that you do not stop your dose too quickly, we will reduce your current dose slowly. At the end of this visit, we will give you **2 bottles of medication**. One bottle will be marked with todays and tomorrows dates, and will contain 2 of the same pills that you have been taking. One bottle will contain more pills. These pills are your next treatment, and you should not start taking them yet. **You will take one-pill from the bottle with today and tomorrows date. Once you have taken this pill, do not take any more pills for the rest of the day.** 
Apr 3rd The day after your visit 	**IN THE AFTERNOON, TAKE ONE PILL FROM THE BOTTLE MARKED WITH TODAY's DATE.** **Do not take any more medication for the rest of the day** 

Apr 4th Washout **No pills**	**DO NOT TAKE ANY MEDICATION TODAY**
Apr 5th Washout **No pills**	**DO NOT TAKE ANY MEDICATION TODAY**
Apr 6th Start your new medications today 	Start taking your next batch of study pill again, three times a day (every six hours) Morning dose: 8:00 AM Afternoon dose: 2:00 PM Evening dose: 8:00 PM Be sure to keep your diary with your symptoms  We will call you in the morning at 10:00 AM to check how you responded to your first dose of new medication. If you do not feel well, we will ask you to come into the Center.
Apr 7th Continue your three a day schedule 	Take your study pills three times daily until May 4th, when you come to the Clinic. Remember to fill your diary
Apr 12th Phone call Safety bloods  	We will contact you by phone to check in on how you are feeling. We will arrange for you to have a blood test to check your health status.
May 3rd Day before next visit 	Repeat ambulatory monitoring and urine collection. We will send you new instructions with dates and times.
May 4th Next visit # X	Plan to attend your next study visit at the clinic ...

If you feel unwell at any time, call the Dysautonomia Center at: 212-263-7225.

AMBULATORY MONITORING: INSTRUCTIONS FOR PATIENTS

DYSAUTONOMIA CENTER
NYU
https://dysautonomia.nyumc.org
Tel: 212-263-7225

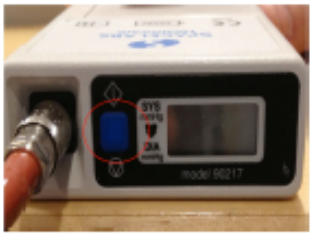
You have been asked to wear an ambulatory blood pressure monitor. This will measure your blood pressure for 24-h so that we can better understand of how it changes over a typical day. Some patients may need to wear the monitor for longer.

Step 1: Fitting the cuff

1. Place the cuff on your non-dominant arm (the one you don't write with). So it does not restrict movement, it should be on your upper arm, completely above the elbow. The cuff should be tight enough to not fall down, but not uncomfortable. The orange tubing needs to face UP. The word "ART" and arrow should on the inside of your elbow.
2. The monitor itself can be worn on a belt or carried in a bag. Make sure you do not drop it.
3.

Step 2: Starting the recording

1. Turn the monitor on by flipping the black switch from OFF (O) to ON (I). You should see numbers or letters on the display screen when the monitor is initially on.
2. Once the monitor is on press the BLUE button to start the readings.
3. The cuff will inflate and start taking your blood pressure



Step 3: Measuring your blood pressure

1. The cuff will inflate every 30 minutes to take your blood pressure. The screen may not show your blood pressure readings. The monitor will store your readings in its internal memory.
2. When the cuff is inflating, make certain you keep you arm relaxed and still. If the machine cannot measure your blood pressure it will automatically inflate another time and try again.
3. You can press the blue button again to take extra blood pressure measurements if you are feeling unwell.
4. You should fill out the diary. Make a note of the position you are in (sitting, standing, laying).
5. Include your medications, meal times and sleep times.

Step 3: Measuring your blood pressure

1. The cuff will inflate every 30 minutes to take your blood pressure. The screen may not show your blood pressure readings. The monitor will store your readings in its internal memory.
2. When the cuff is inflating, make certain you keep you arm relaxed and still. If the machine cannot measure your blood pressure it will automatically inflate another time and try again.
3. You can press the blue button again to take extra blood pressure measurements if you are feeling unwell.
4. You should fill out the diary. Make a note of the position you are in (sitting, standing, laying). Include your medications, meal times and sleep times.

Step 3: Ending a recording.

1. You should arrive at the Center on the morning of your visit still wearing the monitor.