Autonomic neuropathy, gastrointestinal motility, and inflammation in HIV
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Mount Sinai	Protocol Name:	Autonomic neuropathy, gastrointestinal motility, and inflammation in HIV
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Brief Summary of Research:

HIV-infected patients commonly develop autonomic neuropathy (HIV-AN), which is a heterogeneous disorder characterized by varying degrees of both sympathetic and vagal dysfunction. We hypothesize that the vagal component of HIV-AN contributes to chronic inflammation, both directly via loss of cholinergic activity, and indirectly via effects on the GI tract, and that these effects will be treatable using the acetylcholinesterase inhibitor pyridostigmine. The autonomic nervous system controls the inflammatory response to lipopolysaccharide (LPS) via the cholinergic anti-inflammatory pathway. This pathway is mediated by the vagus nerve, and is therefore likely impaired in HIV-AN with vagal dysfunction. Vagal dysfunction also causes slowed GI transit, which could exacerbate LPS-driven inflammation by promoting bacterial overgrowth. However, the anti-inflammatory impact of cholinergic pathways is almost completely unstudied in HIV, despite the known importance of inflammation in HIV disease progression. Therefore, in this exploratory pilot, we seek to establish associations between vagal dysfunction, GI motility and inflammation in virally suppressed, CART-treated individuals with HIV-AN.

Specific Aim 1: To determine whether vagal dysfunction is associated with immune activation in CART-treated participants with HIV-AN, and if so to estimate the extent to which this association is mediated by GI effects (i.e. slowed motility, bacterial overgrowth, microbial translocation) versus direct effects of vagal dysfunction.

Specific Aim 2: In a subset of participants who have both vagal and GI dysfunction, to investigate whether 8 weeks of pyridostigmine: a) reduces immune activation, and b) improves GI motility; and if the immune effect depends on the GI effect.

To achieve these aims, participants with HIV-AN and GI symptoms will be assessed for: vagal dysfunction (heart rate variability); GI dysmotility (gastric emptying scintigraphy); small intestinal bacterial overgrowth (breath testing); microbial translocation (LPS and sCD14); and immune activation (IL-6 and CRP). Participants meeting threshold criteria for both vagal and GI dysfunction will then be treated with pyridostigmine for 8 weeks, after which GI and immune measures will be reassessed.

1) Objectives

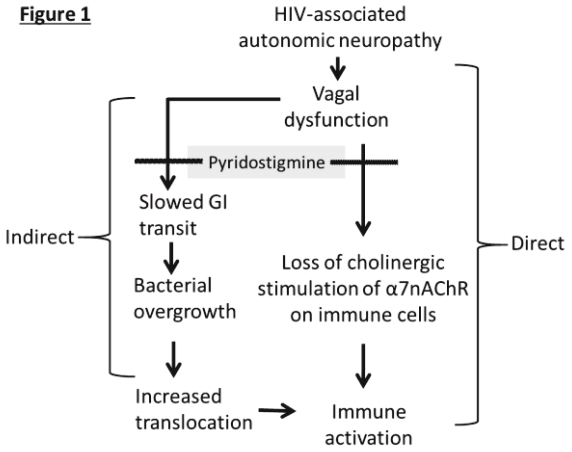
Specific Aim 1: To determine whether vagal dysfunction is associated with immune activation in HIV-infected participants treated with combination antiretroviral therapy (CART), and if so to estimate the extent to which this association is mediated by GI effects (i.e. slowed motility, bacterial overgrowth, microbial translocation) versus direct effects of vagal dysfunction.

Specific Aim 2: In a subset of participants who have both vagal and GI dysfunction, to investigate whether 8 weeks of pyridostigmine: a) reduces immune activation, and b) improves GI motility; and if both effects are present to determine whether the immune effect depends on the GI effect.

2) Background

In this study we propose to generate preliminary evidence that the vagal dysfunction that occurs as part of HIV-associated autonomic neuropathy (HIV-AN) is associated with immune activation in virally suppressed, CART-treated HIV, acting both directly via loss of cholinergic activity, and indirectly via effects on the GI tract, and that these effects will be treatable by increasing the availability of the neurotransmitter acetylcholine (ACh). These are novel hypotheses that have not previously been tested, although there is evidence to support each individual component of them. Should evidence be gathered consistent with these hypotheses, it would constitute a first step in investigating a potentially treatable, non-viral pathway subserving chronic immune activation in CART-treated HIV. Chronic immune activation plays an important role in the progression of HIV, the development of co-morbidities, and accelerated aging.[1] Inflammatory cytokines and biomarkers of hypercoagulability are elevated in HIV, even in the setting of effective CART, and are associated with morbidity, particularly cardiovascular disease, and mortality. Microbial translocation from the GI tract due to disruption of the normal mucosal barrier is an important driver of this immune activation.[2,3] To date, there has been no consideration of autonomic function in maintaining the integrity of this barrier in HIV, either through effects on motility, or via cholinergic stimulation of immune cells. In the following sub-sections we will present evidence to support associations between autonomic function (and in particular vagal function), GI function, and immune activation, and their significance to the proposed study in HIV.

Figure 1



HIV, autonomic neuropathy, and GI effects. We recently reported that HIV-AN is common and typically occurs as part of a more generalized neuropathic process that includes HIV-associated distal symmetric polyneuropathy (HIV-DSP).[4-7] We determined this by performing a comprehensive autonomic assessment on 102 HIV-infected individuals consisting of heart rate response to deep breathing, Valsalva maneuver, tilt table testing, and quantitative sudomotor axon reflex testing. Such testing is considered the diagnostic gold standard,[8,9] and can be summarized as the Composite Autonomic Severity Score (CASS),[10] a validated score with sensitivity and specificity >90%,[8,10] or as the modified CASS (mCASS) a closely related score that we recently developed for use in HIV.[7] Both the CASS and the mCASS contain three sub-scores reflecting different autonomic functions: sudomotor (cholinergic sympathetic innervation of sweat glands), vagal (cardiac cholinergic parasympathetic innervation), and sympathetic (noradrenergic sympathetic innervation of vasculature). We found that 61% of participants met the definition for HIV-AN (CASS $\geq$ 3), but that the deficits were heterogeneous. For example, although nearly 90% had sympathetic deficits (either vascular or sudomotor) only 45% had vagal deficits. These findings suggest that HIV-AN is variable in its anatomic distribution, and therefore likely to be variable in its clinical manifestations. Symptoms potentially attributable to HIV-AN were overall non-specific, including orthostatic dizziness or fainting, dry eyes or mouth, urinary and sexual dysfunction, changes in

sweating, and nausea. The only symptom that was significantly associated with HIV-AN was constipation ($p<.001$), suggesting possible slowed GI transit time**.**

The end-organ effects of HIV-AN are currently unknown, although in the broader field of autonomic dysfunction, GI dysmotility is a well-recognized effect,[11-14] which is characterized clinically by symptoms of nausea, vomiting or bloating especially after meals, and constipation and/or diarrhea.[15,16] In addition to these bothersome symptoms, GI dysmotility can promote bacterial overgrowth,[17-22] and increased translocation across the gut wall.[23-25] There is a single anatomical study demonstrating loss of autonomic fibers in jejunal biopsies from individuals with HIV,[26] and a few studies suggesting GI dysmotility in HIV,[27-30] however these studies were all pre-CART. Only one study attempted to correlate gastric motility with HIV-AN, however autonomic function was characterized solely by spectral analysis of heart rate variability which is not considered to be the diagnostic gold standard.[30]

Autonomic neuropathy, cholinergic anti-inflammatory pathways, and pyridostigmine. Outside of the realm of HIV research, autonomic effects on inflammation, particularly in response to lipopolysaccharide (LPS), have been extensively studied and dubbed the "cholinergic anti-inflammatory reflex."[31] Both the afferent and efferent limbs are contained within the vagus nerve. In rodent models, the afferent limb is demonstrated by intra-abdominal administration of IL-1 to vagotomized animals, which fails to produce the normal fever and sickness behavior.[32] The efferent limb is demonstrated in models of sepsis, where vagal nerve stimulation attenuates LPS-induced hypotension, and vagotomy has the opposite effect.[33] In humans, it has been observed that trauma patients undergoing vagotomy experience greater rates of sepsis, ulcers, and mortality when compared to those without vagotomy.[34] Additionally, in septic humans, decreased heart rate variability, a marker of vagal dysfunction, is correlated with inflammatory markers (e.g. IL-6, CRP) and poor outcomes including progression to shock and death.[35,36]

The vagus nerve regulates inflammation via release of ACh, which can bind the nicotinic ACh receptor subunit α7 (α7nAChR) present on monocytes, macrophages, B cells, T cells and dendritic cells. In the GI tract, vagal action on macrophages and dendritic cells is thought to be mediated through stimulation of the myenteric plexuses, and subsequent release of ACh to immune effectors in the muscularis.[37,38] In the spleen, vagal stimulation of the celiac ganglion may result in adrenergic stimulation of T cells, a subset of which release ACh to stimulate macrophages.[31] In both gut wall and circulating monocytes/macrophages, the effect of α7nAChR activation is to induce intracellular signaling which suppresses the transcription of pro-inflammatory cytokines and mediators, dependent upon upregulation of heme oxygenase 1 (HO-1).[39] In dendritic cells and monocytes/macrophages, activation of α7nAChR also inhibits secretion of high mobility group box 1 (HMGB1), an alarmin essential to the innate immune reponse.[40] While most observations of cholinergic effects have been in murine models, there is experimental evidence that similar effects may be seen in man (in addition to the clinical observations described above). In primary human macrophages, ACh inhibits the LPS-stimulated release of TNF-α, IL-1β, IL-6, and IL-18, but has no effect on IL-10.[33,41] In human monocytes, stimulation of α7nAChR suppresses LPS-associated expression of CD14, TLR4, CD40, ICAM-1, and B7.1.[42]

In our study (described in the sub-section immediately above), we found that the severity of HIV-AN was correlated with the Veteran's Aging Cohort Study (VACS) index, which itself has been shown to be correlated with inflammation.[5] This suggests that HIV-AN could also be associated with inflammation. To our knowledge, a potential role for cholinergic anti-inflammatory effects in HIV has been tested directly by only one small proof-of–concept study examining 19 HIV-infected patients, who were not CART-treated.[43] Participants were randomized to receive a low dose of the acetylcholinesterase inhibitor (AChEI) pyridostigmine (30mg PO tid) or placebo. Participants who received pyridostigmine exhibited a reduction of CD4+ T cell activation (defined as the percent of cells expressing CD69), as well as in vitro reduction of T cell proliferation, decrease of IFN-γ production, and increase of IL-10 production. Autonomic indices were not measured in this study. Pyridostigmine is a peripherally acting AChEI that has been FDA approved for the treatment of myasthenia gravis since 1955. It is generally well tolerated, with a well-known side effect profile that is largely dose dependent. Although it is an old drug that is very familiar to neurologists, there has been considerable recent investigation surrounding its use. In addition to the single study in HIV described above, pyridostigmine has been studied for autonomic symptoms including orthostatic hypotension and constipation.[44-46]

Summary. There is substantial evidence that chronic inflammation is important in HIV, and that vagal dysfunction may contribute to inflammation directly via loss of cholinergic activity or indirectly via effects on GI motility. We have demonstrated that HIV-AN is common in CART-treated individuals and commonly involves vagal dysfunction. However other than a single study of 19 participants,[43] there has been no attempt to study the intersection of these important and interrelated conditions, in order to determine whether vagal dysfunction contributes to the pro-inflammatory state seen with chronic HIV.

3) Setting of the Human Research

All research procedures will be conducted at Mount Sinai. All potential participants will be identified and recruited at Mount Sinai. There will be no active recruitment outside of Mount Sinai, however if patients or providers at other sites become aware of the study we may accept referrals. All study procedures will be conducted on the main campus of Mount Sinai, 1468 Madison Avenue, Annenberg building, Second Floor with the exception of the gastric emptying scintigraphy and the hydrogen breath testing. Gastric emptying scintigraphy is performed in the main campus of Mount Sinai, 1468 Madison Avenue, Guggenheim Building, MC level. Hydrogen breath testing is performed in the main campus of Mount Sinai, either at 1468 Madison Avenue, Annenberg building, Second Floor; or at 17 East 102nd Street 5th Floor (depending on appointment availability).

4) Resources Available to Conduct the Human Research

As described below, the study has two phases. Participants in phase 2 will be a subset of participants in phase 1. Our goal is to recruit 80-100 participants for phase 1, and from this group to have 35-45 participants eligible for phase 2. This is based on the estimate that 45% of phase 1 participants will be eligible for phase 2.

As described in greater detail in the "Recruitment Methods" section, we plan to recruit patients already known to us from previous and ongoing research studies and/or clinical care, and patients not previously known to us from the Jack Martin Fund Clinic (JMFC). Eligibility rests in part on the results of autonomic testing. We are currently aware of about 30 eligible patients meeting eligibility criteria including the appropriate autonomic testing results. Based on our prior studies in this population, we estimate that about 15% of JMFC patients will meet study criteria, and that this will be somewhat higher (~20%) in our research population since these patients are pre-selected for criteria (such as older age) that makes AN more likely. There are currently about 1500 patients in active care at JMFC, and about 200 participants actively participating in our longitudinal neuroAIDS studies. Thus we estimate that we have access to an eligible population of about 265, from which we will need to recruit 50-70.

Eight persons will be involved with the study: Jessica Robinson-Papp MD MS who is the PI and will oversee the study as a whole and interpret and oversee the autonomic testing; Susan Morgello, MD who will oversee all the non-routine laboratory testing (e.g. cytokines); Gina Sam, MD who will oversee the hydrogen breath testing; Josef Machac MD who will oversee the gastric emptying scintigraphy; Emma Benn PhD who will assist Dr. Robinson-Papp with the statistical analyses; research manager Mary-Catherine George; research coordinator Alexandra Nmashie MD; and Sandeep Sharma, Dr.PH. The last three staff members will oversee the coordination of the project.

Dr. Morgello is the PI of the Manhattan HIV Brain Bank (MHBB), and is Dr. Robinson-Papp's research mentor. She has extensive experience in neuroAIDS research over decades and will use the infrastructure of the MHBB to help Dr. Robinson-Papp with the laboratory aspects of this project. Dr. Sam is a gastroenterologist with additional sub-specialty training in neurogastroenterology and GI motility. She has extensive clinical experience diagnosing and treating GI dysmotility. Dr. Machac is board certified in internal medicine, nuclear medicine, and cardiology, and has been an integral part of the nuclear medicine department at Mount Sinai for over 20 years, during which time he has served as director of the Clinical PET and Nuclear Medicine Center at Mount Sinai. He has published in the area of neuroAIDS, and has collaborated on previous studies of dysautonomia. Dr. Benn is a professor of biostatistics at Mount Sinai. She has experience analyzing clinical research data, as well as teaching medical and graduate students to do so. Ms. George is a senior research coordinator and program manager with extensive experience in neuroAIDS research spanning nearly two decades. She has provided organizational oversight and guidance for all Dr. Robinson-Papp's prior studies. Dr. Nmashie has worked with Dr. Robinson-Papp as a research coordinator on multiple prior studies, including a recent study which examined autonomic function in HIV.

In order to ensure that all persons involved with the study are adequately trained, Dr. Robinson-Papp will review the protocol in detail with all study staff prior to beginning any study procedures, and as needed throughout the study. Questions and an open dialog among the study staff will be encouraged.

5) Study Design

a) Recruitment Methods

Participants will be drawn from three main sources: the Jack Martin Fund Clinic (JMFC), where Dr. Robinson-Papp is the attending neurologist, other research studies in which Dr. Robinson-Papp is an investigator, and the FPA Neurodiagnostic Center, which is the other location (in addition to JMFC) where Dr. Robinson-Papp's sees patients. We will also consider patients referred by health care providers or other patients, but do not have an active recruitment plan for these sources.

JMFC patients will be initially pre-screened using a Mount Sinai Data Warehouse (MSDW) Search. We will create a de-identified dataset from the MSDW that includes all JMFC patients with at least1 visit in calendar year 2015, who are being treated with CART (N≈700). The dataset will include the following information relevant to pre-screening for this study: HIV-1 viral load, urine toxicology screening, smoking status, diagnosis of diabetes, diagnosis of COPD or asthma, diagnosis of peptic ulcer disease, diagnosis of PN, treatment with opiate medication.

Using this dataset, we will identify a subset of potentially eligible patients, and will request the patients' names and medical record numbers, and the name of the last prescriber of the CART, who is very likely to be the primary care provider. A waiver of informed consent will be requested for this portion of the study. This information will be requested without linkage of this new identifying information to the pre-existing de-identified dataset, so as to minimize the amount of PHI obtained. It is likely that some of these patients will be patients of Dr. Robinson-Papp, and those patients will be contacted directly. For patients who are not patients of Dr. Robinson-Papp, we will initially contact the provider, and ask whether in their opinion the patient would qualify for the research, and might be interested in participating. If the provider thinks the patient would qualify, might be interested, and would not find contact intrusive, the patient will be contacted through the provider (typically physician or nurse practitioner or member of the clinic staff). Initial contact will be the minimum required to ascertain whether the patient is willing to hear about the study. More detailed information will be provided only if the patient assents. With the patient's permission, this will include mailing a copy of the informed consent to the patient in advance of the screening visit so that they have time to review it, discuss it with others and prepare questions for the study team. The research team will not review the subject's charts for HIV information prior to obtaining informed consent.

Patients who are known to Dr. Robinson-Papp (either clinically or through research) who she believes to be eligible and open to participation in research will be contacted directly. Patients who are referred to the study will also be contacted directly.

There is also a previously IRB approved printed recruitment flyer which is included with this submission.

b) Inclusion and Exclusion Criteria

Inclusion criteria are:

- ≥18 years old
- Documented evidence of HIV-1 infection
- Stable CART therapy for ≥3 months
- Most recent HIV-1 viral load ≤100 copies/ml (value must be within the past six months)
- English speaking
- Able to tolerate autonomic testing (e.g. able to stand, able to perform Valsalva maneuver).
- If using nicotine-containing products willing to refrain from use for 24 hours prior to all testing procedures (autonomic reflex screen, breath testing, and gastric emptying)
- ≥1 GI symptom on the Survey of Autonomic Symptoms (SAS)[47]

Exclusion criteria are:

- Diagnosis known to cause autonomic dysfunction other than HIV (e.g. Parkinson's disease, diabetes)
- Diagnosis known to cause GI dysfunction other than HIV (e.g. peptic ulcer disease, infectious diarrhea)
- Current use of any of the following classes of medications (due to potential for significant autonomic or GI effects, interaction with pyridostigmine, or interference with one or more of the testing procedures)
    - Prokinetics (e.g. metoclopramide)
    - Anti-diarrheals (e.g. loperamide)
    - Antibiotics
    - Mefloquine
- Medical or psychiatric conditions precluding safe participation in study procedures or deemed likely to result in hospitalization during the study period.
- The presence of one or more of the following diagnoses which render the Valsalva maneuver relatively or absolutely contraindicated: uncontrolled glaucoma, aortic stenosis, myocardial infarction in the last 6 months, other retinopathy or unclipped cerebral aneurysm.
- The presence of one or more of the following diagnoses which impede interpretation of autonomic testing: cardiac arrhythmias or pacemakers.
- An allergy to eggs (contraindication to gastric emptying scintigraphy)
- Any of the following laboratory results:
    - Positive pregnancy test (administered to women of childbearing potential only)
    - Urine toxicology screen positive for stimulants (e.g. amphetamines, cocaine) or opiates/opioids.

Screening procedure:

No research procedures will be performed prior to obtaining informed consent. The majority of inclusion/exclusion criteria are ascertainable from the patient and the medical record, with the following exceptions (in order of increasing complexity): the SAS which is a questionnaire; pregnancy and toxicology screening which requires a urine sample; and the CASS which requires autonomic testing. In order to limit participant exposure to unnecessary testing, the screening will be performed in a tiered manner. The simplest criteria (i.e. those items obtainable from the medical record) will be assessed first, and the most involved (i.e. the autonomic testing) will not be performed unless all other criteria have been satisfied. The screening process will end at the first evidence of ineligibility.

c) Number of Subjects

We intend to pre-screen about 700 JMFC patients using our automated MSDW search. We will also pre-screen patients known to Dr. Robinson-Papp or referred to the study. We will enroll (i.e. obtain informed consent from) up to 250 participants as required to identify up to 100 participants eligible for full baseline assessment, and up to 45 participants to enter the intervention phase.

d) Study Timelines

The maximum expected duration of individual subject participation is 6 months for participants who complete both phase 1 and 2. For patients who complete phase 1 but are found to be ineligible for phase 2, study participation will be much shorter, typically 1-2 weeks. The duration anticipated to enroll all study subjects is 18 months. The estimated date for the investigators to complete this study (complete primary analyses) is 24 months.

e) Endpoints

The study endpoints are: vagal sub-score of mCASS (vagal dysfunction, calculated from autonomic testing), GE T½ (GI dysmotility, calculated from gastric emptying scintigraphy), CRP and IL-6 (immune activation), LPS (bacterial translocation), total hydrogen excretion in the breath (ppm x min) over 2 hours following ingestion of 50g glucose (SIBO). Secondary measures include: vagal baroreflex sensitivity; Valsalva ratio; heart rate variability with deep breathing; percent gastric emptying at 1-4h; CRP; sCD14.

Participants who are eligible for the intervention phase of the study in which they will receive treatment with pyridostigmine will be monitored for adverse events. Adverse events, including anticipated medication side effects, may be grounds for discontinuation of study drug, based on the judgment of the PI and the preferences of the participant. If the study drug is to be discontinued early, and it is a non-emergent discontinuation, effort will be made to perform follow-up measurements (see intervention visit 3 in the subsequent section) prior to discontinuation of the drug.

f) Procedures Involved in the Human Research

The study design has two phases. In the first phase (baseline) cross-sectional data will be collected for correlational analyses, and for determination of participant eligibility for phase two. In the second phase, the subset of eligible participants will be treated with pyridostigmine for 8 weeks, at the end of which the measures will be repeated to assess for drug effect. A summary of study visits is provided here, followed by a detailed description of each of the individual procedures. All procedures are performed solely for the research, and not for clinical purposes. However all tests to be performed are standardized tests which are performed clinically.

Screening visit: All participants will be seen for a screening visit which consists of informed consent and assessment of eligibility including: documentation of medical history and current medications; the SAS questionnaire; brief neurologic examination; urine pregnancy and toxicology screening; and autonomic function testing. All procedures, including the informed consent process will take place on Annenberg 2 in a private exam room. The informed consent process will take place prior to any research related activities. Participants meeting all inclusion/exclusion criteria will then proceed to the baseline visit. The screening visit will last about approximately 4 hours unless there is a test or questionnaire that reveals ineligibility, in which case the screening process will be stopped and so will be shorter in duration.

Baseline visit: The baseline visit may occur over several days (7-10 days) and will include hydrogen breath test (approximately 3.5 hours), blood collection (approximately 2-3 tablespoons), gastric emptying scintigraphy (approximately 4 hours), and questionnaires as listed below (approximately 30 minutes).

Participants will then move on to additional visits if they meet ALL of the following criteria:

1) CASS $\geq$ 3 OR at least one abnormal vagal parameter on the autonomic function testing (vagal baroreflex sensitivity; Valsalva ratio; heart rate variability with deep breathing).
2) Abnormality in gastric emptying scintigraphy (retention at 30 min: <70%; 60 min:<30%, >90%; 120 min: >60%; 180 min: >30%; 240 min: >10%).
3) No use of pyridostigmine within the past 6 months
4) No contraindication to pyridostigmine including current: mechanical intestinal or urinary obstruction, known hypersensitivity to anticholinesterase agents, bradycardia, cardiac arrhythmias, or asthma or chronic obstructive pulmonary disease.

Participants with an abnormality in hydrogen breath testing suggestive of small intestinal bacterial overgrowth (SIBO), will be informed of this finding (which may warrant treatment). Permission will also be sought from the participant to convey this information to their primary care provider (PCP). The participant will not have any further study visits until a course of treatment has been decided upon.

If the PCP chooses to treat the participant with a course of oral antibiotics, the hydrogen breath test will be repeated after the treatment is complete. If the test is still abnormal, and the above criteria 1, 3 and 4 are met, the participant will proceed to intervention visit 1. If the test is normal the participant will proceed to intervention visit 1 only if all the above criteria are met (1-4).

If the PCP decides that oral antibiotics are not indicated, and the above criteria 1, 3 and 4 are met then the participant will proceed to intervention visit 1.

For participants not meeting the criteria, study participation will end with the baseline visit.

Intervention visit 1: Participants will begin treatment with pyridostigmine 30mg PO tid. This visit will last about 1 hour.

Phone contact: Participants will be contacted by phone two days after pyridostigmine initiation to assess for adverse events and adherence.

Intervention visit 2: Approximately 4 weeks after initiation of study drug, participants will repeat all questionnaires and be assessed for adverse events and adherence. This visit is expected to last less than one hour.

Intervention visit 3: Approximately 8 weeks after initiation of study drug, participants will be seen for blood collection, gastric emptying scintigraphy, hydrogen breath test, questionnaires, and assessment for adverse events and adherence. Urine toxicology and pregnancy test (for women of childbearing potential) will also be repeated at this visit prior to other testing. This visit will be conducted in essentially the same manner as the baseline visit. Pyridostigmine will be discontinued at the end of this visit.

Intervention visit 4: Approximately 10 weeks after initiation of study drug (which is approximately 2 weeks after cessation of study drug), participants will repeat all questionnaires and be assessed for adverse events. This visit is expected to last less than one hour.

Autonomic function testing is performed under the supervision of Dr. Robinson-Papp. This is a standard battery of non-invasive physiologic tests to assess autonomic nervous system function.[10,49] The patient is asked to lie supine on the tilt table for all the tests. Quantitative Sudomotor Axon Reflex Testing (QSART) is performed by placing a capsule containing acetyl choline (ACh) on the skin in four standardized locations (forearm, proximal leg, distal leg and foot). The capsule is attached to a computer system which delivers a small continuous electrical stimulus to the capsule causing iontophoresis of ACh into the skin. The ACh causes a reflexive sweat response which is collected by the capsule. The total sweat volume is measured by the computer system and compared to standardized values. Following the QSART, a non-invasive continuous beat-to-beat blood pressure monitoring device is attached to the participant's finger (Nexfin system; www.bmeye.com) and a 3-lead surface electrocardiogram is attached to the chest. Continuous blood pressure (BP) and heart rate (HR) are recorded during Valsalva

maneuver (VM; forced exhalation to a pressure of 40 mmHg for 15 seconds) and during a standardized paced deep breathing exercise (heart rate response to deep breathing; HRDB). BP and HR responses during VM are analyzed manually according to the Composite Autonomic Scoring Scale (CASS). For the HRDB test, the average change in HR from peak inspiration to expiration is calculated for 5 consecutive cycles of breath and compared to standardized values. Finally the participant is secured to the tilt table and tilted to the upright position. Continuous HR and BP are recorded for 10 minutes (as tolerated). Criteria for immediate return to the supine position are: 1) the patient becomes symptomatic or asks to end the test; 2) there is hemodynamic evidence of orthostatic intolerance (HR increase of >30 bpm, systolic BP decrease >20 mmHg, diastolic BP decrease >10 mmHg); 3) the clinician's discretion.

The hydrogen breath test is currently in clinical use for the detection of small intestinal bacterial overgrowth (SIBO) and is performed under the supervision of Dr. Sam.[51] Breath samples are obtained throughout the testing by having participants exhale into a plastic bag. The hydrogen content of the samples is measured using a commercially available analyzer (Quintron; http://www.breathtests.com). A baseline breath sample is collected. The participant then ingests 50g of glucose after which breath samples are collected every 10 minutes for three hours.

Gastric emptying scintigraphy is a clinically established diagnostic test performed under the supervision of Dr. Machac.[52] It is used to study the mechanical and autonomic innervation integrity of the upper GI tract. The total examination scan time is 4 hours. The subject is asked to fast overnight. The study begins when the subject, in an upright sitting position, ingests a standard meal, consisting of four ounces (118 mL) of liquid egg whites (equivalent of 2 eggs) (e.g., Eggbeaters [ConAgra Foods, Inc.] or equivalent generic liquid egg whites), mixed with 0.5 mCi 99mTc sulfur colloid, cooked to a firm consistency, served together with toasted two slices of white bread and 30 g of jam or jelly. The subject drinks 4-8 oz of water after ingesting the meal. Additional water may be given if any residual activity is seen in the mouth or esophagus is seen on the gamma camera persistence scope on the first frame of the image set. Standard anterior and posterior 128x128 matrix images are aquired for 60 seconds at four time points (0, 1, 2, 3 and 4 hours) on the same gamma camera. The subject must remain in the upright sitting or standing position the entire 4 hours. If vomiting occurs at any point, the study is ended. The acquired images are displayed for review by a nuclear medicine physician. A nuclear medicine technologist draws a region of interest around the stomach in both anterior and posterior views. The total counts in the anterior and posterior regions of interest are multiplied, and a square root is obtained to yield the geometric mean at that time point, which is plotted for all time points. The total body absorbed radiation dose from the ingested 0.5 mCi activity is 0.45 mSv. The organ receiving the largest exposure is the large intestine, which receives 2.1 mSv.

Routine blood tests (CRP, T cell enumeration (CD4/CD8), HIV-1 viral load) will be performed by Mount Sinai's clinical laboratory services. Blood for experimental assays

(IL-6, LPS, sCD14) will be processed in the laboratory of Dr. Susan Morgello, utilizing the infrastructure of the MHBB. Additional markers may also be added based on the availability of funding (e.g. IFN-γ, IL1-β, IL-10, IL-18, TNF-α, sCD163, IL-18, HMGB-1, HO-1 and TLR4 mRNA quantitation).

The questionnaires will include: Survey of Autonomic Symptoms,[47] the Composite Autonomic Symptom Score (COMPASS),[56] Medical Outcomes Study (MOS-HIV) quality of life questionnaire.[57]

Every effort will be made to minimize risk to participants as follows. Risks to privacy will be minimized by conducting all procedures in a calm and private environment staffed by experienced and professional personnel, and by carefully explaining all procedures before they are performed, and by minimizing physical exposure. The confidentiality of participant data will be preserved by never removing research records from Mount Sinai, separating identifiable data from the source documents, physically locking up all paper source documents, physically and password locking all computer data, and restricting access to data to study personnel who need such access to do their job. The risks associated autonomic testing are minimized by excluding participants for whom transient alterations in blood pressure might be dangerous, for example those with certain medical conditions such as recent myocardial infarction. Syncope and pre-syncope during tilt-table testing are minimized by continuous monitoring by experienced personnel, and by clear instructions to the participant to report any symptoms. The risk of radiation exposure during the gastric emptying scintigraphy is minimized by performing the procedure according to established protocols, and by performing a pregnancy test in women of childbearing potential prior to the study. The risks associated with pyridostigmine are minimized by using a low dose, and by performing a careful medical evaluation prior to initiating therapy, including assessment for drug-drug interactions and contraindications. In addition, Dr. Robinson-Papp and study staff will make certain that participants know that they should contact the study team with any safety concerns at any time, and ensure that they know how to do so.

g) Specimen Banking

Blood samples identified by study ID only, will be stored within the laboratories of the MHBB. Other data will include the specimen type and the date of collection. This is not optional because specimens must be analyzed in batches in order to minimize cost. In addition, PBMCs from the blood samples will be frozen viably after Ficoll-Hypaque separation (as per MHBB protocol), so that HO-1 and TLR4 mRNA quantitation can be undertaken via rtPCR when funding becomes available to do so. These specimens may be stored until such funding is available. MHBB laboratory staff will have access to these specimens (as necessary to do their jobs) which will be clearly marked as separate from MHBB specimens. Specimens will not be released to investigators outside this study.

h) Data Management and Confidentiality

Source documents will be created during each study visit for each participant and stored in Dr. Robinson-Papp's locked office. Only study staff will have access to these paper records, and only as required to perform their roles. The participant demographics form and the informed consent document, which contain identifying data will be separated from the other documents. All other documents will be identified by the participant's study identification (ID) number only. De-identified data will be entered into a password protected database that is housed on a secure, encrypted, Mount Sinai network drive. No data will be sent out or received. All GI testing data will be reviewed for quality by Drs. Machac or Sam as appropriate. All laboratory data will be reviewed for quality by Dr. Morgello or her delegate. All autonomic testing data and all data overall will be reviewed by Dr. Robinson-Papp to assure its quality, and also by Dr. Benn who will look particularly for statistical evidence of errors e.g. outliers. In addition the database will be programmed to only accept data within an expected range, which minimizes error.

Eighty participants in phase 1 provide >80% power (α=0.05) for detecting a "Medium" effect size for both "paths" depicted in figure 1, where "Medium" corresponds to an effect accounting for 13% of the variance (as per Cohen's criteria). However since all phase 2 participants are drawn from phase 1, the sample size requirements of phase 2 and the expected percent eligibility must also be considered in choosing the phase 1 sample size. Forty phase 2 participants provide at least 80% power to detect a standardized difference of 0.45 in the change scores on any outcome. However this is a conservative calculation, since it is based on a paired t-test, and the mixed model to be used will reduce the estimate of the standard error by estimating the correlation between the repeated outcomes from the same subjects, thus providing a more powerful test. Thus our phase 2 enrollment target is about 40 participants. Based on our previous study we expect 45% of participants with HIV-AN to have vagal dysfunction, and based on a prior study of HIV-infected participants with GI symptoms, we expect a similar percentage with abnormal gastric emptying. Although the extent of overlap between these two groups is not known, in diabetics 80% of those with vagal dysfunction have gastric emptying abnormalities. Thus we estimate that about 35-40% of phase 1 participants will meet criteria for phase 2, and therefore 80-85 phase 1 participants should be sufficient. However we recognize that these estimates contain significant uncertainty, thus planning for up to 100 phase 1 participants provides a safety margin.

Primary measures for the analyses will be: vagal sub-score of mCASS (vagal dysfunction), GE T½ (GI dysmotility), IL-6 (immune activation), LPS (bacterial translocation), total hydrogen excretion in the breath (ppm x min) over 2 hours following ingestion of 50g glucose (SIBO). Secondary measures include: vagal baroreflex sensitivity; Valsalva ratio; heart rate variability with deep breathing; percent gastric emptying at 1-4h; CRP; sCD14. The remainder of the cytokines and inflammatory mediators described above will be used in exploratory analyses to generate hypotheses regarding the function of the cholinergic anti-inflammatory pathway and the effects of pyridostigmine in HIV. Covariates to be included in all analyses as appropriate are: age, gender, duration of known HIV infection,

current and nadir CD4+ cell count. All analyses will be two-tailed, conducted at the $\alpha = 0.05$ level, and adjusted for multiple comparisons as appropriate.

Specific Aim 1 is to determine whether vagal dysfunction is associated with immune activation in CART-treated participants with HIV-AN, and if so to estimate the extent to which this association is mediated by GI effects (i.e. slowed motility, SIBO, microbial translocation) versus direct effects of vagal dysfunction. Initially, Spearman rank correlations will be performed pairwise between IL-6, the vagal sub-score of mCASS, GE T½, breath hydrogen excretion, and LPS. If correlations are demonstrated we will use the method of direct acyclic graphs (DAG) to test the paths hypothesized in figure 1. This method can also be used to test whether any of the secondary measures of the constructs in the DAG support the hypothesized paths. The rules for testing the various paths in a DAG for significance are based on regression coefficients obtained from statistical models constructed using the particular DAG under investigation. Statistical testing of the regression coefficients will be carried out using PRODCLIN software. Power for the effects of mediation variables in the DAGs is estimated using tables provided by Fritz and MacKinnon. Since different DAGs have different numbers of variables in them, with many distinct possible paths, power is calculated for any given "triangle" formed by a mediator (M) between a predictor (X) and an outcome (Y). The power is given in terms of Small, Medium, and Large effect sizes for the paths.

Specific Aim 2 is to investigate whether pyridostigmine reduces immune activation and/or improves GI function, and if so to estimate the extent to which the reduction in immune activation is mediated by GI effects (i.e. improved motility, SIBO, microbial translocation) versus direct cholinergic effects. Following appropriate transformation of non-normally distributed variables, linear mixed models for longitudinal data will be used to evaluate the longitudinal trend in each of the following outcomes in participants who receive pyridostigmine: IL-6, GE T½, breath hydrogen excretion and LPS. In these models, the dependent variable will be the outcomes listed above, and the independent variables will be time (fixed effect) and subject (random effect). We will also construct a multivariate mixed linear model to estimate the contribution of each variable's change to the change in IL-6. In secondary analyses we will perform similar calculations using secondary measures of immune activation, GI motility, and translocation.

i) Provisions to Monitor the Data to Ensure the Safety of Subjects

Part I: Elements of a Data and Safety Monitoring Plan

1. Dr. Robinson-Papp is the principal investigator and will be the principle monitor responsible for monitoring the data to ensure the safety of all the participants in the study.

MSSM Principal Monitor:
Principal Investigator
Last Name: Robinson-Papp

First Name: Jessica
Academic Title: Assistant Professor
Department: Neurology
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Phone: 212-241-8390
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E-mail: jessica.robinson-papp@mssm.edu

MSSM Additional Monitor:
Independent
Last Name: Rodriguez-Caprio
First Name: Gabriela
Academic Title: Assistant Professor
Department: Medicine, Infectious Diseases
Mailing Address: Mount Sinai Medical Center, [redacted], New York, NY 10029
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2. Dr. Robinson-Papp is a neurologist with special expertise in caring for patients with HIV/AIDS and patients with neuromuscular disorders. She has experience with the study population and with the study drug and will be involved with the day to day activities of the study and is therefore the most suitable person to be the primary safety monitor, particularly since this is an open label study and so there is no difficulty regarding blinding. Dr. Rodriguez-Caprio is an internist and an infectious disease specialist, focusing in HIV/AIDS treatment. She is the medical director of the clinic from which a large proportion of the research participants will be drawn. Dr. Rodriguez-Caprio and Dr. Robinson-Papp work together at the clinic and have several mutual patients. The rationale for selecting Dr. Rodriguez-Caprio was that as the director of the clinic, and as a clinician not involved in the research, her primary interest is in the safety and wellbeing of the patient population. We also wanted an independent monitor with a general medical background so as to complement Dr. Robinson-Papp's more focused neurologic expertise.

3. Adverse events (AE) will be monitored for safety, including those events related and unrelated to the study procedures. All such events will be characterized as described in item 7, and documented in the AE log. In addition to AEs, self-reported adherence to study drug will be queried including the reason for any missed doses. Any drop-outs from the interventional phase will also be recorded, along with the reason.

4. Internal monitoring of subject participation will occur as the data is collected. With each study visit, at least one member of the team will have knowledge of all events occurring on the date of evaluation, and will alert the PI in real time to any AEs. In addition, the research team will meet each week for detailed review of all subjects seen that week. Informed consent documentation and subject eligibility is monitored and verified for each enrolled subject. Accumulated safety information will be reviewed by the PI and the additional monitor at least semi-annually.

5. Should an unanticipated event occur related to drug, the study will be halted while the need for modification is considered. A report will be provided both to the IRB and funding agency. If modifications are deemed necessary, these will also be presented to the IRB and funding agency for review and approval.

6. There are no dose selection procedures. The same dose (30mg PO tid) will be used for all participants throughout the entire 8-week interventional period of the study. This is a commonly used starting dose for pyridostigmine in clinical practice, and lower doses are not practicable due to the lack of smaller pill sizes. This low dose has been chosen to minimize toxicity.

7. AEs will be classified as mild, moderate or severe based upon the following criteria:
   - Mild: symptoms do not alter the subject's normal functioning.
   - Moderate: symptoms produced some degree of impairment to function but are not dangerous, uncomfortable or embarrassing to the subject.
   - Severe: symptoms are dangerous to the subject's well-being and cause significant impairment of function or incapacitation.

The relationship of the AE to the treatment will be classified as follows:
   - Related: there is good reason and sufficient evidence to assume that there is a causal relationship with the treatment.
   - Not related: there is good reason and sufficient evidence to exclude a causal relationship with the treatment.

All AEs will require the investigator to obtain adequate information to determine the outcome of the AE and to assess whether it meets criteria as a serious adverse event (SAE), which requires immediate notification to the funding agency and IRB. The investigator will obtain sufficient information to determine the causality of the AE (i.e. treatment or other cause); and provide an opinion on causal association (i.e., whether they consider the AE related or not related to the study intervention). Subjects will receive follow up until the AE either resolves completely or stabilizes to a level acceptable to the PI.

All SAEs will be reported within 24 hours of the investigator's knowledge of the event to the IRB and/or funding agency. An SAE is any AE that fulfills one of the following criteria:

- Results in death
- Is life threatening and places the subject at immediate risk of death
- Results in hospitalization
- Results in significant disability/incapacity, where the disability is a substantial disruption of a person's ability to conduct normal daily life functions
- Is an important medical event that may not result in death, be life threatening, or require hospitalization, but which based upon the appropriate medical judgment may require medical and/or surgical intervention to prevent one of the outcomes listed above

8. All GI testing data will be reviewed for quality by Drs. Machac or Sam as appropriate. All laboratory data will be reviewed for quality by Dr. Morgello or her delegate. All autonomic testing data and all data overall will be reviewed by Dr. Robinson-Papp to assure its quality (i.e. accuracy and completeness), and also by Dr. Benn who will look particularly for statistical evidence of errors e.g. outliers. In addition the database will be programmed to only accept data within an expected range, which minimizes error.

9. Should a temporary or permanent suspension of the study occur, this will be reported to the PPHS/IRB and the funding agency.

A data safety monitoring board will not be formed.

j) Withdrawal of Subjects

A subject may be withdrawn from the research without their consent if they exhibit behavior that is disruptive to study procedures. Prior to such a termination, the patient will be assessed by Dr. Robinson-Papp, and then informed of the termination in a calm, secure and private setting, and the encounter documented in writing. If termination occurs further data collection will not continue. However we anticipate that our familiarity with this patient population, close collegial relationship with JMFC primary care providers and the screening process will help us avoid this scenario. Participants may also be withdrawn without their consent if continuation in the study is deemed inappropriate due to a change in medical condition, for example if a subject becomes acutely ill and is hospitalized.

Participants may voluntarily withdraw from the protocol at any time. A member of the study staff will meet privately with any participant wishing to withdraw in order to determine the reason if possible, and also to determine if the participant would be willing to consider partial withdrawal, which might include termination of the intervention, but completion of the remaining study assessments.

6) Risks to Subjects

The procedures involved in the research include: documentation of medical history, physical/neurologic exam, answering of questionnaires, autonomic testing, gastric

emptying scintigraphy, hydrogen breath testing, collection of blood and urine, answering of questionnaires, and for a subset of participants taking pyridostigmine 30mg PO tid for 8 weeks.

We do not foresee significant psychological, social, financial, legal or economic risks. The main risks of documentation of medical history, physical/neurological exam, and answering of questionnaires relate to issues of privacy and confidentiality. This is discussed in sections 5h and 9.

The potential physical risks to subjects include those associated with venipuncture, gastric emptying scintigraphy, hydrogen breath testing, autonomic testing and in a subset of participants: pyridostigmine. Risks associated with venipuncture include pain and bruising. The discomfort associated with the insertion of the needle is likely to be experienced by most or all participants, more significant pain is unlikely. Mild bruising may occur in a minority of participants, more significant bruising is unlikely. The main risk of gastric emptying scintigraphy is that of radiation exposure. All participants will be exposed, but the small amount of radiation involved is not considered to be clinically significant. There is essentially no significant risk associated with hydrogen breath testing. There are some physical risks associated with autonomic testing. Valsalva maneuver causes brief (< 1 minute) alterations in blood pressure which are unlikely to be of clinical significance. Tilt-table testing may cause hypotension in subjects with autonomic dysfunction leading to unpleasant pre-syncopal symptoms or rarely, syncope. Pre-syncopal symptoms may occur in a minority of participants, syncope is considered unlikely because subjects are immediately returned to the supine position if there are changes in blood pressure or pre-syncopal symptoms. Other potential risks of autonomic testing include mild discomfort associated with lying supine for a prolonged period of time and local skin discomfort or irritation from the quantitative sudomotor axon reflex testing. These events are considered moderately likely, but not very burdensome. Loss of confidentiality is also a potential risk with these procedures but is considered unlikely, and of low burden.

Pyridostigmine has been in clinical use for over 50 years, primarily in the treatment of myasthenia gravis (MG). The dose used in this study is a common starting dose, the maintenance dose used clinically is usually substantially higher and is taken for life. MG is a neurologic autoimmune disease that typically requires long-term treatment with immunosuppressive medications and can affect children and adults of all ages. MG patients often experience medical co-morbidities including other autoimmune conditions and the effects of long term iatrogenic immunosuppression. The population in this study is similar to the MG population in that they are medically complicated adults with varying degrees of immunosuppression. However well-controlled HIV is an inclusion criterion for this study, and so we will not have participants with advanced immunosupression.

According to the FDA, there are no known long-term side effects of pyridostigmine (http://www.fda.gov/Drugs/EmergencyPreparedness/BioterrorismandDrugPreparedness/uc

m130343.htm#8). Possible short term side effects included in the prescribing information are: stomach cramps, gas, diarrhea, nausea, increased urge to urinate, drooling, sweating, headaches, dizziness, eye tearing, blurred vision, runny nose, shortness of breath, acid stomach, including heartburn or reflux, tingling, muscle twitching, weakness, or cramping. The incidence of these side effects is not reported in the prescribing information as it would be for a more recently developed drug, however clinical experience suggests that side effects are fairly infrequent at the low dose used in this study. When side effects do occur, they are short-lived, resolving within a few hours or less. We expect the dose used in this study to be well-tolerated. An excerpt from the drug brochure regarding adverse effects is included below for completeness:

"The side effects of pyridostigmine bromide are typically of two varieties, muscarinic and nicotinic. Muscarinic side effects include abdominal cramps, bloating, flatulence, diarrhea, emesis, increased peristalsis, nausea, hypersalivation, urinary incontinence, increased bronchial secretion, diaphoresis, miosis, and lacrimation. Nicotinic side effects are comprised chiefly of muscle cramps, fasciculations, and weakness. Pyridostigmine is a quaternary ammonium compound and does not readily cross the blood-brain barrier. Compared to the peripheral effects of pyridostigmine bromide, central nervous system manifestations are less frequent and less serious, primarily consisting of headache and vertigo, with minor and clinically insignificant changes in heart rate, blood pressure, and respiratory function. Extremely high doses may produce CNS symptoms of agitation, restlessness, confusion, visual hallucinations, and paranoid delusions. Electrolyte abnormalities, possibly resulting from high serum bromide concentrations, also have been reported. Death may result from cardiac arrest or respiratory paralysis and pulmonary edema.

"In a controlled study of 90 healthy volunteers comparing pyridostigmine 30 mg every 8 hours to placebo for 21 days, the following incidence of adverse events was reported.

Table 1 Incidence of Adverse Events

Event:	% Pyridostigmine N = 60	% Placebo N = 30
Diarrhea	7	0
Abdominal Pain	7	0
Dysmenorrhea	5	0
Twitch	3	0
Myalgia	2	0
Dry Skin	2	0
Urinary Frequency	2	0
Epistaxis	2	0

Amblyopia	2	0
Hypesthesia	2	0
Neck pain	2	0

Other less common adverse events seen during controlled and uncontrolled clinical trials for pyridostigmine include the following:
· Pulmonary: Exacerbation of acute bronchitis and asthma
· Cardiovascular: Elevated blood pressure, decreased heart rate (4-6 beats per minute), chest tightness
· Eyes: Change in vision, eye pain
· Neurologic: Headache, hypertonia, difficulty in concentrating, confusion, disturbed sleep, tingling of extremities, numbness of the tongue
· Skin: Increased sweating, rash, alopecia
· Digestive: Vomiting, borborygmi, nausea, bloating, flatulence
· General: Warm sensation, lethargy/drowsiness, depressed mood

During safety studies at the recommended dosage, there were two reports of loss of consciousness, one of which also included urinary and fecal incontinence, stiffness of the upper torso and arms, post syncopal skin pallor, post syncopal confusion, and post syncopal weakness (suggesting a seizure event).As with any compound containing bromide, a skin rash may be observed in an occasional patient, which usually subsides promptly upon discontinuance of the medication."

7) Provisions for Research Related Harm/Injury

The study team will be available for the participant should any anticipated or unanticipated injury occur. The team will assess the patient and facilitate referral to the appropriate medical care. All necessary care will be billed in the manner customary for clinical services, i.e. to the patients' insurance and/or the patients themselves as applicable.

8) Potential Benefits to Subjects

Individual participants may experience no benefit. Those treated with pyridostigmine may benefit from reduction in inflammation or amelioration of GI symptoms. Others may experience satisfaction from contributing to scientific research in HIV. Some participants may benefit if undiagnosed neurologic or GI conditions are revealed by study related procedures.

9) Provisions to Protect the Privacy Interests of Subjects

As described above in the recruitment section, participants will be sought from three main sources: the Jack Martin Fund Clinic (JMFC), other research studies in which Dr. Robinson-Papp is an investigator, and Dr. Robinson-Papp's clinical practice. In recruiting subjects, consideration must be given to a person's desire to control how, and with whom, they interact and communicate.

Thus it is recognized that not all potentially eligible patients may wish to communicate with the study team. Dr. Robinson-Papp will pre-screen potential participants who are known to her to determine whether (based on previous interactions) such participants are likely to be eligible and to welcome contact with the study team. For potential participants not known to Dr. Robinson-Papp, the first point of contact will be health care provider, who will be asked to make a preliminary assessment of eligibility and of willingness to be contacted. In all cases the initial contact will seek first to establish interest in hearing about the study, prior to initiating a more lengthy communication regarding the details. Care to respect the participants' privacy will be taken in this, and all subsequent communications. No specific information will ever be left with family members or in voice messages.

Throughout the study, ensuring that participants feel comfortable with the study staff and procedures will be a high priority. It is our practice to carefully review all procedures (including timing and the necessary space, staff and equipment) for each study visit in advance so that the visits are well organized. An organized and punctual visit helps put participants at ease. All procedures will be conducted in a calm and private environment staffed by experienced and professional personnel. All procedures will be carefully explained before they are performed, and physical exposure will be minimized. Staff always endeavors to be caring and respectful in their communications with participants.

It is acceptable and appropriate for members of the research team to approach prospective participants because we are very familiar with the population and have expertise in recruiting here in an inoffensive manner. Furthermore, it is our experience that this population usually enjoys participating in research, but also feels empowered to decline if the subject is not of interest to them.

10) Economic Impact on Subjects

In the event of a study-related adverse event or injury, subjects will be responsible for any expenses not covered by health insurance.

11) Payments to Subjects

Participants will be reimbursed $150 for completing the screening and baseline visits. If prior to completing the baseline visit they are deemed a screen failure then this payment will be prorated as follows: $20 for screen failure prior to autonomic testing, $50 for screen failure after autonomic testing. Participants who enter the interventional phase will receive $20 for visits 1, 2 and 4 and $100 for visit 3. All reimbursements are paid in cash at each visit.

12) Consent Process

Typically initial contact with a potential participant will be by phone. Once the potential participant expresses interest in the study, an informed consent document will be mailed to

their home for their review (with their permission), and an in person visit will be scheduled. The informed consent document will contain an explanation of all study procedures, including risks and benefits, explained in lay language. The informed consent process will be performed at the time of the study visit by the PI or a delegate. The delegate must have fulfilled all the mandatory regulatory requirements and training, have had additional specific training in the protocol, and have undergone a period of observation by the PI. At the time of the visit, the informed consent document will be reviewed with the participant in a quiet, private setting to ensure understanding of the protocol and willingness and ability to adhere to the study procedures. This is done by engaging the potential participant in a dialogue about the study, asking the participant to reiterate protocol procedures, and encouraging the participant to ask questions. This process is then documented in a narrative progress note and in the signed consent document. The team will adhere to "SOP HRP-090 Informed Consent Process."

The research will not include children, or cognitively impaired adults who are unable to provide informed consent for themselves.

Non-English Speaking Subjects

In general subjects will be required to read and understand English, but should a participant be eligible whose primary language is Spanish, a Spanish consent form would be provided to the PPHS for review in order to include these participants in the study. Also, a translator would be arranged for all of the study visits, because the study team does not have a member fluent in Spanish. We do not have significant numbers of patients in our population who speak neither English nor Spanish.

Waiver or Alteration of the Consent Process

We are requesting a waiver of consent (and a HIPAA waiver of authorization) to access data for the purposes of pre-screening from the Mount Sinai Data Warehouse (MSDW). As described above in the recruitment section, we currently have a de-identified dataset from the MSDW that includes all JMFC patients (N≈700) with at least 3 visits between 11/1/13-10/31/14, who are treated with CART. The dataset, which was assembled for an unrelated project, includes several variables which are useful for pre-screening for this study. We are requesting a waiver of informed consent in order to obtain from the MSDW a list of potentially eligible patients, which will include names, medical record numbers, and the name of the prescriber of their HIV-medications (who is very likely to be the primary care provider) for the patients in our list who have an undetectable HIV-1 viral load, no urine toxicology positive for opiates/opioids or other illicit substances, no diagnosis of diabetes, no diagnosis of peptic ulcer disease, and no prescriptions for opiate/opioid medications. This information will be requested without linkage of the identifying information to the de-identified information so as to minimize the amount of PHI obtained. Also we are using the search to exclude patients who have certain characteristics (e.g. a particular diagnosis, test result, or medication), and so we will have very little specific information about the patients who are included on the list. The process of contacting the patients identified by this process is described above in the recruitment section.

We believe that this portion of the protocol meets the requirements set forth in criteria set #2 of the HRP-415 checklist, namely:

- This portion of the research is not FDA-regulated.
- This research does not involve non-viable neonates.
- This portion of the research involves no more than minimal risk to the subjects, namely a contact from the research team, after obtaining permission from their healthcare provider.
- The waiver will not adversely affect the rights and welfare of the subjects.
- The research could not be practicably carried out without the waiver. In order to be able to identify a sufficient number of potential participants, while remaining within the resources of the project, an automated pre-screening process is necessary.
- There will be no new/timely information discovered by this process that would be pertinent to reveal to participants.

13) Process to Document Consent in Writing

Consent will be documented in writing using the standard PPHS consent template. The team will adhere to "SOP HRP-091 Written Documentation of Consent."

14) Vulnerable Populations

As indicated in the table below, vulnerable populations will not be included.

Include	Exclude	Vulnerable Population Type
	X	Adults unable to consent
	X	Individuals who are not yet adults (e.g. infants, children, teenagers)
	X	Wards of the State (e.g. foster children)
	X	Pregnant women
	X	Prisoners

15) Multi-Site Human Research (Coordinating Center)

N/A.

16) Community-Based Participatory Research

N/A.

17) Sharing of Results with Subjects

Results that are deemed to be clinically relevant by the PI will be shared with the participant. Permission will also be sought from the participant to share such results with their primary care provider.

18) External IRB Review History

N/A.

19) Control of Drugs, Biologics, or Devices

The Mount Sinai research pharmacy will provide the study drug to the participants. Accountability and destruction will also be managed by the research pharmacy.

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