Protocol Title: Does N-Acetylcysteine Decrease Spontaneous Oxidation of Central Neural Dopamine in Parkinson's Disease?
Abbreviated Title: NAC Protocol
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Human Research Protections Program Investigator and Staff Training: For this protocol, the following "Just in time" human subjects protection training courses are required for investigators and staff: None.

Total requested accrual
35 Patients
6 Healthy Volunteers

Project Uses Ionizing Radiation: ☐ No ☒ Yes
- ☐ Medically-indicated only
- ☒ Research-related only
- ☐ Both

IND/IDE ☒ No ☐Yes

Durable Power of Attorney ☒ No ☐ Yes
Multi-institutional Project ☒ No ☐ Yes
Data and Safety Monitoring Board ☒ No ☐ Yes

Technology Transfer Agreement ☒ No ☐ Yes

Samples are being stored ☐ No ☒ Yes

Flesch-Kincaid reading level of consent form: 8.8

Précis:

Objective: This study is to test whether N-acetylcysteine (NAC) inhibits the spontaneous oxidation of central neural dopamine as indicated by the cerebrospinal fluid (CSF) concentration of 5-S-cysteinyl-dopamine (Cys-DA) in patients with Parkinson's disease (PD).

Study population: The study population comprises up to 35 patients with early (≤ 5 years from diagnosis), mild, levodopa-untreated PD. The patients will be on an inhibitor of monoamine oxidase (MAO) that is prescribed for their disease. There is also a pilot study of healthy volunteers (HVs), to measure biological variability of CSF Cys-DA levels.

Design: The study has a one group, pretest-posttest design. Each patient undergoes a lumbar puncture (LP) as an inpatient at the NIH Clinical Center to obtain cerebrospinal fluid (CSF) for assays of Cys-DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and related biochemicals. The second LP is done after the patient has taken at least 5 doses of NAC (2 grams orally twice per day). The LP takes place about 2 hours after the last NAC dose. For the purpose of establishing biological variance and thereby carrying out a meaningful power analysis, we will study up to 6 HVs who undergo 2 LPs as inpatients, with 48 hours between LPs and no NAC treatment. In the study of HVs the main outcome measure is the biological variation of CSF Cys-DA between the 2 LPs.

Outcome measures: The main outcome measure is the CSF concentration of Cys-DA. Other outcome measures are levels of other catecholamine-related neurochemicals or of indices of oxidative stress. Depending on the results, an exploratory study may be done involving NAC at 1 gram orally twice per day. In the pilot study of HVs the main outcome measure is the biological variation of CSF Cys-DA between the 2 LPs.

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List of Abbreviations

ALDH = aldehyde dehydrogenase

AR = aldehyde/aldose reductase

CC = NIH Clinical Center

CSF = cerebrospinal fluid

DA = dopamine

DAT = cell membrane dopamine transporter

Cys-DA = 5-S-cysteinyldopamine

Cys-DOPA = 5-S-cysteinylDOPA

DOPAC = 3,4-dihydroxyphenylacetic acid

DOPAL = 3,4-dihydroxyphenylacetaldehyde

IT = Information Technology

IRB = Institutional Review Board

LAAAD = L-aromatic-amino-acid decarboxylase

LP = lumbar puncture

MAO = monoamine oxidase

MTA = Material Transfer Agreement

NAC = N-acetylcysteine

PD = Parkinson's disease

VMAT2 = Type 2 vesicular monoamine transporter

1. Introduction and Background

PD is a major public health burden. Parkinson's disease (PD) is one of the most prevalent neurodegenerative diseases, affecting about a million people in the United States. PD is aging related, and the incidence of PD is likely to increase as the population ages. PD therefore poses a public health burden that likely will grow over time. Mortality ratios in PD are about twice those in the general population.

Dopamine deficiency causes the movement disorder in PD. The proximate cause of the movement disorder that characterizes PD results from deficiency of the catecholamine, dopamine, in the nigrostriatal system—especially in the putamen (1). Effective symptomatic treatments for PD work directly or indirectly by countering the effects of nigrostriatal dopamine deficiency. Levodopa, the precursor of dopamine, combined with a peripheral inhibitor of L-aromatic-amino-acid decarboxylase to attenuate conversion of levodopa to dopamine outside the central nervous system, is the mainstay of treatment.

There is no known means to slow the catecholaminergic neurodegeneration in PD. The mechanisms of nigrostriatal dopamine depletion PD are incompletely understood, and there is no proven treatment or prevention strategy.

The catecholamine autotoxicity theory proposes a third determinant of catecholaminergic neurodegeneration besides genes and environment. It is widely presumed that PD is caused by complex interactions between genes and environment. An additional element may be catecholamine "autotoxicity" (Figure 1). According to the autotoxicity theory, catecholamines can be turned into "suicide chemicals" that kill the neurons that contain them (2).

There are two general proposed mechanisms of autotoxicity. Autotoxicity as applied to dopaminergic neurons in PD occurs by two routes (Figure 1). The first is via spontaneous oxidation of cytoplasmic dopamine to form reactive oxygen species and dopamine-quinone (DA-quinone). DA-quinone can undergo rearrangement to form aminochrome and can bind covalently with glutathione or cysteine to form 5-S-cysteinyl-DA (Cys-DA); both aminochrome and Cys-DA are toxic (3, 4).

The second—and likely predominant—route of autotoxicity is via enzymatic oxidation of cytoplasmic dopamine, catalyzed by monoamine oxidase (MAO), to form hydrogen peroxide and 3,4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL can bind covalently to DA to form tetrahydropapaveroline (5), react with hydrogen peroxide and divalent metal cations to produce hydroxyl radicals (6) ,or oxidize spontaneously to form reactive oxygen species and DOPAL-quinone (7). DOPAL-quinone, in turn, readily forms adducts with lysine residues in proteins, thereby altering their functions (8) or causing them to oligomerize (9).

The catecholaldehyde hypothesis is a derivative of the autotoxicity theory. All of the enzymatic metabolism of intra-neuronal dopamine passes through DOPAL. DOPAL, like

other intracellular aldehydes, is extremely toxic but is detoxified by ALDH to form the non-toxic acid, 3,4-dihydroxyphenylacetic acid (DOPAC), which is actively pumped out of the neuron. Post-mortem neurochemical studies have shown that patients with PD have decreased ALDH activity and DOPAL buildup in the putamen (10). The catecholaldehyde hypothesis proposes that DOPAL buildup causes or contributes to the death of the nigrostriatal dopaminergic neurons in PD (11).

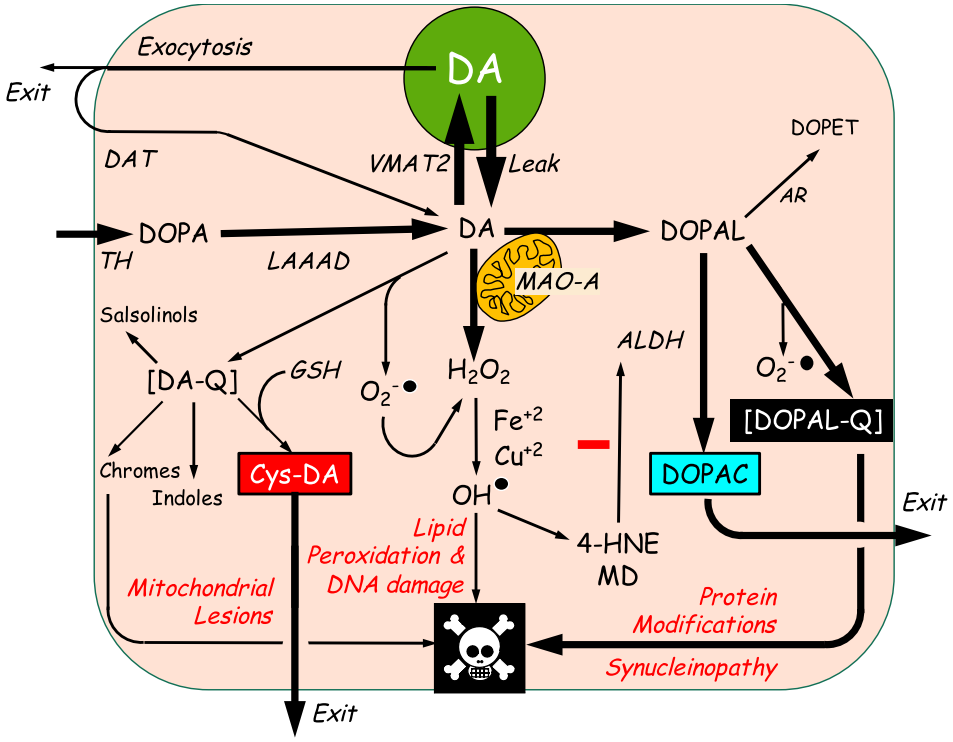


Figure 1: Overview of the catecholamine autotoxicity theory. *The theory explains PD in terms of toxic effects of products of enzymatic and spontaneous oxidation of cytoplasmic dopamine. Levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-S-cysteinyldopamine (Cys-DA) in cerebrospinal fluid reflect these processes.*

DOPAL toxicity depends on its spontaneous oxidation. Accumulating evidence indicates that DOPAL toxicity depends importantly on its spontaneous oxidation to DOPAL-quinone (9, 12). Inhibiting DOPAL formation, in series with inhibiting DOPAL oxidation, would then be expected to decrease catecholamine autotoxicity and slow the loss of catecholaminergic neurons.

MAO inhibition decreases DOPAL formation. Examination of the concept diagram in Figure 1 leads straightforwardly to the prediction that inhibiting MAO should decrease DOPAL formation, and this is the case (13). There are two isoforms of MAO, MAO-A and MAO-B, and dopaminergic neurons exclusively express MAO-A. At first glance, testing the catecholaldehyde hypothesis would seem to require an MAO-A inhibitor. We wish to avoid using MAO-A inhibitors, because these drugs predispose to paroxysmal

hypertension upon ingestion of common tyramine-containing dietary constituents such as red wine and hard cheese (the "cheese effect").

MAO-B-inhibitors decrease MAO-A activity in humans. The MAO-B inhibitors selegiline and rasagiline are not associated with the cheese effect (14). These drugs decrease plasma levels of DOPAC and 3,4-dihydroxyphenylglycol, consistent with MAO-A inhibition in sympathetic nerves (13, 15). Selegiline also decreases CSF levels of DOPAC (16), and when administered clinically as a transdermal patch or effervescent tablet, selegiline decreases brain MAO-A activity in humans (17). We have found preliminarily that rasagiline decreases CSF DOPAC (unpublished observations). Therefore, a clinical trial to test the catecholaldehyde hypothesis could be based on MAO-B inhibition.

Treatment with an MAO inhibitor alone might trade off one form of toxicity for another. Large multi-center clinical trials of MAO-B inhibitors have failed to show convincing evidence that this treatment slows the progression of symptomatic PD (18). A variety of explanations have been offered for this failure (19). A relatively recent one is based on our finding that MAO inhibition, while decreasing DOPAL formation, increases spontaneous oxidation of dopamine (13). Treatment with an MAO inhibitor alone might be ineffective because of concurrently decreased enzymatic oxidation and increased spontaneous oxidation of cytoplasmic dopamine.

N-Acetylcysteine (NAC) is promising as an anti-oxidant that may be bioavailable to central dopamine neurons. NAC is a glutathione precursor and well established anti-oxidant. NAC is an FDA approved drug to treat acetaminophen overdose and is marketed in the United States as a dietary supplement. Clinical trials of NAC in PD are under way (20). In humans NAC increases glutathione levels in the brain (21), and orally administered NAC appears unchanged in the cerebrospinal fluid (CSF) (22). Basic cellular studies have shown that NAC or glutathione attenuates DOPAL-induced protein reactivity (12) and virtually prevents DOPAL-induced oligomerization of alpha-synuclein (9). A small clinical trial of NAC in PD has preliminarily noted clinical and laboratory improvement (20).

NAC could mitigate dopamine autotoxicity (Figure 2). NAC should decrease the toxicity of DOPAL by inhibiting formation of DOPAL-quinone. Moreover, NAC should attenuate the increase in spontaneous oxidation of dopamine to form Cys-DA and increase availability of DOPA to form dopamine.

Whether NAC is bioavailable to central dopaminergic neurons in humans and exerts an anti-oxidant effect in those neurons is unknown. This "proof of concept" study is designed to test the hypothesis that NAC decreases spontaneous oxidation of central neural dopamine in patients with PD.

Testing the main hypothesis of this study requires a means to assess spontaneous oxidation of dopamine *in vivo* in the human brain.

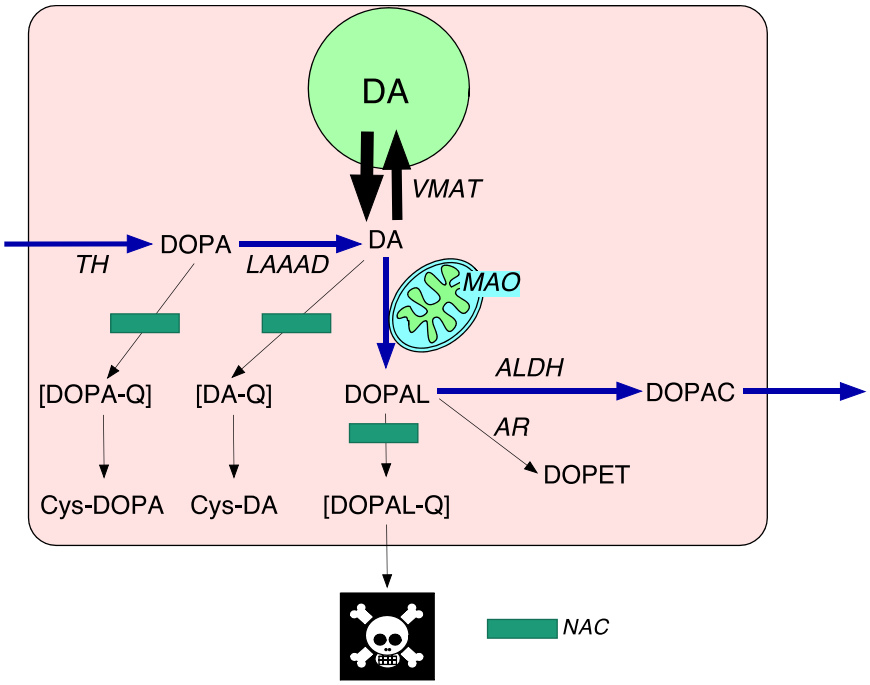


Figure 2: Possible effects of NAC-related anti-oxidation in central dopaminergic neurons. *N-Acetylcysteine (NAC) should inhibit the spontaneous oxidation of DOPA to DOPA-quinone (DOPA-Q), dopamine to dopamine-quinone (DA-Q), and DOPAL to DOPAL-quinone (DOPAL-Q). DOPA-Q is converted to 5-S-cysteinyldopa (Cys-DOPA), and DA-Q is converted to 5-S-cysteinyldopamine (Cys-DA). The level of Cys-DA in cerebrospinal fluid provides a biomarker of spontaneous oxidation of dopamine in the brain.*

Cys-DA in cerebrospinal fluid (CSF) provides a biomarker of spontaneous oxidation of central neural dopamine. Cys-DA is produced from the spontaneous oxidation of cytoplasmic dopamine. We have found that Cys-DA is present in the CSF of healthy humans and patients with PD (23). Our laboratory has the unique capability of simultaneously measuring CSF levels of Cys-DA and of DOPAC. Since DOPAC is produced by the action of MAO on cytoplasmic DA, measuring CSF Cys-DA and DOPAC enables a way to track both spontaneous and enzymatic oxidation of DA. Cys-DA in CSF is probably not derived from released DA, because released DA is highly efficiently metabolized by catechol-O-methyltransferase. Since the vast preponderance of DA production in the brain is in the striatum, most of the Cys-DA in CSF probably reflects a basal ganglia source. Measurement of Cys-DOPA/DOPA is another way to examine oxidative stress in central catecholaminergic neurons.

In this study we will test whether NAC at a dose of 4,000 mg per day decreases CSF Cys-DA levels. The following information is provided as background to justify the chosen dosing regimen of NAC.

NAC is available in different forms and doses. Effervescent and liquid forms of NAC have equivalent plasma bioavailability (24); however, liquid forms have a bad odor and are less well tolerated.

Cetylev™ (500 mg and 2.5 grams), a brand of effervescent, quick-dissolving, flavored tablets, is a trademark of Arbor Pharmaceuticals (Atlanta, GA). Cetylev is FDA approved as an antidote for acetaminophen overdose. Since Cetylev is FDA approved, the company manufactures the drug according to Good Manufacturing Practices (GMP), and Arbor can provide a Certificate of Analysis to verify the identity and potency of the drug. For the clinical indication of acetaminophen overdose, a loading oral dose of 140 mg/kg is given, followed by maintenance doses of 70 mg/kg every 4 hours for a total of 17 doses. Cetylev is available as 500 mg and 2.5 g tablets.

An alternative source is PharmaNAC™ (900 mg), another brand of effervescent, quick-dissolving, flavored tablets. PharmaNAC™is a trademark of BioAdvantex Pharma (Mississauga, Canada). BioAdvantex Pharma voluntarily complies with essential elements of GMP, from the production of raw materials to the packaging of supplements for distribution. BioAdvantex Pharma has a warehouse in Buffalo, NY.

In the study by Katz et al. (22), the FDA approved liquid form of NAC (20% solution, USP verified) was purchased from a contracting pharmaceutical distributor. A capsule form was compounded by UCSF Medical Center's Drug Product Services Laboratory in the Department of Clinical Pharmacy, with NAC active ingredient purchased in powder form (USP verified) from Professional Compounding Centers of America (the PCCA Baltimore Distribution Center is in Elkridge, MD). Oral NAC doses of 7 mg/kg, 35 mg/kg, or 70 mg/kg for two days, twice each day, were given to PD patients (22).

In the study by Monti et al. (20), both IV and oral NAC was given to PD patients for 3 months. The oral dose was 600 mg tablets twice per day on the days that patients did not receive IV NAC.

In the clinical trial, "N-Acetylcysteine for Neuroprotection in Parkinson's Disease (NAC for PD) by Shungu et al. (ClinicalTrials.gov NCT01470027), 1,800 or 3,600 mg doses of effervescent NAC tablets are given for 30 days.

A recent review and meta-analysis summarized dosing regimens in clinical trials using NAC for a variety of conditions such as schizophrenia, bipolar disorder, attention deficit hyperactivity disorder, post-traumatic stress disorder, amyotrophic lateral sclerosis, impulse control disorder, and Alzheimer's disease (but not PD) (25). A wide range of NAC doses have been given, over periods from several days to more than 2 years. All the clinical trials have used doses far less than those used for acetaminophen toxicity. Some clinical trials have given NAC on a per kg basis; most have given a constant dose regardless of body mass. Of 50 clinical trials, 8 used <1 gram of NAC per day, 12 1-2 grams/d, 17 2-3 grams/d, 7 3-4 grams/d, and 6 ≥4 grams/d.

The study by Hoffer et al. (26) used a 4 gram load, then 2 grams bid for 4 days. Since we want to use a relatively high dose to minimize the chance of false negative results, we will use the regimen as in Hoffer et al., except without the 4 gram loading dose.

The biological variabiity of CSF Cys-DA is unknown. An estimate of test-retest variation in CSF Cys-DA is needed in order to carry out an informed power analysis.

Preliminary information: CSF Cys-DA data from 2 patients treated with NAC and 4 HVs who underwent LPs on 2 different occasions under a different protocol are shown in Figure 3. Across the 6 subjects, the correlation coefficient (r value) for the scatter plot relating CSF Cys-DA for the second LP vs. CSF Cys-DA for the first LP was 0.99 (p=0.0002). We predict that in the present study the r value will be even closer to 1 because of the more tightly controlled situation for the 2 LPs. Based on this preliminary information, the reproducibility of CSF Cys-DA is excellent.

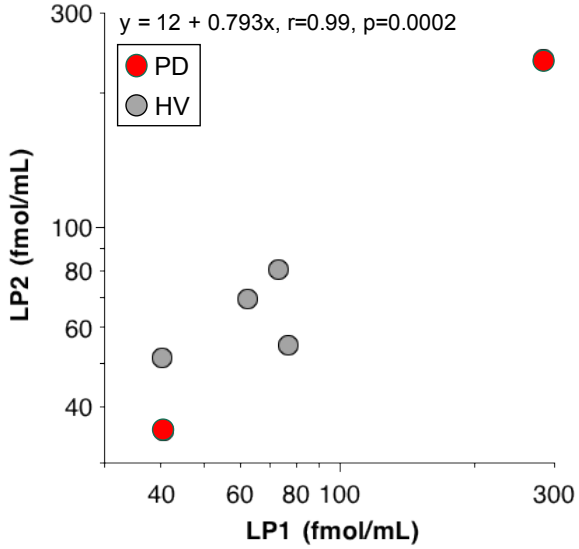


Figure 3: Scatter plot relating CSF Cys-DA in a second LP to that in a first LP in 2 PD patients (red) and in 4 HVs studied under a different protocol (gray). *The linear regression equation for the line of best fit, r value, and p value are shown. Note excellent reproducibility of CSF Cys-DA.*

2. Study Objectives

This study is designed to test whether NAC decreases spontaneous oxidation of central neural dopamine in patients with PD.

2.A. Primary objective: The primary objective is to test whether NAC at a dose of 4,000 mg per day decreases CSF Cys-DA levels.

2.B. Secondary objectives: A secondary objective is to evaluate levels of CSF Cys-DA and other catecholamine-related compounds or indices of oxidative stress in CSF or plasma before and after NAC administration. We will also explore whether at a lower dose (2,000 mg per day) NAC produces similar results to those seen with the 4,000 mg per day dose. In a pilot study of HVs we will assess the test-retest variability of CSF Cys-DA.

3. Subjects

3.A. Description of study populations: The study population is patients with PD who are taking an MAO inhibitor for their disease and are not on levodopa. The Planned Enrollment Report for this small study takes into account demographic differences in the prevalence of the disease under study.

The pilot study includes adult HVs.

3.A1. Accrual ceiling: 41.

3.A2. Target number of completers: 10 patients, 6 HVs. Withdrawals/dropouts will be replaced.

3.A3. NIH employees may participate. NIH employees who are subordinates/relatives/co-workers of investigators may not participate.

3.B. Inclusion criteria:

3.B.a. Inclusion criteria for PD Patients

- PD diagnosed within the past 5 years
- Taking a monoamine oxidase (MAO) inhibitor
- Able to provide consent
- At least18 years old

3.B.b. Inclusion criteria for Healthy Volunteers

- Able to provide consent
- At least18 years old

3.C. Exclusion criteria:

3.C.a. Exclusion criteria for PD Patients

- Taking levodopa in any form
- Known allergy to NAC
- A condition that would increase risk from a lumbar puncture (e.g., symptomatic spinal stenosis or myoclonus)
- History of a post-spinal headache that required treatment with a blood patch
- On a prescribed anti-coagulant (e.g., Coumadin, Plavix)
- Pregnant or breast-feeding
- History of alcohol or drug abuse
- Any medical condition that could put subjects at increased risk. Potential participants are excluded who have evidence of bone marrow, liver, or kidney failure based on abnormal screening lab results.
- On a medication that could interfere with the scientific results. An example of an exclusionary drug is the catechol-O-methyltransferase inhibitor Entacapone, Tricyclic anti-depressants are another type of exclusionary drug.

3.C.b. Exclusion criteria for Healthy Volunteers

- A condition that would increase risk from a lumbar puncture (e.g., symptomatic spinal stenosis or myoclonus)
- History of a post-spinal headache that required treatment with a blood patch
- On a prescribed anti-coagulant (e.g., Coumadin, Plavix)
- Pregnant or breast-feeding
- History of alcohol or drug abuse
- Any medical condition that could put subjects at increased risk. Potential participants are excluded who have evidence of bone marrow, liver, or kidney failure based on abnormal screening lab results.

3.D. Eligibility Checklists: Eligibility Checklists for Patients and for HVs based on the inclusion/exclusion criteria are attached **(25.B.).** The checklists will be used by investigators at the time of pre-screening communications by phone or secure e-mail or at the time of screening for admission to the protocol. E-mail correspondence with potential participants for pre-screening will be via a secure system.

4. Study Design and Methods

4.A. Study overview: The site is the NIH Clinical Center.

4.A.1. Study overview for patients: There is a screening visit and a single inpatient stay. Subjects are studied as inpatients, while on a prescribed MAO inhibitor. Each patient undergoes a lumbar puncture (LP) as an inpatient. A second LP is done after the patient has taken at least 5 doses of NAC over at least 48 hours. The inpatient stay is for up to 8 days. The inpatient stay is less than 8 days if the participant does not develop a post-spinal headache after the second LP. The patient continues his or her MAO inhibitor treatment throughout the hospital stay. The same dose and regimen of MAO inhibitor are used throughout the study in a given patient. This study is not related to other protocols. Subjects are not required to participate in another protocol. There are no follow-up visits under this protocol.

4.A.2. Study overview for HVs: There is a screening visit and an inpatient stay. Each HV undergoes a lumbar puncture (LP) as an inpatient, followed 2 days later by a second LP as an inpatient. While on study, HV subjects are not allowed to participate in another protocol outside the protocols of the Clinical Neurocardiology Section. There are no follow-up visits under this protocol.

4.B. Recruitment: Accruing sufficient patients to meet the Primary Objective within 1 year of initiation of the study will require an effective recruitment plan.

4.B.1. NINDS PD Clinic: The first source of participants will be patients who are in the NINDS PD Clinic database. Personnel of the NINDS PD Clinic will contact

possibly eligible patients to determine whether the patients are interested in participating in the study.

4.B.2. PRPL: The Patient Recruitment and Public Liaison Office (PRPL) will be used for recruitment. The PRPL will distribute recruitment information via the internet to neurology clinics, patient support groups, relevant websites, or other institutions or individuals. A protocol description for lay people is appended **(Appendix 25.C.)**. A screening questionnaire is appended **(Appendix 25.D.)**. Recruitment strategies to be utilized by the PRPL will include flyers, a Public Service Announcement (PSA), NIH newsletters, ResearchMatch, Clinical Center Facebook, Twitter, CC News/NIH Record, and LISTSERVs. The recruitment flyer is appended (**Appendix 25.K**).

4.B.3. Recruitment ad: A sample recruitment ad is appended **(Appendix 25.E.)**. The ad may be mailed or transmitted electronically to patient support groups, academic medical centers, local neurology practices, or other institutions or individuals. Flyers containing the ad may be posted or handed out at public events or places.

4.B.4. Recruitment video: Recruitment may be done via a video ad. The script of the video ad is attached **(Appendix 25.F.)**. The ad may be modified based on recommendations by the PRPL, without amending the protocol. The video ad may be disseminated via YouTube or any of the strategies in the section on Recruitment (4.B.). The link to the YouTube video is https://www.youtube.com/watch?v=bbUcuxtVo6o.

4.B.5. Weblink ad: Non-NIH website(s) may link to information for this protocol. The weblink ad is appended (**Appendix 25.G.).** The planned placements of the link include pdtrials.org, the National Parkinson Foundation at www.parkinson.org, the Michael J. Fox Foundation for Parkinson's Research at www.michaeljfox.org, the American Parkinson Disease Association at www.apdaparkinson.org, the Parkinson's Disease Foundation at pdf.org, the Parkinson Foundation of the National Capital Area at parkinsonfoundation.org, and the Parkinson's Pipeline Project at pdpipeline.org.

4.B.6. List-Serv ad: For e-mail announcements sent to an individual's e-mail address, the use of a listserv for solicitation will be approved in advance by the listserv manager. We will abide by any listserv rules. The text of the List-Serv ad is appended **(Appendix 25.H)**. We do not plan to mail solicitation letters to those on commercial mailing lists for recruitment.

4.B.7 NINDS Office of Communications and Public Liaison: The NINDS Office of Communications and Public Liaison will be used for recruitment, including via the NINDS Spotlights. The text for carrying the protocol in the NINDS Spotlights is appended (**Appendix 25.L)**.

4.B.8. Pre-Screening: Candidate participants are pre-screened by phone, secure e-mail, or during attendance at the NINDS PD Clinic. Pre-screening questions for PRPL or other recruiters are appended **(Appendix 25.D.)**.

4.B.8.a. Steps to minimize confounding by antioxidant drugs and supplements on study measurements: Many antioxidant drugs and supplements are available over the counter, and their effects on levels of neurochemicals in CSF are unknown. If a patient is taking an antioxidant drug or supplement and is willing to stop it temporarily, then the first LP will be done after the antioxidant drug or supplement has been held for at least 7 days. If a patient wishes to continue the antioxidant drug or supplement, then the patient will take the antioxidant drug or supplement at the same dose and time of day for both LPs.

4.B.9. No Direct Solicitation: There will be no direct solicitation by supervisors or coworkers.

4.B.10. Google AdWords: Google AdWords may be used to enhance recruitment, via a text ad or image ad. (**Appendices 25.M. and 25.N.).** An image ad contains an image and text. The image in an image ad can be animated (up to 30 seconds). The image may be animated, using a clip from the IRB-approved video ad for the study. The image may be colorized rather than black and white as in the video ad. The colors will be the same as in the image for the image ad.

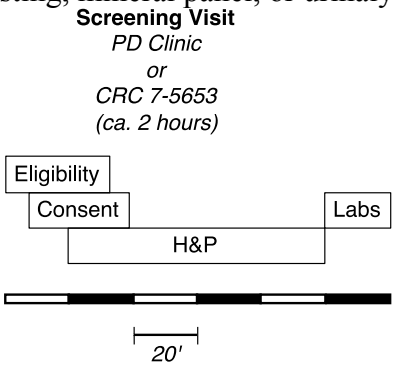
In terms of the strategy, we are granting Google AdWords permission to place the text ad or image ad at any of the websites related to Parkinson's Disease. Initially the placement will be based on keywords and target organizations that we supply. Text ads appear with the search results when keywords are used. We may add keywords and target websites as experience with the Google AdWords campaign proceeds.

Regarding how the ads appear on websites, when someone does a search and goes to one of the websites listed by us or chosen by Google AdWords for placement, the text ad or image ad appears on the person's computer desktop. This is called an "impression." The frequency and location of impressions depend on payment to Google AdWords. Clicking on the text ad or image ad will bring the person to either the IRB-approved YouTube video or to the NIH CC webpage that describes the study ((https://go.usa.gov/xXSsQ).

The written advertisements (and the images within the video ad) will be used in color as submitted or may be printed in black and white. The color of the ads may vary. Color changes will not be used to change the emphasis of an ad. The size of the ads (including images in the video ads) may vary, but all parts of the ads, including fonts and pictures, will be changed proportionately to the rest of that ad. Disproportionate changes in size will not be used to change the emphasis of an ad.

4.C. Screening: Consent is obtained before any study procedures, including screening procedures, are done. Each subject gives written informed consent and is accrued at the time of on-site screening. The location of screening is the 5th floor clinic, the Patient Testing Room in CRC 7-5653, or other room in the NIH Clinical Center. Screening will be done by the Principal Investigator or by clinical staff of the Clinical Neurocardiology Section who are authorized to obtain informed consent. The screening is to confirm eligibility criteria, by records review and history and physical examination. A Uniform

Parkinson's Disease Rating Scale (UPDRS), University of Pennsylvania Smell Identification Test (UPSIT) or Montreal Cognitive Assessment (MoCA) may be done. Screening labs may include a complete blood count, electrolytes, glucose, BUN and creatinine, liver function testing, mineral panel, or urinalysis.



Flow Diagram (Screening): *The main purposes of the screening are to confirm eligibility, obtain consent, and carry out screening tests such as a medical history and physical examination. Rating scales or clinical pathology labs may be done.*

4.D. Study procedures (see Flow Diagrams)

4.D.1 History and physical examination: An admission history and physical examination are done.

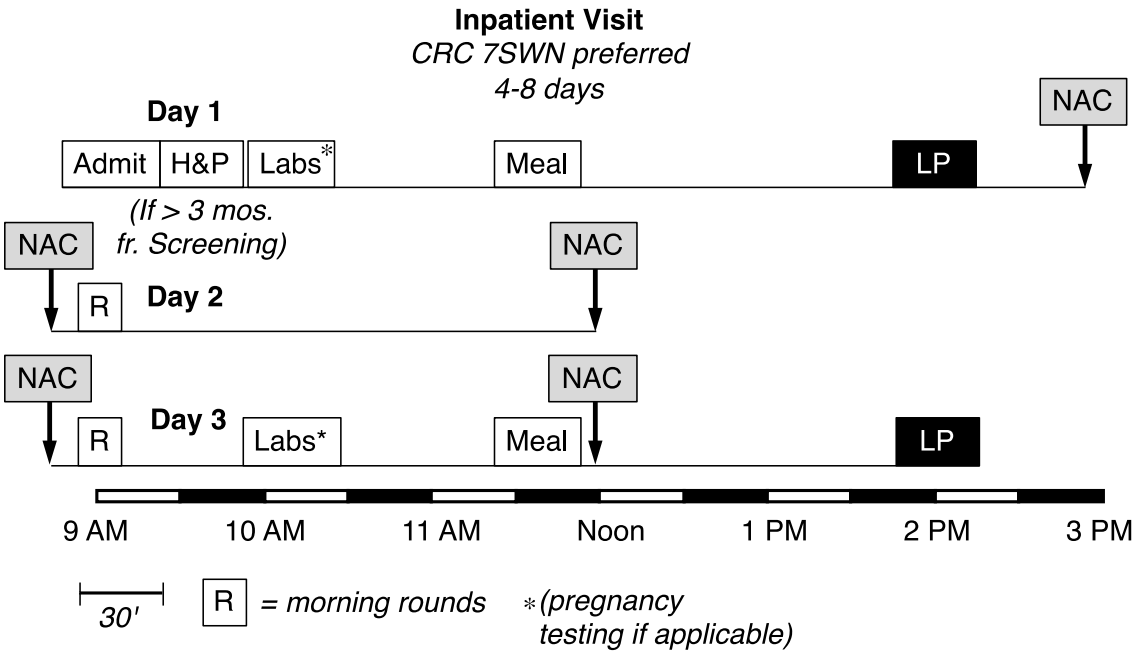
4.D.2. Venipuncture: Venipuncture is done to obtain blood via a needle or catheter.

4.D.3. Blood drawing: Blood is drawn for screening laboratory tests.

4.D.4. Lumbar puncture: In this study, lumbar puncture (LP) is done for research purposes, in order to obtain cerebrospinal fluid (CSF). The LP is done independently of other protocols.

On the day of a LP, the patient may receive a pre-arranged breakfast. The patient receives a pre-arranged lunch in the late morning, to be finished by about 2 hours before the LP. On the day of a LP the patient should have no other oral intake except for medications and water before the LP. When the patient is on NAC, as soon as the lunch is finished, the patient takes 2,000 mg of NAC in water.

At about 2 hours after the pre-arranged lunch meal, a LP for research purposes is done to obtain CSF. For the LP, the subject sits upright (alternatively the subject may be lying sideway with knees curled up toward the chest). The lower back will be cleansed. For local anesthesia, lidocaine without epinephrine will be injected into and through the skin of the back. A needle will be inserted through the anesthetized skin between two lumbar vertebral processes. A total of 6 1-mL aliquots of CSF will be obtained. Each aliquot will be placed immediately on dry ice. It usually takes 5 to 10 minutes to collect the CSF. After the fluid is collected, the needle will be removed.



Flow Diagram (Inpatient Visit): *A lumbar puncture (LP) is performed before and after 5 doses of N-acetylcysteine (NAC). The length of the inpatient stay at the NIH Clinical Center will depend on when during the week the patient is admitted and whether the patient has a post-spinal headache. After the second LP, the patient is observed at least overnight (discharge on Day 4). The inpatient visit may last up to 8 days, for insertion of a blood patch and observation in the event of a post-spinal headache after the second LP.*

After 5 NAC doses and about 48 hours of NAC administration, the LP for research purposes is repeated. Again, the patient receives a pre-determined lunch at about 12 noon. After the second LP, the subject is observed overnight and is discharged the next day if there is no post-spinal headache.

As noted below, the lumbar puncture may be done under fluoroscopic guidance by a neuroradiologist in the X-ray department.

In HVs, LPs are done also at about 2 hours after ingestion of a pre-determined meal. NAC is not given.

4.D.5. N-Acetylcysteine: In patients, beginning after the first LP, N-acetylcysteine (NAC) is begun at a 2,000 mg by mouth twice daily. HVs do not receive NAC.

4.D.6. Radiation (fluoroscopy): If technical difficulty is experienced or anticipated, the LP may be done under fluoroscopic guidance by a neuroradiologist, for research purposes. The anticipated Effective Dose is 0.023 rem per fluoroscopy (up to 2 fluoroscopies). The radiation exposure is for research purposes only.

In the event of a post-spinal headache after the first LP, a blood patch is inserted by an anesthesiologist, the patient is observed as an inpatient as needed until the headache is resolved, and the patient is then discharged and withdrawn from the study.

In the event of a post-spinal headache after the second LP, a blood patch is inserted by an anesthesiologist, the patient is observed as an inpatient as needed until the headache is resolved, and the patient is then discharged from the study.

4.E. End of participation: At the end of study participation the patient is transferred to the care of his or her primary physician or neurologist. There is no medical care offered at completion of study procedures. There are no follow-up visits under this protocol

After completing study participation, the patient will receive a letter summarizing the results of the research tests they underwent and their interpretation, recognizing that the data should not be used for clinical diagnosis or management purposes. The patient will be free to share the letter with health care providers; however, the letter will be mailed only to the patient. HVs do not receive summary letters describing their research results.

5. Management of Data and Samples

Data from this study will be entered in spreadsheets of the Clinical Neurocardiology Section (CNCS) or onto the Section's clinical research FileMaker Pro database, until the planned CTDB database under construction for the CNCS is active.

5.A Storage: In this study, CSF samples are obtained and stored for neurochemical assays. The sample tubes are labeled coded without personal identifying information. The coded samples are stored in locked freezers of the Clinical Neurocardiology Section (CNCS). Residual samples will continue to be stored, so that groups of samples can be re-analyzed as needed.

5.A.1. Future use: The Consent Forms state that the subject allows future use of his/her data and samples by the Clinical Neurocardiology Section (CNCS) or other components of intramural NIH. If the subject does not wish to have his/her data and samples used in future studies, the subject should not participate.

5.A.2. Transfer to Repository Protocol 08-N-N090: Upon termination of this protocol data and samples will be transferred to repository protocol No. 08-N-N090. Data without personal identifying information will continue to be stored on secured computers, on encrypted electronic media, or in notebooks of the CNCS. Coded samples will continue be stored in locked freezers of the CNCS. Personnel of the CNCS will have access to the stored samples and data. Any loss or destruction of stored data or samples will be reported to the IRB.

5.B. Data and sample sharing plan

This protocol is not subject to the Genomic Data Sharing (GDS) policy.

The Consent Forms state that the samples and data will not be shared with those outside NIH or a non-NIH repository.

6. Additional Considerations

6.A. Research with investigational drugs or devices: This study does not involve an investigational drug or device.

6.A.1. N-Acetylcysteine (NAC): will be obtained from a commercial supplier of NAC. The obtained NAC will be assayed and a Certificate of Analysis obtained before the NAC is administered. NAC will be dispensed through the NIH Clinical Center Pharmacy Department.

6.A.2. MAO inhibitor: The prescribed MAO inhibitor the patient takes will be identified by the NIH Clinical Center Pharmacy Department and dispensed by inpatient nursing personnel.

6.B. Gene therapy: This study does not involve gene therapy.

7. Risks and Discomforts

An admission history and physical examination are done. This does not involve risk.

7.A. History and physical examination: Having a history and physical examination done does not involve increased medical risk.

7.B. Venipuncture: There may be some discomfort and bruising from the needle insertion. Some people feel light-headed or faint.

7.C. Blood drawing: The amount of blood to be drawn for laboratory tests (up to about 30 mL) should not involve increased medical risk.

7.B. Lumbar puncture: Lumbar puncture may produce brief pain or a tingling sensation in the legs if the needle brushes against a nerve. If this happens, the needle will be adjusted. There may be a mild backache at the site of needle insertion. About one-third of people have a headache for a few days after a lumbar puncture. In the event of a post-spinal headache a blood patch will be inserted by an anesthesiologist.

7.C. N-Acetylcysteine: NAC is likely safe for most adults, when used as a prescription medication. It can cause gastrointestinal side effects such as nausea or abdominal pain, as well as headache and hypersensitivity reactions. Based on the mechanism of anti-oxidant effects of NAC, no potential drug interactions would be anticipated in PD patients on an MAO inhibitor.

We anticipate that gastrointestinal side effects will be dose-related. If these symptoms occur at the initial dose, they will be considered to be expected non-serious adverse events and reported as such, and the dose will be decreased by one-half. If the symptoms occur at the lower dose, then the NAC will be stopped, the patient will be withdrawn from the study, and the patient will observed as an inpatient until the symptoms resolve.

Rarely, NAC can cause rashes, fever, headache, drowsiness, low blood pressure, or liver problems. If a patient experiences these symptoms or signs or others that in the opinion of the Principal Investigator are the result of NAC, the patient will be withdrawn from the study.

7.D. Radiation (fluoroscopy): This research study involves exposure to radiation from fluoroscopy if technical difficulty is anticipated or experienced with the lumbar puncture. This radiation exposure is not required for medical care and is for research purposes only. The estimated Effective Dose, **0.05 rem** (2 fluoroscopy procedures), is below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee.

There is no direct evidence that the amount of exposure received from participating in this study is harmful. Pregnancy or lactation are exclusionary in this study.

8. Subject Safety Monitoring

8A. Identify monitors during participation in study procedures: In this study the monitors during participation in study procedures are the Principal Investigator, Research Nurse, or Nurse Practitioner.

8.B. Parameters to be monitored: The parameters to be monitored include symptoms and signs. During all study procedures, subjects are monitored by clinically credentialed personnel of the Clinical Neurocardiology Section (Physician, Registered Nurse, or Nurse Practitioner) or authorized personnel of the NIH Clinical Center (e.g., neuroradiologist).

8.C. Toxicity criteria to be used: The Protocol requires monitoring of procedures and possible symptomatic side effects of NAC or the development of a post-spinal headache. The NIH's Common Terminology Criteria for Adverse Events (version 4.0) will be used. The patient is queried about any new symptoms at morning rounds on Day 2 and Day 3 during NAC treatment. If the patient reports gastrointestinal side effects at the initial dose, the dose will be halved. All procedures involving more than minimal anticipated risk are administered only by experienced, qualified personnel or personnel.

8.D. Criteria for stopping individual procedures: The PI has the discretion of stopping individual procedures if the medical or psychological risk outweighs the anticipated scientific benefit.

8.E. Criteria for individual subject withdrawal: If a patient develops a post-spinal headache requiring a blood patch, the subject is withdrawn. Subjects may refuse certain tests or procedures, or may withdraw from participation early, without loss of benefits to which they were previously entitled. The Principal Investigator may also exclude a subject from further participation, such as in the event of known or suspect falsification of medical history information or refusal to undergo planned tests or procedures, without loss of benefits to which the subject was previously entitled. If NAC at the initial dose produces gastrointestinal symptoms, the dose will be halved; and if the gastrointestinal symptoms persist after decreasing the NAC dose, then the patient will be withdrawn from the study. If a patient experiences other symptoms that in the opinion of the Principal Investigator are the result of NAC, the patient may be withdrawn from the study. If a woman becomes pregnant while on study, she will be withdrawn.

8.E.1. Withdrawal definition: For record-keeping purposes, "withdrawal" refers to cessation from any further participation in the study, based on the decision of the participant or the Principal Investigator, before the endpoint for participation has been reached. The endpoint for participation is after obtaining CSF while the patient is on N-acetylcysteine. Reaching the study endpoint for participation does not constitute withdrawal.

9. Outcome Measures

In this study outcome is not related to measures of symptoms or signs or a disease but to CSF neurochemical findings.

9.A. Primary outcome measure

The primary outcome measure is the relative change in CSF Cys-DA levels between pre and post-NAC treatment, which is calculated as: the difference of (CSF Cys-DA at pre-treatment – CSF Cys-DA at post-treatment) divided by CSF Cys-DA at pre-treatment. The change will be dichotomized as decrease vs. no decrease with a threshold decrease to be determined based on the pilot study of HVs.

9.B. Secondary outcome measures

9.B.1. CSF levels of other biochemicals besides Cys-DA. For instance, CSF Cys-DOPA/DOPA ratios may provide an index of oxidative stress in central catecholaminergic neurons.

9.B.2. Percent decrease in CSF Cys-DA expressed as a continuous variable.

9.B.3. In an exploratory study, NAC may be given at 2,000 mg per day.

9.B.4. In a pilot study, reproducibility of CSF Cys-DA will be assessed in a group of HVs studied as inpatients. In this group 2 LPs will be done, separated by 2 days, without NAC treatment prior to either LP. The LPs will be done at about the same time of day, after ingestion of the same pre-determined meal.

10. Statistical Analysis

10.A. Analysis of data/ study outcomes:

For the primary objective, a one-sample t-test (t-test for dependent means) or Wilcoxon signed rank test will be performed to evaluate the difference between pre- and post-NAC treatment for CSF Cys-DA. The proportion of the patients with decreased CSF Cys-DA levels between pre- and post-NAC treatment will be evaluated by the exact binomial test.

For the secondary objectives, one-sample t-tests (t-tests for dependent means) or Wilcoxon signed rank tests will be performed to evaluate the differences between pre- and post-NAC treatment for each secondary outcome.

10.B. Power analysis: Based on the results of the study of HVs, a power analysis will be outlined and approved by the PIRC. The power analysis will be used to justify altering the accrual ceiling for PD patients. The power analysis will be performed using the primary outcome, the difference in CSF Cys-DA between pre- and post-NAC treatment. A one-sample t-test will be applied to estimate the sample size using the standard deviation of the difference calculated from the data in the HVs.

10.C. Accrual ceiling: Based on power analysis, 10-11 patients with complete datasets would be required to achieve the Primary Objective. Considering the possibility of withdrawals, 15 subjects would undergo inpatient testing. In the exploratory study, up to 20 subjects may be needed to test whether NAC at a 2,000 mg per day decreases CSF Cys-DA levels. Therefore, the accrual ceiling for patients in the study as a whole is 35.

The pilot study about biological variability of CSF Cys-DA levels in HVs will include up to 6 HVs with complete datasets. Dropouts will be replaced.

Therefore, the total accrual ceiling under the protocol is 35 + 6 = 41 participants.

11. Human Subjects Protection

11.A. Subject selection:

11.A.1. Equitability: Participation in this study is offered to eligible subjects, without regard to race, ethnicity, sex, disability, nationality, religion, handedness, age, or English-language ability. Non-English speakers may be included. In this situation, the short-form consent process will be used.

11.B. Justification for exclusion of children: PD is a disease of middle aged or elderly people.

11.C. Justification for exclusion of other vulnerable subjects: This study does not involve pregnant or lactating women, due to the risks involved. Since this research study involves more than minimal risk without direct benefit, those who are unable to provide informed consent are excluded.

11.D. Justification for sensitive procedures: This study does not involve a placebo, medication withdrawal, provocative testing, or deception.

11.E. Safeguards for vulnerable populations:

11.E.1. NIH staff employee protections: NIH staff and members of their immediate families may participate in this NIH intramural research unless prohibited by the NINDS or excluded by the criteria of the study.

Protections for employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff. Independent consent monitoring will be provided by the NIH HSPU.

The research will be conducted consistent with the *Guidelines for the Inclusion of in NIH Intramural Research Studies (March, 2012)* and the requirements of NIH Policy Manual 2300-630-3 – *Leave Policy for NIH Employees Participating in NIH Medical Research Studies https://oma1.od.nih.gov/manualchapters/person/2300-630-3*, as set forth in SOP 14F of the Intramural NIH Human Research Protection Program (HRPP), website https://ohsr.od.nih.gov/OHSR/pnppublic.php.

11.E.2. Pregnancy testing: Pregnancy testing is done in all women of child-bearing potential.

11.F. Qualifications of investigators

David S. Goldstein, MD PhD, is the Principal Investigator. He directs the Clinical Neurocardiology Section (CNCS) in intramural NINDS. He has been a tenured Senior Investigator since 1984 and will be active in all aspects of this study. He obtains consent.

Jamie Cherup, DNP, CRNP, USPHS, is the CRNP of the CNCS. She carries out histories and physical examinations, places orders, manages patients, generates notes for medical records, and carries out procedures for clinical research protocols of the CNCS. She has substantial experience in lumbar puncture to obtain CSF and will be doing this as part of this study. She obtains consent.

Irene Dustin, PhD CRNP, USPHS, is a CRNP in the NINDS PD Clinic. She assists with recruitment. She does not obtain consent.

Debra Ehrlich, MD, directs the NINDS PD Clinic. She assists with recruitment. She does not obtain consent.

Janna Gelsomino, RN, is the Research Nurse of the CNCS. She is in charge of the Patient Observation Room of the CNCS. She assesses protocol eligibility, arranges travel, enters data into the CNCS research database, and carries out procedures. She obtains consent.

Codrin Lungu, MD, Staff Clinician, has been the director of the NINDS PD Clinic. He is a neurologist with expertise in movement disorders. He knows the workings and patients of the PD Clinic and will help recruit patients into this study. He does not obtain consent.

Tianxia Wu, PhD, is a statistician in the Clinical Trials Unit, OCD/DIR/NINDS. She has expertise and experience in experimental design and statistics. She will help analyze the data from this study. She does not obtain consent.

The Principal Investigator has verified that all individuals working on this protocol required to take HRPP training under OHSRP SOP 25 (Training requirements for the NIH Human Research Protections Program) have completed all required training.

12. Anticipated Benefit

Protocol subjects benefit indirectly, in that the study is likely to yield generalizable knowledge.

13. Classification of Risk (for the study as a whole)

13.A. For adults*:* More than minimal risk.

13.B. For adults without consent capacity: This study does not involve adults without consent capacity.

13.C. For children: This study does not involve children.

13.D. Overall risk and benefit consideration: The risks are reasonable in relation to anticipated benefit.

14. Consent Documents and Process

14.A. Designation of those obtaining consent: Study investigators designated as able to obtain consent in section **11.F.** above, will obtain informed consent. All study investigators obtaining informed consent have completed the NIMH HSPU 'Elements of Successful Informed Consent' training.

14.B. Consent procedures: All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to review carefully the written consent form and ask questions regarding this study prior to signing. Coworkers will not consent one another.

14.C. Consent document: The consent form contains all required elements. the consent document submitted with this protocol is for patients.

14.D. Short form process for non-English speaking participants: If a non-English speaking participant is unexpectedly eligible for enrollment, the NIH Clinical Center standard short written consent form in the appropriate language and a written summary of what the investigator will say to the participant will be used as part of an oral consent process. The IRB approved English written consent form will serve as the written summary if the short form process is used. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Interpreters provided by the NIH Clinical Center will be used whenever possible. The interpreters will translate the current IRB-approved English version of the consent verbatim and facilitate discussion between the participant and investigator.

The written summary will be signed by the investigator obtaining consent and a witness to the oral presentation. The short written consent form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of the signed form will be provided to the participant to take home.

The investigator obtaining consent will document the consent process in the participant's medical record, including the name of the interpreter. Further, all instances of use of the short form process will be reported to the IRB at the time of continuing review. If the Clinical Center Short Written Consent form is used three times or more for the same language within an IRB approval period, this will be reported to the IRB immediately.

Interpreters will also be present for the other protocol procedures as necessary.

15. Data and Safety Monitoring

15. Data and Safety Monitoring

15.A. Data and safety monitor: Data and safety will be monitored by the PI.

15.B. Data and safety monitoring plan: Parameters to be monitored are those in the appended **Clinical Investigation Monitoring Form (Appendix 25.I.**). At the end of study participation, for each protocol participant, the Clinical Investigation Monitoring Form is completed and signed by the PI. Data and safety monitoring is conducted at the time of de-briefing of each protocol subject prior to discharge. Data and safety monitoring for the entire study is conducted via review by the PI of the notebook containing the Clinical Investigation Monitoring Forms at 6-month intervals beginning from the date of de-briefing of the first protocol participant.

15.C. Criteria for stopping the study or suspending enrollment or procedures: The study will be stopped if 2 of the same Serious Adverse Events occur related to NAC administration.

16. Quality Assurance (QA)

16.A. Quality assurance monitor: The PI and NINDS Quality Assurance Audit Committee will monitor the study.

16.B. Quality assurance plan: This protocol will undergo periodic review by the NINDS Quality Assurance (QA) Audit Committee as outlined in the NINDS QA Standard Operating Procedure (SOP). The purpose of the QA audit is to assess compliance with applicable regulatory requirement, good clinical practice guidelines, and NINDS policy, as well as to provide recommendations for improving the management of clinical research data. The protocol will be audited according to the decision algorithm as described in the NINDS SOP. Since the study involves more than minimal risk, the protocol will undergo an audit during the first year and then a minimum of every 3 years thereafter.

17. Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will be determined by the PI in consultation with the CD.

Serious unanticipated problems and serious protocol deviations will be reported to the IRB and CD as soon as possible and in writing not more than 7 days after the PI first learns of the event. Not serious unanticipated problems and not serious deviations will be reported to the IRB and CD as soon as possible and in writing not more than 14 days after the PI first learns of the event. Written reports will be submitted in PTMS.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review.

For reporting purposes, toxicity criteria in the Common Terminology Criteria for Adverse Events (CTCAE) will be used.

18. Alternatives to Participation

In this study, subjects do not receive any treatment to treat symptoms of PD or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

The patients will be taking an MAO inhibitor under the care of their own physicians.

NAC is marketed in the United States as a dietary supplement. Patients can take NAC on their own.

19. Privacy

All research activities will be conducted in as private a setting as possible.

20. Confidentiality

20.A. For research data and investigator medical records: Procedure notes, medical histories and physical examinations, and results of clinical laboratory tests will be kept in the subject's NIH medical chart and handled according to policies and procedures of the Medical Records Department, CC, NIH. Data from this protocol will be kept in password-protected computers or on electronic media or in data notebooks in offices or laboratories of the CNCS that are locked when unoccupied.

20.A.1. De-identified results from this study will be posted on cctrials.gov.

20.B. For stored samples: Samples and data will be stored using codes that assigned by the PI or personnel of the CNCS. Data will be kept in password-protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data.

20.C. Special precautions: Research staff will be trained to respect the privacy and confidentiality of NIH employees/staff. Such training may be accomplished through the Collaborative Institutional Training Initiative (CITI) course, "Vulnerable Subjects - Research Involving Workers/Employees."

21. Conflict of Interest

21.A Distribution of NIH guidelines: NIH guidelines on conflict of interest have been distributed to all investigators.

21.B. Conflict of interest: There are no conflicts-of-interest to report for NIH investigators. Non-NIH investigators will abide by the conflict-of-interest policies of their own institutions.

21.C. Role of a commercial company or sponsor: There is no commercial sponsor for this study.

22. Technology Transfer

No technology transfer agreement is in place for this protocol.

Arbor Pharmaceuticals, LLC may provide NAC for this study under a Material Transfer Agreement (MTA), pending review of the IRB-approved protocol.

23. Research and Travel Compensation

Patients in this study will be paid for time and research-related inconveniences. Otherwise eligible patients would likely not participate unless reimbursed, due to the demanding protocol regimen and lack of anticipated personal benefit. Although PD is a common disease, a rare specific subgroup is required (recent onset, levodopa-untreated, MAO inhibitor-treated).

23.A. Amount of compensation: The maximum total compensation for a given subject is $500. A planned **Payment Schedule** is appended **(Appendix 25.J)**. There is no payment for the screening visit. Compensation will be prorated for parts completed if subjects do not complete the study. Payment is sent at the end of the study. Travel/lodging compensation will be provided. Travel/lodging compensation for a medical escort may be provided if deemed necessary for safety by the PI.

23.B. NIH employees/staff participants: NIH employees or staff who participate during work hours must have permission from their supervisor. NIH employees or staff must either participate outside of work hours or take leave in order to receive compensation.

24. References

(1) Kish, S.J., Shannak, K. & Hornykiewicz, O. Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. *N Engl J Med* **318**, 876-80 (1988).

(2) Goldstein, D.S., Kopin, I.J. & Sharabi, Y. Catecholamine autotoxicity. Implications for pharmacology and therapeutics of Parkinson disease and related disorders. *Pharmacol Ther* **144**, 268-82 (2014).

(3) Montine, T.J., Picklo, M.J., Amarnath, V., Whetsell, W.O., Jr. & Graham, D.G. Neurotoxicity of endogenous cysteinylcatechols. *Exp Neurol* **148**, 26-33 (1997).

(4) Linsenbardt, A.J., Wilken, G.H., Westfall, T.C. & Macarthur, H. Cytotoxicity of dopaminochrome in the mesencephalic cell line, MN9D, is dependent upon oxidative stress. *Neurotoxicology* **30**, 1030-5 (2009).

(5) Kobayashi, H., Oikawa, S. & Kawanishi, S. Mechanism of DNA damage and apoptosis induced by tetrahydropapaveroline, a metabolite of dopamine. *Neurochem Res* **31**, 523-32 (2006).

(6) Li, S.W., Lin, T.S., Minteer, S. & Burke, W.J. 3,4-Dihydroxyphenylacetaldehyde and hydrogen peroxide generate a hydroxyl radical: possible role in Parkinson's disease pathogenesis. *Brain Res Mol Brain Res* **93**, 1-7 (2001).

(7) Anderson, D.G., Mariappan, S.V., Buettner, G.R. & Doorn, J.A. Oxidation of 3,4-dihydroxyphenylacetaldehyde, a toxic dopaminergic metabolite, to a semiquinone radical and an ortho-quinone. *J Biol Chem* **286**, 26978-86 (2011).

(8) Jinsmaa, Y. *et al.* Dopamine-derived biological reactive intermediates and protein modifications: Implications for Parkinson's disease. *Chem Biol Interact* **192**, 118-21 (2011).

(9) Follmer, C. *et al.* Oligomerization and membrane-binding properties of covalent adducts formed by the interaction of alpha-synuclein with the toxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL). *J Biol Chem* **290**, 27660-79 (2015).

(10) Goldstein, D.S. *et al.* Determinants of buildup of the toxic dopamine metabolite DOPAL in Parkinson's disease. *J Neurochem* **126**, 591-603 (2013).

(11) Burke, W.J., Li, S.W., Williams, E.A., Nonneman, R. & Zahm, D.S. 3,4-Dihydroxyphenylacetaldehyde is the toxic dopamine metabolite in vivo: implications for Parkinson's disease pathogenesis. *Brain Res* **989**, 205-13 (2003).

(12) Anderson, D.G., Florang, V.R., Schamp, J.H., Buettner, G.R. & Doorn, J.A.

Antioxidant-mediated modulation of protein reactivity for 3,4-dihydroxyphenylacetaldehyde, a toxic dopamine metabolite. *Chem Res Toxicol* **29**, 1098-107 (2016).

(13) Goldstein, D.S., Jinsmaa, Y., Sullivan, P., Holmes, C., Kopin, I.J. & Sharabi, Y. Comparison of monoamine oxidase inhibitors in decreasing production of the autotoxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde in PC12 cells. *J Pharmacol Exp Ther* **356**, 484-93 (2016).

(14) Elsworth, J.D. *et al.* Deprenyl administration in man: a selective monoamine oxidase B inhibitor without the 'cheese effect'. *Psychopharmacology* **57**, 33-8 (1978).

(15) Eisenhofer, G. *et al.* Simultaneous liquid-chromatographic determination of 3,4-dihydroxyphenylglycol, catecholamines, and 3,4-dihydroxyphenylalanine in plasma, and their responses to inhibition of monoamine oxidase. *Clin Chem* **32**, 2030-3 (1986).

(16) Heinonen, E.H., Savijarvi, M., Kotila, M., Hajba, A. & Scheinin, M. Effects of monoamine oxidase inhibition by selegiline on concentrations of noradrenaline and monoamine metabolites in CSF of patients with Alzheimer's disease. *J Neural Transm* **5**, 193-202 (1993).

(17) Fowler, J.S. *et al.* Evidence that formulations of the selective MAO-B inhibitor, selegiline, which bypass first-pass metabolism, also inhibit MAO-A in the human brain. *Neuropsychopharmacology* **40**, 650-7 (2015).

(18) de la Fuente-Fernandez, R., Schulzer, M., Mak, E. & Sossi, V. Trials of neuroprotective therapies for Parkinson's disease: problems and limitations. *Parkinsonism Relat Disord* **16**, 365-9 (2010).

(19) de la Fuente-Fernandez, R., Schulzer, M., Mak, E. & Sossi, V. Trials of neuroprotective therapies for Parkinson's disease: problems and limitations. *Parkinsonism Relat Disord* **16**, 365-9 (2010).

(20) Monti, D.A. *et al.* N-Acetyl Cysteine May Support Dopamine Neurons in Parkinson's Disease: Preliminary Clinical and Cell Line Data. *PLoS One* **11**, e0157602 (2016).

(21) Holmay, M.J. *et al.* N-Acetylcysteine boosts brain and blood glutathione in Gaucher and Parkinson diseases. *Clin Neuropharmacol* **36**, 103-6 (2013).

(22) Katz, M. *et al.* Cerebrospinal fluid concentrations of N-acetylcysteine after oral administration in Parkinson's disease. *Parkinsonism Relat Disord* **21**, 500-3 (2015).

(23) Goldstein, D.S., Holmes, C., Sullivan, P., Jinsmaa, Y., Kopin, I.J. & Sharabi, Y. Elevated cerebrospinal fluid ratios of cysteinyl-dopamine/3,4-dihydroxyphenylacetic acid in parkinsonian synucleinopathies. *Parkinsonism Relat Disord* **31**, 79-86 (2016).

(24) Greene, S.C., Noonan, P.K., Sanabria, C. & Peacock, W.F. Effervescent N-Acetylcysteine Tablets versus Oral Solution N-Acetylcysteine in Fasting Healthy Adults: An Open-Label, Randomized, Single-Dose, Crossover, Relative Bioavailability Study. *Curr Ther Res Clin Exp* **83**, 1-7 (2016).

(25) Deepmala *et al.* Clinical trials of N-acetylcysteine in psychiatry and neurology: A systematic review. *Neurosci Biobehav Rev* **55**, 294-321 (2015).

(26) Hoffer, M.E., Balaban, C., Slade, M.D., Tsao, J.W. & Hoffer, B. Amelioration of acute sequelae of blast induced mild traumatic brain injury by N-acetyl cysteine: a double-blind, placebo controlled study. *PLoS One* **8**, e54163 (2013).

25. Attachments/ Appendices

25.A. Flow Diagrams
25.B. Eligibility Checklists
25.C. Protocol description for lay people
25.D. Screening questionnaire for Patient Recruitment Office (PRPL)
25.E. Recruitment ad
25.F. Recruitment video script
25.G. Weblink ad
25.H. List-Serv ad
25.I. Clinical Investigation Monitoring Form
25.J. Payment schedule
25.K. Recruitment flyer
25. L. Text for NINDS Spotlights
25.M Text for Google AdWords
25.N. Image for Google AdWords

26. Consent Form (Adult Patient, Adult Volunteer)