A randomized, double-blind, placebo-controlled trial of urate-elevating inosine treatment to slow clinical decline in early Parkinson's disease

Study Chair
Michael A. Schwarzschild, MD PhD
Chair, Parkinson Study Group Executive Committee
Neurologist, Massachusetts General Hospital

Study Co-Chair
Alberto Ascherio, MD DrPH
Professor of Epidemiology and Nutrition
Neuroepidemiologist, Harvard School of Public Health

Data Coordinating Center (DCC) Director and Biostatistician
David Oakes, PhD
Professor of Biostatistics
Department of Biostatistics, University of Rochester

Clinical Coordinating Center (CCC) Biostatistician
Eric A. Macklin, PhD
Statistician, Parkinson Study Group Executive Committee
MGH Biostatistics Center, Massachusetts General Hospital

Supported by
The National Institute of Neurological Disorders and Stroke (NINDS)

Sponsor (Sponsor-Investigator)
Michael A. Schwarzschild
IND # 100,896

Version
4.0

Date
November 28 2017

SITE INVESTIGATOR AGREEMENT

Protocol #: INO-PD-P3-2014
Version 4.0
28 November 2017

IND # 100896

Title: **A randomized, double-blind, placebo-controlled trial of urate-elevating inosine treatment to slow clinical decline in early Parkinson's disease**

I have carefully read this protocol including all appendices and agree that it contains all the necessary information for conducting the study safely.

I will conduct this study in strict accordance with this protocol and according to the current Good Clinical Practice (GCP) regulations and guidelines and local and national regulatory requirements. Any changes in procedure will only be made if necessary to eliminate immediate hazards or to protect the safety, rights or welfare of subjects.

I will provide copies of the protocol and all other information relating to the pre-clinical and prior clinical experience, which were furnished to me, to all physicians and other study personnel responsible to me who participate in this study. I will discuss this information with them to assure that they are adequately informed regarding the study drug and conduct of the study.

I will ensure that the drugs supplied to me for this study will be used only for administration to subjects enrolled in this study protocol and for no other purpose.

I agree to keep records on all subject and study information (case report forms, informed consent statements, drug shipment, drug return forms, and all other information collected during the study) in accordance with the current GCP, local and national regulations.

PSG Site Number: _____

Print Site Name: _____

Print Site Investigator Name: _____

Site Investigator Signature: _____ Date: _____

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ABBREVIATIONS

8-OHdG	8-hydroxy-deoxyguanosine
AE	Adverse Event/Experience
AT	As-treated
BBB	Blood Brain Barrier
BID	Twice per day
BL	Baseline
BMI	Body Mass Index
BP	Blood Pressure
C	Celsius
CBC	Complete Blood Count
CCC	Clinical Coordinating Center
CDE	Common Data Element
CHD	Coronary Heart Disease
Chem	Chemistry
CI	Confidence Interval
CNS	Central Nervous System
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CTCC	Clinical Trials Coordinating Center
CVD	Cardiovascular Disease
DAT	Dopamine Transporter
DATATOP	Deprenyl and Tocopherol Antioxidative Therapy of Parkinson's Disease
DBP	Diastolic Blood Pressure
DCC	Data Coordinating Center
DS1	DAT scan 1 (screening)
DS2	DAT scan 2 (optional)
DSMB	Data and Safety Monitoring Board
dL	Deciliter
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eDC	Electronic Data Capture
eDE	Electronic Data Entry
FDA	Food and Drug Administration
FRAP	Ferric Reducing Antioxidant Power (or Ferric Reducing Ability of Plasma)
GCP	Good Clinical Practice
GDS-S	Geriatric Depression Scale – Short version
GFR	Glomerular Filtration Rate
HDFP	Hypertension Detection and Follow-up Program
HDPE	High-density polyethylene
HIPAA	Health Insurance Portability and Accountability Act

HPFS	Health Professionals Follow-up Study
HR	Hazard Ratio
Hr	Hour
ICH	International Conference on Harmonization
ID	Identification
IEC	Independent Ethics Committee
IMP	Inosine monophosphate (a.k.a. inosinic acid or inosinate)
IND	Investigational New Drug
IRB	Institutional Review Board
K	Potassium
kg	Kilogram
LFT	Liver Function Test
MAO	Monoamine Oxidase
MCID	Minimum clinically important difference
MDS-UPDRS	Movement Disorders Society UPDRS
MedDRA	Medical Dictionary for Regulatory Activities
mEq	Milliequivalent
mITT	Modified Intent-to-treat
mL	Milliliter
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MRFIT	Multiple Risk Factors Intervention Trial
MS	Multiple Sclerosis
NET-PD	National Institutes of Health Exploratory Trials in PD
NHS	Nurses Health Study
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
OTC	Over-the-counter
PD	Parkinson(’s) disease
PDQ-39	Parkinson’s Disease Questionnaire – 39 item version
pH	‘power of Hydrogen’ (negative log of hydrogen ion concentration)
PK	Pharmacokinetics
PAU	Persistently acidic urine
PI	Principal Investigator
PO	By mouth
PRECEPT	Parkinson Research Examination of CEP-1347 Trial
PRO	Patient-reported outcome
PSG	Parkinson Study Group
PW	Premature Withdrawal
QA	Quality Assurance
QC	Quality Control
QID	Four times per day
QoL	Quality of Life
RBD	REM (Rapid Eye Movement) Behavior Disorder
SBP	Systolic Blood Pressure
S&E ADL	Schwab and England Activities of Daily Living Scale
SAE	Serious Adverse Event/Experience
SAP	Statistical Analysis Plan
SC	Steering Committee
SC1 (SC2)	Screening Visit 1 (or 2)
SD	Standard Deviation

Smart*4*SURE	Smartphone substudy and app for the SURE-PD3 trial
SPECT	Single Photon Emission Computerized Tomography
SU	Serum urate
SURE-PD	Safety of URate Elevation in Parkinson's Disease
SURE-PD3	Study of URate Elevation in Parkinson's Disease, phase 3
SV	Safety Visit (at month 27)
SWEDD	Scans Without Evidence of Dopaminergic Deficit
TE1 (or TE2)	Telephone Evaluation 1 (or 2)
TID	Three times per day
TSH	Thyroid-Stimulating Hormone (thyrotropin)
UAC	Uric acid crystalluria
UPDRS	Unified Parkinson's Disease Rating Scale
VS	Vital Signs

SYNOPSIS

Study Title: A randomized, double-blind, placebo-controlled trial of urate-elevating inosine treatment to slow clinical decline in early Parkinson's disease

Study Acronym: SURE-PD3 (Study of URate Elevation in Parkinson's Disease, phase 3)

Study Objectives

Primary – To determine whether oral inosine dosed to moderately elevate serum urate (from ≤5.7 mg/dL to 7.1-8.0 mg/dL) over 2 years slows clinical decline in early PD.

Secondary – To determine:

a) safety and tolerability of urate-elevating inosine treatment,

b) short-term (3 mo) wash-in and wash-out effects on MDS-UPDRS and other outcomes,

c) time to disability warranting dopaminergic medication, and

d) long-term (2 yr ±3 mo of wash-out) effects on:

i) need for initiating dopaminergic medication changes,

ii) specific non-motor measures of cognitive, mood and autonomic function (vital signs and orthostatic changes), and

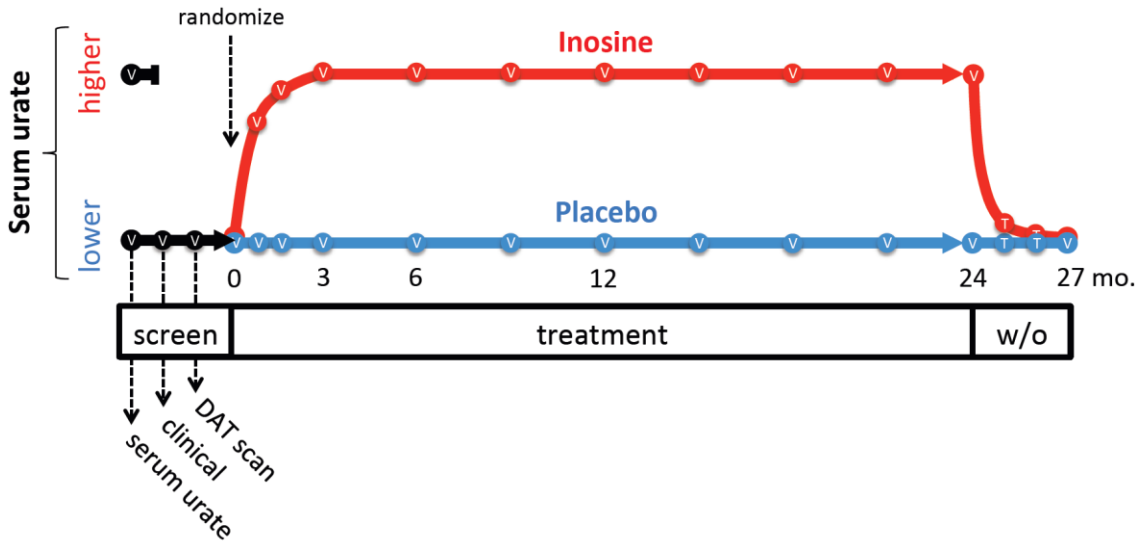
iii) quality of life and functional disability measures.

Design and Outcomes

Multicenter, randomized, double-blind, placebo-controlled trial with clinical decline assessed as change in the primary outcome variable of the Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS), a composite scale comprising patient- and clinician-reported outcomes.

Secondary outcome variables include safety, disability warranting initiation of dopaminergic therapy, quality of life and functional disability measures, and specific non-motor measures of cognition, mood and autonomic function.

Diagram of Timeline & Treatment



Interventions, Evaluations and Duration
Capsules containing 500 mg of inosine (active drug) or lactose (placebo) will be taken orally up to two capsules three times per day (i.e., up to 3 gm/day) for 24 months. In the inosine-treated group the number of capsules taken per day will be titrated to serum urate levels – measured at trough at all study visits (**V**'s in the above diagram) no more than three months apart – in order to achieve concentrations of 7.1-8.0 mg/dL. Initial dosing will be tailored to individualized factors including gender and pretreatment serum urate, and then advanced gradually toward the projected target dose. Adjustments in dosing of placebo capsules in the control arm will be algorithm-based to match dosing of inosine capsules in the active drug arm.

Following study drug discontinuation all subjects will be followed during a 3-month wash-out period (**w/o**) by telephone calls (**T**'s in the above diagram) and a final study visit. All study visits after screening will include measurement of the primary outcome variable (MDS-UPDRS) and most will include secondary outcome variables: adverse events, dose adjustments, disability warranting initiation of dopaminergic therapy, Quality of Life in Neurological Disorders (Neuro-QOL), 39-item Parkinson's Disease Questionnaire (PDQ-39), Schwab & England Activities of Daily Living (S&E ADL) scale, Montreal Cognitive Assessment (MoCA), and orthostatic vital signs.

Study Population
To enroll ~270 subjects with early PD (not yet requiring treatment with levodopa or dopamine agonist) and with lower serum urate, ~667 people diagnosed with probable early PD will be consented at ~60 US Parkinson Study Group (PSG) clinical sites. Initial screening for serum urate levels at or below 5.7 mg/dL (approximate population median) and other clinical eligibility criteria (e.g., to exclude those at high risk of adverse effects of urate elevation) will identify 300 probable candidates. They will undergo neuroimaging of dopamine transporter (DAT) ligand binding uptake to enhance diagnostic confidence by excluding ~10% who have scans without evidence of dopaminergic deficit (SWEDDs). The resultant cohort of ~270 subjects with lower serum urate predictive of more rapid clinical progression will be well suited to study whether urate-elevating inosine treatment can slow clinical progression in early PD.

Sample Size
Power for the primary outcome of rate of change in MDS-UPDRS over 24 months is based on a random slopes model with shared baseline. The model will include fixed effects of time, treatment x time, gender, gender x time, baseline MAO-B inhibitor use, and baseline MAO-B inhibitor use x time and random site- and subject-specific intercepts and slopes. Use of a shared baseline adjusts for baseline MDS-UPDRS score in addition to the adjustment for gender and baseline MAO-B inhibitor use. Other baseline covariates may be included in the final model based on any revision in our understanding of predictors of PD progression prior to finalizing the analysis plan and breaking the blind. MDS-UPDRS assessments completed after a subject has initiated dopaminergic therapy will be censored. Based on applying the same primary analysis model to data from SURE-PD, the following variance components were estimated for MDS-UPDRS trajectories (assuming a 1.29x conversion factor from UPDRS scores): site-level variance (intercept = 12.6, slope = 0.0159 / month, covariance = -0.446), subject-level variance (intercept = 99.8, slope = 0.297 / month, covariance = 3.70), and residual variance = 17.9. Similarly informed by experience in SURE-PD we assumed that 70% of subjects would initiate dopaminergic therapy plus up to 8% additional loss to follow-up prior to initiating dopaminergic therapy. With quarterly follow-up for MDS-UPDRS, the variance and censoring estimates above, a final two-sided test at alpha = 0.046, allowing for two non-binding interim analyses at one-sided alpha = 0.001 each, the study would have 80% power with n = 270 subjects randomized 1:1 to placebo or urate elevation if the true effect of treatment were to reduce 2-year increase in MDS-UPDRS by 6.3 points, a 20% reduction in the expected rate of progression. This is robust to variable gender-specific enrollment rates and treatment efficacy as long as the average effect of treatment across genders in the ratio enrolled is 6.3 points over 2 years.

1. STUDY OBJECTIVES

1.1 Primary Objective

To determine whether oral inosine dosed to moderately elevate serum urate (from ≤5.7 mg/dL to 7.1-8.0 mg/dL) over 2 years slows clinical decline in early PD.

We hypothesize that inosine will reduce the two-year change in total MDS-UPDRS score by 6 points versus *placebo, representing a 20% reduction in rate of progression.*

1.2 Secondary Objectives

To determine:

a) safety and tolerability of urate-elevating inosine treatment,

b) short-term (3 mo) wash-in and wash-out effects on MDS-UPDRS and other outcomes,

c) time to disability warranting dopaminergic medication, and

d) long-term (2 yr ± 3 mo of wash-out) effects on:

i) need for initiating dopaminergic medication changes,

ii) specific non-motor measures of cognitive, mood and autonomic function (vital signs and orthostatic changes), and

iii) quality of life and functional disability measures.

2. BACKGROUND

2.1 Rationale

2.1.1 Rationale for Elevating Urate in Parkinson's Disease

As an apparent consequence of mutations in the *urate oxidase* gene during primate evolution,[1] urate (a.k.a. uric acid) in humans circulates at high concentrations near the limits of its solubility and constitutes the end product in the metabolism of purines such as adenosine. (Fig. 1.) Urate has an antioxidant efficacy comparable to that of ascorbate[2] and accounts for most of the antioxidant capacity in human plasma.[3] Thus its high level may serve as one of our major defenses against oxidative damage caused by reactive oxygen and nitrogen species. Urate also displays potent iron-chelating actions independent of its direct antioxidant properties.[4] Because oxidative and nitrative stress[5,6] (including that accelerated by oxidized free iron) and possibly adenosinergic mechanisms[7,8] are thought to contribute to the loss of dopaminergic neurons in individuals with Parkinson's disease (PD), urate or its precursors could be an important determinant of disease susceptibility and progression in PD. Indeed a potential role for urate is consistent with the demonstration of low levels of urate in cerebrospinal fluid[9] and post-mortem substantia nigra and striatum[10,11] of patients with PD. Moreover, in cellular models of PD administration of urate reduced oxidative stress, mitochondrial dysfunction and cell death in dopaminergic cell lines exposed to the pesticide rotenone, MPP^+, 6-hydroxydopamine, glutamate, H_2O_2 or

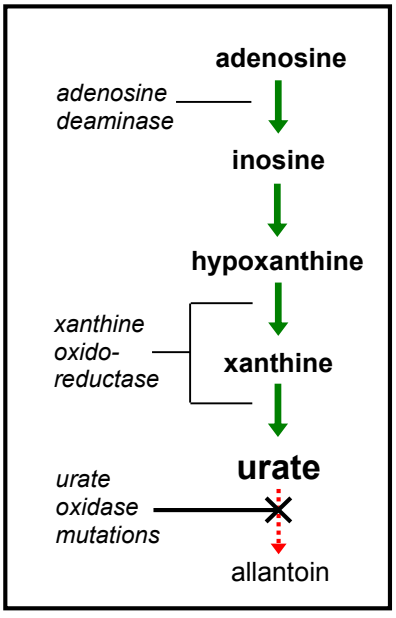


Fig. 1. Metabolism of purines (e.g., adenosine) in humans. Enzymatic oxidation of urate does not occur in humans due to *urate oxidase* gene mutations.

iron ions,[12-15] and conferred protection against dopaminergic neuron death in primary cell cultures[16,17]. Animal models of PD have provided direct evidence that urate and its elevation protect nigrostriatal dopaminergic neurons *in vivo*.[18,19] Interestingly, several laboratories have independently reported that neuroprotection by urate may rely more on indirect rather than its direct antioxidant properties, as the protective actions of urate can require nearby astrocytes[14,17,216,217] as well as the activation of the Nrf2 transcriptional antioxidant response pathway[217,218].

A) ***Blood urate and PD risk*** – Our epidemiology research group led by Alberto Ascherio (Co-chair of the SURE-PD3 trial Steering Committee) has confirmed and extended earlier observations [20,21] that lower blood urate levels in healthy individuals are a risk factor for developing PD later in life. In our prospective study[22, 23] of a larger population -- 18,000 men followed for more than 8 years in the Health Professionals Follow-up Study (HPFS) -- we found that men in the top quartile of plasma urate concentration had a 55% lower risk of PD than men in the bottom quartile (Fig. 2A). The decrease in PD risk among men with high levels of urate was stronger among men with blood collected at least four years before the diagnosis of PD (Fig. 2B), suggesting that the lower serum urate among individuals with PD precedes the onset of neurological symptoms and is thus unlikely to be a consequence of changes in diet, behavior, or medical treatment early in the course of the disease. Further, this inverse association was independent of age, body mass index (BMI), smoking, caffeine consumption and other aspects of lifestyle that have been related to both PD and uricemia. Taken together in a meta-analysis[23] the available prospective data on urate and PD risk demonstrated a substantially lower risk of PD in people who have higher plasma urate levels, with a 20% reduction in the pooled rate ratio of PD for each standard deviation (1.3 mg/dL) increase in blood urate concentration ($p < 0.0001$).[23] With more recent prospective cohort studies[28,212,213] the evidence for serum urate as an inverse risk factor for PD has overall strengthened, particularly in men.[28] In women the link appears weaker,[28,212,213] possibly reflecting the substantially lower levels of serum urate in women rather than the absence of a relationship (see below). Moreover, in numerous case-control

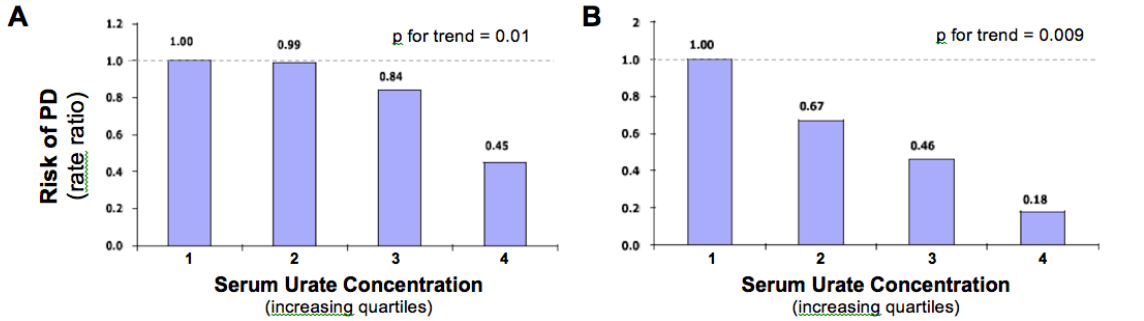


Figure 2. Serum urate is associated with reduced risk of PD. A. Rate ratio (RR) for PD by quartile of urate concentration among controls adjusted for age, pack-years of smoking, and quintile of caffeine intake in HPFS study. **B**. Only cases whose blood was drawn >4 years before PD diagnosis and matched controls were included. RR is indicated above each bar. (Adapted from ref. # 23.)

studies lower serum urate has been linked to PD in both women and men.[214] Similarly, the risk of PD was reduced in people with gout as shown in two independent prospective cohort studies, [253,254] though not in a third study[255].

In addition to blood urate its genetic and environmental (dietary) determinants have also been linked to PD risk or diagnosis, supporting the possibilities of causality and modifiable risk. SLC2A9 is a urate transporter and variation in its gene is statistically the strongest known genetic determinants of blood urate levels in humans.[205] *SLC2A9* polymorphisms predictive of higher serum urate have been linked to a later age at onset in PD.[206] Similarly, a composite genetic index of lower serum urate comprising single nucleotide polymorphisms in *SLC2A9* and other genes linked

to serum urate was significantly higher in PD than in control subjects,[207] though in another study single nucleotide polymorphisms of *SLC2A9* were largely unassociated with PD[208]. Complementing these 'urate gene' links to PD, dietary sources of higher urate were found to be strongly associated with a reduced risk of PD in the prospectively followed HPFS cohort.[139]

B) ***Blood urate and PD progression*** – This epidemiological association with PD *risk* in healthy populations prompted us to investigate whether urate might also be linked to PD *progression* amongst those already diagnosed with PD. Working with the Parkinson Study Group (PSG), a non-profit, cooperative group of PD clinicians from medical centers in the United States and Canada who are dedicated to improving treatment of the disease, we have been able to address this question in two long-term, rigorously managed clinical trials. One is known as PRECEPT (for Parkinson Research Examination of CEP-1347 Trial)[30] and the other as DATATOP (for Deprenyl and Tocopherol Antioxidative Therapy of Parkinson's Disease).[31-34] Together these cohorts comprise over 1600 cases of early PD. In our analyses with the biostatistics group of David Oakes (serving as PI of the the Data Coordinating Center [DCC] at Univ. of Rochester for the trial) we have found that higher blood urate levels are indeed strongly associated with a slower progression of PD symptoms and signs, both clinically and radiographically.[35,36] Eight hundred and six (806) patients with early PD were enrolled in the PRECEPT study, a two-year double-blind, placebo-controlled, randomized trial of CEP-1347 (a c-Jun N-terminal kinase pathway inhibitor).[30] Serum urate was measured as a component of safety monitoring. The hazard ratio (HR) of reaching the primary study endpoint, i.e. the development of disability sufficient to require dopaminergic therapy, declined with increasing serum urate (p for trend <0.0001).[35] The age- and gender-adjusted HR for individuals in the top quintile of uricemia (>6.7 mg/dL) compared with those in the bottom quintile (<5.1 mg/dL) was 0.51 (95% confidence index: 0.37 to 0.72; p<0.0001). Interestingly, in an analysis by gender, an even stronger and progressive reduction in the hazard of reaching the endpoint with increasing urate concentration was found in men (trend p<0.0001), but no significant association was appreciated in women (p, trend=0.33). The evidence for interaction was suggestive but not statistically significant (p for interaction [sex x urate] = 0.15).

Moreover, a similar robust inverse association[35] was

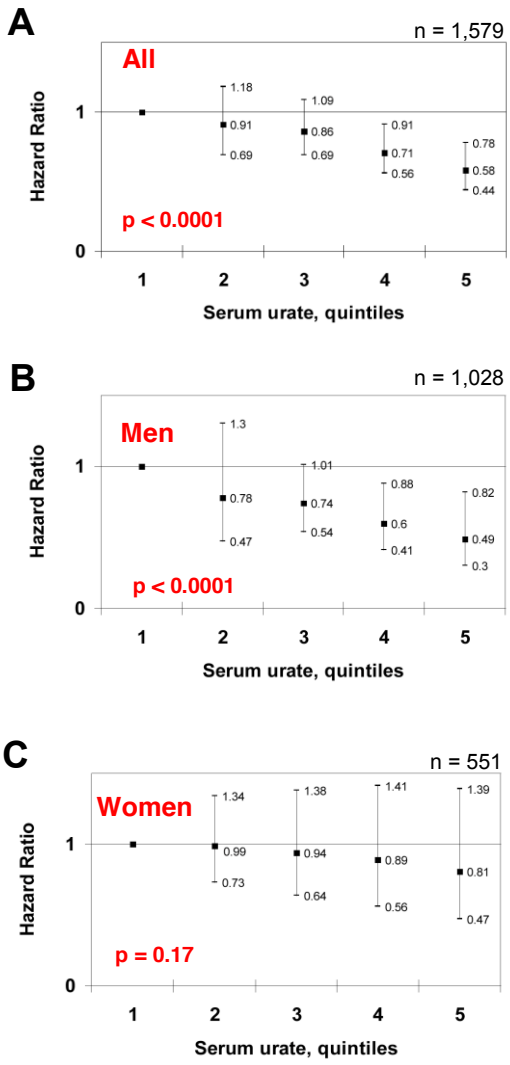


Figure 3. Serum urate as a predictor of PD disability progression in the pooled PRECEPT[35] and DATATOP[36] cohorts. Tabulated hazard ratios of reaching the primary endpoint of disability sufficient to require dopaminergic therapy according to quintiles of serum urate for **All** subjects (**A**), **Men** (**B**) or **Women** (**C**). Data are presented as means and 95% confidence intervals. P values are for trends across quintiles. For men (B) n's for increasing quintiles = 84, 170, 245, 258, and 271; for women (C) n's for increasing quintiles = 255, 127, 81, 46, and 42.

observed between baseline urate and loss of striatal iodine-123–labeled 2-β-carboxymethoxy-3-β-(4-iodophenyl)tropane ([^{123}I]β-CIT) uptake, a marker for the presynaptic dopamine transporter, in a subset of PRECEPT subjects. Overall, the mean change was –2.5% among patients in the top quintile of serum urate *versus* –9.3% for those in the bottom quintile (p=0.002). Just as for the clinical endpoint, a highly significant association was found in men but not in women.[35]

Analysis of serum urate values in the DATATOP cohort[31-34] enrolled 25 years ago also showed that higher blood urate at baseline was associated with slower rates of PD progression (again particularly amongst men) as assessed by the same clinical endpoint used in PRECEPT (n=776; trend p=0.002),[36] allowing for pooled analysis and substantiation of a decreasing rate of disability progression as a function of serum urate levels early in PD (Fig. 3). Interestingly, serum urate was highly predictive of slower rate of clinical decline among those not randomized to vitamin E (e.g., as measured in total UPDRS change, with those in the highest serum urate quintile declining at one third the rate of those in the lowest; p<0.001). By contrast, among those randomized to receive vitamin E serum urate was not related to total UPDRS change, with no difference between extreme quintiles (p=0.9), consistent with a competitive interaction between putative protective effects of urate acting as an endogenous antioxidant and vitamin E administered as an antioxidant. Indeed, in contrast to the null primary DATATOP trial results for the full cohort, among those in the lowest quintile of serum urate vitamin E treatment appeared to significantly slow the rate of clinical progression (p<0.01)[36] albeit in a *post hoc* secondary analysis without adjustment for multiple comparisons.

Importantly, it remains unclear whether there exists a true gender difference in the relationship between urate and PD progression, or whether the relationship is simply less apparent in women due to gender differences in urate levels. Of note, a significant interaction between gender and urate was not appreciated in either of these populations (e.g., p for interaction = 0.5)[36]. Indeed, a relationship would be expected to appear weaker in women not only because there were half as many women as men in the DATATOP and PRECEPT cohorts (Fig. 3), but also because women have a substantially lower mean serum urate level than men. Consequently, women in both of these studies comprise a very small proportion (≤15%) of the two highest population quintiles of serum urate (see Fig. 3 legend for n's by quintile for each gender). Because the inverse association between urate and PD is appreciable only above the population mean (i.e., the curve describing the relationship appears relatively plateaued below the mean and much more steeply sloped downward above the mean; Fig. 3) – as was similarly found for PD risk (Fig. 2A)[23] – it can be expected that if a similar inverse urate-PD progression relationship exists in women and men, then it should be much less apparent in women. Interestingly, if the inverse association were in fact due to a neuroprotective effect of urate at serum concentrations above ~ 6 mg/dL, one could reasonably argue that a higher proportion of women with PD than men with PD would stand to gain from a protective urate elevation strategy of boosting serum urate to the 7-8 mg/dL range (i.e., because a much higher proportion of men already reside at these levels), consistent with preliminary data from the SURE-PD phase 2 study (see below).

Newly reported data from a third independent cohort of prospectively followed de novo PD (i.e., those newly diagnosed and not yet required antiparkinsonian medication) has shown that baseline serum urate is also a predictor of favorable progression of multiple non-motor features of the disease, which included cognitive, mood and autonomic function domains.[219] As disability from PD is increasingly appreciated to result from non-dopaminergic neuron involvement[220] producing progressive non-motor features[221,222] in addition to dopaminergic neuron degeneration and deficits, these findings further support the potential impact of pursuing the hypothesis that the 'urate-PD' association is causal.

To address the causality of the link between higher serum urate and slower progression of PD we conducted a Mendelian randomization study[223] of 735 DATATOP and PRECEPT participants with available DNA, using a genetic variant of the urate transporter SLC2A9 (see preceding Sec. 2.1.1A) as

an unconfounded proxy for serum urate concentrations. Consistent with prior population-based studies, variations in *SLC2A9* were strongly associated with serum urate levels. The number of *SLC2A9* alleles linked to lower serum urate also correlated with a faster clinical progression. Specifically, for a 0.5 mg/dL genetically conferred decrease in serum urate, the hazard ratio (HR) for developing disability warranting dopaminergic therapy was 1.27 (95% CI = 1.00–1.61, p=0.05). Of note, the marginal statistical significance notwithstanding, the magnitude of the apparent benefit of the urate-lowering *SLC2A9* variant appears substantial, corresponding to a 76% reduction in the rate of clinical progression for a 3.0 mg/dL increase in serum urate, which is the average inosine-induced increase expected in SURE-PD3 (based on a mean baseline urate of 4.5 mg/dL[102] and a mean target of 7.5 mg/dL). Overall, these results strengthen but do not prove the hypothesis that higher serum urate can slow clinical progression in early PD.

C) ***CSF urate and PD progression*** – Because cerebrospinal fluid (CSF) more closely reflects the microenvironment of degenerating neurons than does blood,[37] we measured urate concentration in stored baseline CSF samples from the DATATOP study[31] to conduct a preliminary investigation of whether higher urate CSF levels also predict a slower rate of clinical disease progression in PD. Urate was measured by HPLC with electrochemical detection.[33] Despite some two decades of storage at -80 ^{o}C, CSF urate measured in 2008 in samples collected in 1987-1988 from DATATOP subjects at baseline correlated with that analyzed after only ~3 years of storage in 1991 (r^2 = 0.52), and with serum urate measured fresh during the trial in 1987-1988 (r^2 = 0.51) (K. Xu, W. Matson, A. Ascherio and M. Schwarzschild, unpublished data). Higher baseline levels of CSF urate were found to be predictive of a slower rate of clinical decline (assessed either by time to disability warranting to initiation of symptomatic dopaminergic medication, or by change in UPDRS scores), with a significantly slower decline in those in the highest quintile of CSF urate compared to the lowest.[36] As noted above for serum urate the CSF urate-progression relationship was even stronger among those not receiving vitamin E and absent in those randomized to receive it.[36]

D) ***Significance for neurotherapeutics*** The convergence of these clinical and epidemiological as well as laboratory data has implicated urate as an attractive candidate neuroprotectant for PD. Growing reservations over reliance on animal models of PD to predict therapeutic potential are being raised[24,38,39] in the wake of clinical failures of several candidate neuroprotectants. For example, anti-apoptotic (CEP-1347 and TCH346), anti-excitotoxic (riluzole), lipophilic antioxidants (α-tocopherol and coenzyme Q_{10}), neuroimmunophilin (GPI-1485), neurotrophic (GDNF) and mitochondrial stabilizing (creatine) agents have shown no disease-modifying efficacy in randomized controlled trials for PD. Of note, all these compounds appeared promising based on their prior vetting in preclinical PD models, though none was associated with improved clinical outcomes in PD. Thus the development of a urate-elevating strategy for PD – based in part on the unprecedented availability of human data linking urate to favorable PD progression – is particularly timely and compelling.

2.1.2 Rationale for Selecting Inosine as a Means to Elevate Urate

We considered three general pharmacological strategies to elevate urate in PD patients. First and most intuitive is **administration of urate itself**. Although urate may not freely cross the blood-brain barrier (BBB),[40] the main prohibitive factor is poor oral bioavailability. In humans oral urate up to 4 gm/day does not significantly elevate blood urate levels due to its degradation in the gastrointestinal tract, likely by uricase-expressing bacteria.[41]

Second, although enzymatic degradation of urate does not occur in humans (due to our *urate oxidase* mutations)[1] and thus cannot be targeted, the clearance of urate can in fact be reduced by **enhancement of renal urate resorption**. For example, commonly taken thiazide diuretics reliably elevate serum urate

through this mechanism.[42,43] However, thiazide diuretics generally produce only mild urate elevations (< 1 mg/dL) at doses that cause significant reductions in blood pressure, which could be a particular liability in PD patients who are at increased risk of orthostatic hypotension. An additional theoretical concern over serum urate-elevating agents like thiazides that rely on transporter-blocking mechanisms[44] is the uncertainty over the directionality of their effects on urate transport across the BBB and into or out of relevant CNS or cellular compartments.

Third, **urate precursor administration** can provide a simple mass action approach to elevating urate levels. The nucleoside inosine, the immediate deamination product of adenosine metabolism (see Figs. 1 and 4), was a particularly attractive candidate because it is orally bioavailable and CNS-penetrant.[40] In addition, substantial human experience had shown inosine to be effective at chronically, stably and relatively safely elevating serum urate – at least in young athletes and patients with multiple sclerosis (MS).[41,45-48] Inosine's reputation as an athletic performance enhancer has led to its widespread marketing and use as a nutritional supplement. However, exercise physiology studies on healthy subjects under laboratory conditions have not substantiated any enhancement in athletic performance.[48-50] In these studies inosine doses as high as 10 gm/day x 10 days[50] produced no apparent adverse effects.

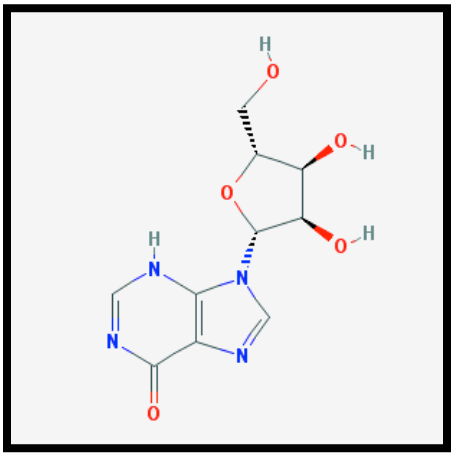


Figure 4. Structural formula of inosine (hypoxanthine 9-β-D-ribofuranoside) from http://pubchem.ncbi.nlm.nih.gov/compound/inosine.

Based on these pharmacological and toxicological considerations oral inosine was selected as the most rational initial strategy for elevating serum and CSF urate in PD patients.

2.2 Supporting Data

2.2.1 Human Nutritional and Clinical Experience with Inosine

A) Nutritional Supplement Use of Inosine

1) 'Performance Enhancer'

Reflecting its common *intended* use in the US as an athletic performance enhancer,[51] inosine is defined in a US President's Council on Physical Fitness and Sports informational publication posted by the US Department of Health and Human Services:[52] "*Inosine is a nucleoside with a variety of proposed ergogenic effects, including enhancement of aerobic endurance performance by facilitating the delivery of oxygen to the muscles during exercise. Although scientific research is limited, two well-controlled* [authors' phrasing] *studies did use the recommended supplementation protocol* (see B1 below in this section, 2.2.1) *for endurance athletes and reported no beneficial effects of inosine on cardiovascular-respiratory or metabolic functions during submaximal or maximal exercise, nor was there any effect on time to complete a simulated three mile race on a treadmill. Both studies actually suggested inosine could be ergolytic for certain athletic endeavors involving anaerobic glycolysis (Starling, R., et al., 1996; Williams, M., et al., 1990*)."[48-50] More succinctly, a major health care professional resource Thomson Healthcare states that, "*Inosine is a nucleoside (one of the basic compounds comprising cells) that is used for tiredness and to increase athletic performance. There are, however, no clinical studies that support the use of Inosine for any condition*."[53]

Although the extent of its use for this purpose in the US is difficult to reliably document, its common listing and wide availability on its own or as a component of athletic performance supplements by nutritional supplement retailers (e.g., 8 listed for inosine in multiple formulations on Amazon.com)[54] are consistent with its common use in the US.

Similarly, because it has not been regulated as a drug in the US, information or statistics on its safety in common use is limited. General statements of its relative safety as a nutritional supplement at doses tested in the exercise physiology literature (see below, 2.2.1-A.2.a.) reflect the lack of major adverse effects from acute or subacute dosing with multiple grams-per-day usage. For example, "*The Health Professional's Guide to Dietary Supplements"* notes, "*In general, supplemental inosine appears to be safe in doses of 5-6 grams for several weeks.*"[55]

2) **Food Additive/Component (Umami Taste of Inosine Monophosphate)** Inosine is the natural dephosphorylation product of inosine monophosphate (IMP; also known as inosinic acid or inosinate) as well as the deamination product of adenosine metabolism. IMP is commonly used as a food additive because of its flavor enhancing properties. IMP is a key component of the 'umami taste',[56] a primary taste (in addition to bitter, salty, sour and sweet) and generally described as beefy, brothy or savory. A substantial portion of dietary IMP is converted to inosine prior to ingestion (during the manufacture process)[57-59] and/or through human metabolism[60] (dephosphorylating nucleotidase activity).

Although exposures to inosine via IMP are generally much lower than that for the SURE-PD3 trial of inosine, oral administration of IMP in safety tests in animals or humans are consistent with a favorable safety profile of inosine at doses relevant to the planned trial. Healthy volunteers were given up to 2.5 gm/day IMP over 7 consecutive days with no ill effects despite a doubling of serum urate from 3.6 to 6.9 mg/dL.[61,62] A publicly posted evaluation of IMP by the United States Food and Drug Administration (FDA) (Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA) stated, "*The Committee concluded that, on the basis of the available data, the combined total daily intake of disodium 5'-guanylate and disodium 5'-inosinate [IMP] is not of toxicological significance,...*",[63] though common exposure to IMP is likely at the sub-gram level.

B) Human Research Studies

1) **Studies of Inosine Use in Exercise Physiology**
Published studies of oral inosine effects on athletic performance have all shown that multi-gram doses are well tolerated acutely, but have provided no evidence of putative ergonomic benefit. In order of decreasing cumulative inosine exposure, these are:

- McNaughton L, Dalton B, Tarr J. (1999).[50] Inosine supplementation has no effect on aerobic or anaerobic cycling performance. *Int J Sport Nutr* 9:333-344.
  - 10.0 gm/day (5.0 gm b.i.d.) inosine x 10 days
  - associated with rise in serum urate from 7.3 (baseline) to 13.9 mg/dL
  - no adverse events were reported; however the sample size was small: n=7 healthy fit subjects (all active cyclists training at 340 ± 36 km/wk) followed for 10 days

- Starling, R. et al. (1996).[49] Effect of inosine supplementation on aerobic and anaerobic cycling performance. *Med Sci Sports Exerc* 28:1193-1198.
  - 5.0 gm/day inosine x 5 days

- Williams, M. et al. (1990).[48] Effect of oral inosine supplementation on 3-mile treadmill run performance and VO_2 peak. *Med Sci Sports Exerc*. 22:517-522.
  - 6.0 gm/day inosine x 2 days

2) Clinical Trials of Inosine

i) Inosine for Multiple Sclerosis (US/European trials)

To our knowledge five clinical trials have been undertaken to evaluate the effects of urate-elevating doses of inosine in multiple sclerosis (MS). Inosine has been administered in this population (mean subject age ~40; ~75% women) for prolonged periods (1-3 years) at up to 4 gm/day (*b.i.d*. dosing).

Although the safety results for two of the studies[41,45] noted no adverse effects (including urolithiasis, gout, renal disease and cardiovascular disease), more preliminary and more recently published data from the other 3 trials[25,26,47] indicate an adverse event of urolithiasis has occurred in up to 25% of the study subjects. In a Univ. of Penn. study (IND#57,871)[47] urolithiasis was associated with excessive hyperuricemia (i.e., above this study's planned target range) prior to implementing standard preventative recommendations for kidney stones. In this study 4 of 16 (25%) subjects on inosine developed urolithiasis (4 during active drug treatment), which resolved readily with medical measures.

In a Spanish study,[46] two (11%) of the subjects treated with inosine developed renal colic (personal communication, D. Muñoz), presumably due to renal lithiasis. These episodes resolved without need for hospitalization. In the largest (Belgian) study,[26] urolithiasis also occurred but only in three subjects (3.8%) on inosine therapy, with one subject (1.2%) on placebo developing urolithiasis as well, over two years. Of note, in all five of these prior studies there were no apparent efforts to reduce the risk through screening, monitoring or prophylaxis of low urine pH, a major risk factor in the formation of uric acid urolithiasis.[64]

Elevation of urate levels in MS has been studied under two INDs. In IND #54,969 (Dr. Hilary Koprowski, sponsor), urate was administered to subjects with severe MS. Under INDs #54,969 and #57,871 (Dr. Hilary Koprowski, sponsor) inosine administration to subjects with MS was proposed and conducted in order to elevate serum urate levels. A letter of authorization to cross-reference those INDs was provided by Dr. Koprowski.

ii) Inosine for Other Conditions – The use of inosine has been reported in peer-reviewed literature in other clinical indications, including Tourette syndrome (at doses corresponding to 4-6 gm/day x 1 year)[65] and myocardial infarction (up to 0.8 gm/day x 1 month in subjects with cardiovascular disease [CVD])[66-69]. Preclinical data[70-72] on potential benefits of inosine for spinal cord injury and stroke recovery have been reported; no trials have been registered for these indications.

iii) Inosine Pranobex for Immune Disorders – Inosine pranobex is a drug approved and marketed since 1971 for use now in 43 countries outside the US (including Canada, France, Germany, Italy, Japan and the UK) for the treatment of immune disorders.[73] It comprises inosine together with 2-hydroxypropyldimethylammon-ium 4-acetamidobenzoate in a 1:3 molar ratio (such that 10.0 gm of inosine pranobex contains 2.54 gm inosine). Inosine pranobex is also known as Isoprinosine, Inosiplex and Immunovir (CAS: 36703-88-5). The US National Library of Medicine defines inosine pranobex as, "*An alkylamino-alcohol complex of inosine used in the treatment of a variety of viral infections. Unlike other antiviral agents, it acts by modifying or stimulating cell-mediated immune processes rather than acting*

on the virus directly."[74] The immunological disorders for which inosine pranobex may be an effective treatment are primarily viral illnesses such as HIV infection,[75] herpes simplex virus infections, genital warts,[76] influenza, zoster, viral hepatitis, subacute sclerosing panencephalitis.[77] On September 20, 1988 the FDA designated inosine pranobex as orphan product status for the indication of "treatment of subacute sclerosing panencephalitis".[78] www.clinicaltrials.gov lists seven completed trials of inosine pranobex.[79]

Usual oral dosage of inosine pranobex corresponds to an inosine dose of approximately 0.8-1.8 gm/day,[77,80] which generally produces a mild elevation of serum urate without significant adverse effects. For example, in a study of 3 gm/day inosine pranobex (corresponding to an inosine dose of 0.8 gm/day) serum urate rose from 4.0 to 5.4 mg/dL over 3 months (without gout or urolithiasis).[81] In the European Isoprinosine Trial[82] the equivalent of 1.1 gm/day inosine administered over two years to subjects with multiple sclerosis produced a serum urate elevation of 42% in men (4.4→6.2 mg/dL), and of 15% in women (3.5→4.0 mg/dL) (R. Gonsette, European Isoprinosine Trial PI, personal communication).

Inosine pranobex at typical doses in clinical use is considered to have a good clinical safety profile. A review in *Drug Safety* entitled, "Adverse effects and drug interactions of clinical importance with antiviral drugs" noted that, "Only inosine pranobex is largely free from toxicity."[83] For example, in a major study of the effects of inosine pranobex on the development of AIDS, "No serious side effects were observed" in the 429 subjects taking 3 gm/day inosine pranobex for 24 weeks.[75] However, differences in the dose of inosine itself, the duration of drug exposure and study population age limit the relevance of the comparison between such studies of inosine pranobex to our trial of inosine for PD.

2.2.2 Human Experience with Elevated Urate

A) Epidemiology of Urate and Disease (other than PD)

Higher Serum Urate: Clinical Consequences and Associations – The planned intervention, oral inosine titrated to moderate elevation of serum urate levels is expected to increase risks of gout and uric acid kidney stones, which are caused by local increases in urate or uric acid concentration. As discussed below, the risk of uric acid urolithiasis can be markedly reduced by avoiding or raising low urinary pH, whereas the risk of gout can be minimized but not eliminated by avoiding prolonged elevation of serum urate concentrations above ~8 mg/dL (the upper limit of the target range). In contrast, there is no conclusive evidence that increasing serum urate will affect the risk of cardiovascular events or other diseases. Nevertheless, we will preemptively exclude from the trial individuals at high cardiovascular risk and will closely monitor the subjects for any evidence of harm. Most importantly, we expect that the hypothesized slowing of PD progression produced by urate elevation will more than compensate for the known adverse effects of higher urate levels, which are reversible or treatable.

1) Epidemiology of Urate and Gout

Although it is widely accepted that high levels of serum urate predispose to gout, there are few published prospective epidemiologic studies that have investigated the relationship between prior urate levels and the risk of incident gout.

As part of the Framingham Study, a total of 5,127 individuals were examined biennially for a follow-up of 12 years.[84] The incidence of gout increased with increasing levels of urate. Over this period, gout developed in 18 (1.1%) of 1,615 men with urate < 6 mg/dL; 26 (7.3%) of 354 men

with urate between 6 and 6.9 mg/dL, and 11 (14.2%) of 78 men with urate between 7.0 and 7.9 mg/dL. The corresponding annual incidence rates were thus approximately 0.1%, 0.6%, and 1.1 %. The incidence rate of gout increased sharply at urate levels above 8 mg/dL, with 8 (36%) of 22 men in this range developing gout. In women, gout developed in 3 (0.1%) of 2,405 with urate < 6 mg/dL; 5 (7.0%) of those with urate between 6.0 and 6.9 mg/dL; and 3 (27.2%) of those with urate between 7.0 and 7.9 mg/dL. The corresponding annual incidence rates were thus approximately 0.01%, 0.6%, and 2.6%. Only one woman had a serum urate level above 8 mg/dL and she did not develop gout.

In a study of 2,046 initially healthy men in the Normative Aging Study followed for about 15 years there were 87 incident cases of gout. The annual incidence of gout was 4.9% in men with serum urate values of 9.0 mg/dL or more, compared with an annual incidence of 0.5% for serum urate levels of 7.0 to 8.9 mg/dL, and an annual incidence of only 0.1 % for serum urate levels < 7.0 mg/dL.[85] Among men with serum urate levels ranging between 7.0 and < 8.0 mg/dL (the target interval in SURE-PD3), the annual incidence of gout was 0.4% in the Normative Aging Study.

In a Chinese prospective study among men with hyperuricemia (defined as serum concentrations > 7.0mg/dL at baseline) 42 cases of incident gout were documented during the 5-year follow-up.[86] Annual incidence rates of gout in this study were 2.2% for serum urate levels of 7.0-7.9 mg/dL**,** 5.5% for 8.0-8.9 mg/dL, and 12.2% for > 9.0 mg/dL.

Thus, the associated risk of gout might be considered an acceptable risk if the benefits of increasing urate by administration of inosine can be demonstrated to delay the progression of PD. Based on available epidemiological evidence, one may expect an excess of 0.3 to 2.6 episodes of gout for 100 persons per year in the group targeted to 7.0-8.0 mg/dL of serum urate. However, there was no episode of gout or gout-like symptoms among PD patients with over 25,000 subject-days of exposure to urate-elevating inosine in our phase 2 trial[102], and similarly none were reported among ~200 MS patients in multiple prior trials of urate-elevating inosine (despite numerous kidney stone AEs among these subjects)[25,26,41,45,46]. Also of note, inosine is unlikely to cause gout per se, as inosine-induced hyperuricemia is expected to be reversible and gout is defined as a disease of recurrent episodes of urate crystal-induced arthritis and its degenerative consequences.

2) Epidemiology of Urate and Renal Lithiasis

*Formation of uric acid stones***.** Higher urate generation leads not only to higher urate levels in the serum, but also in the urine (uricosuria) and consequently the risk of kidney stones, specifically those comprising uric acid crystals. However the main determinant of uric acid stone formation is not uricosuria, but an acidic urinary pH. [29,64,87,88] Uric acid stone formation can thus be prevented even in individuals with high urine urate, provided their urinary pH is greater than 5.5.[89] Although cases of nephrolithiasis were reported in some of the previous trials using inosine (see above), no monitoring of urinary pH was implemented in any of those studies. Our strategy for preventing uric acid nephrolithiasis in SURE-PD3 includes exclusion of subjects with acidic urine, monitoring of urinary pH,[90] and raising of urinary pH by administration of potassium citrate or other alkalinization therapy if it falls below 5.5 (or 6.0 in the presence of uric acid crystalluria).[89,91] In the past, concerns were raised that uricosuria could also contribute to the formation of calcium oxalate stones,[92] but this relationship was not supported by the results of further study.[93]

3) Correlations between Serum Urate and Hypertension

A positive association between baseline serum urate and risk of developing hypertension has been consistently observed in numerous longitudinal studies, and seems to be independent from other known risk factors.[94-99] In recent studies in which relative risk estimates were adjusted for baseline body mass index, blood pressure, and other potential confounding factors, the increased risk of

hypertension seems to be linear, without obvious threshold effects. The relative risk associated with a 1.0 mg/dL difference in serum urate concentrations ranged from 1.09 (95% CI: 1.02 to 1.17) among 3,073 men followed for six years as part of the Multiple Risk Factors Intervention Trial (MRFIT)[99] to 1.31 among 2,520 individuals (men and women) followed for 10 years as part of the Beaver Dam Study;[95] the pooled relative risk was 1.13 (95% CI: 1.06 to 1.20) in a 2011 meta-analysis.[100] Results of the same meta-analysis suggested that the association between urate and hypertension tends to be stronger at younger ages and in women. An increase in blood pressure has also been found among individuals who did not develop hypertension during the follow-up. For example, a 1.0 mg/dL increase in serum urate concentration was found to be associated with a 2.5 mm Hg increase in systolic blood pressure (SBP) and a 1.2 mm Hg increase in diastolic blood pressure (DBP) after 5-years of follow-up in the Beaver Dam Study,[95] whereas a smaller average increase was reported over a one year period in the MRFIT (0.33/0.16 mm Hg, SBP/DBP).[99]

However, recent studies in humans with MS[47,101] and PD[102] (see below) on the effects of urate-elevating inosine found no detectable effect on blood pressure (SBP, DBP or their orthostatic changes) of inosine that elevated serum urate by up to 4 mg/dL to achieve chronic stable levels of 7-8 mg/dL. Thus any increase in blood pressure attributable to urate elevation that might be observed in SURE-PD3 would likely be modest. Further, among individuals with PD, blood pressure tends to decline early in the disease course.[103] Nevertheless, blood pressure will be closely monitored during the trial and anti-hypertensive treatment will be initiated or adjusted as needed.

4) Other Potential Adverse Effects of Elevated Serum Urate

The association between serum urate and health outcomes has been investigated in numerous longitudinal studies spanning over five decades, but whether high serum urate is an <u>independent</u> risk factor for chronic diseases other than kidney stones, gout, or hypertension remains controversial. Although individuals with high serum urate in several studies were found to have higher rates of heart disease and stroke, these adverse associations were largely explained by the correlation between urate and other risk factors, including obesity, hypertension, high plasma lipids, renal insufficiency and other markers of increased cardiovascular risk or general poor health. In some studies a positive association between serum urate and cardiovascular morbidity or mortality remained after adjustment for other risk factors, but this adjustment was in all cases incomplete -- potential confounders were either poorly measured or completely ignored. Among the potential confounders not considered are levels of physical activity and dietary factors. A summary of literature on urate and cardiovascular risk is provided below.

i) <u>Coronary heart disease</u> (CHD) – In several rigorous longitudinal investigations, most notably in the Framingham Study,[104] there was no independent relation between baseline serum urate and risk of coronary events (relative risk for highest category of urate *versus* lowest = 0.91; 95% CI: 0.83 to 0.99), coronary mortality (0.95; 0.86 to 1.06), or all cause mortality (0.97; 0.91 to 1.03). In contrast, several other investigations reported an increased risk of cardiovascular outcomes, although the associations were strongly attenuated after adjustment for other risk factors. A meta-analysis of 16 longitudinal studies including 9,458 cases of coronary heart disease and 155,084 controls was published in 2005 -- the relative risk comparing individuals in the highest third of serum urate serum concentrations to those in the lowest third was 1.13 (CI: 1.07 to 1.20), but it was only 1.02 (CI: 0.91 to 1.14) in the eight studies with more complete adjustment for possible confounders.[105] In a separate review, the association between serum urate concentrations and cardiovascular events was reported to be small in healthy individuals, but possibly important in high-risk patients.[106] In 2010 Kim et al.[107] have conducted a new meta-analysis of prospective studies including a total of over 400,000 subjects – relative

risks (RR) were reported for hyperuricemic vs. non-hyperuricemic individuals, with the definition of hyperuricemia varying across studies. The pooled relative risk of incident CHD after adjustment for traditional risk factors (age, sex, hypertension, hypercholesterolemia, obesity, and smoking) was 1.09 (95% confidence interval: 1.03 to 1.16). Sex-specific adjusted relative risks were 1.04 (0.90 to 1.17) in men (based on 7 studies) and 1.07 (0.82 to 1.32) in women (based on 4 studies). The pooled and adjusted RR for CHD mortality was 1.16 (1.01 to 1.30) in men (based on 8 studies) and 1.67 (1.30 to 2.04) in women (based on four studies).

A few studies published after this meta-analysis have reported mixed findings, but most have been too small to substantially impact the overall meta-analysis results.[108-116] The largest studies have been based on historical databases, in which information on potential confounders is most likely incomplete. In a historical cohort based on laboratory and insurance claims among 148,217 patients in the U.S., serum urate was associated with cardiovascular morbidity, particularly among individuals with reduced glomerular filtration rate.[117] Two investigations were conducted in Taiwan. Among 128,569 adults recruited in a cohort in 1994-6 and followed through a National Health Insurance database, the relative risk for ischemic heart disease was 1.25 (1.11 to 1.40) in men with serum urate ≥ 7.0 mg/dL and 1.19 (1.02 to 1.38) in women with serum urate ≥ 6.0 mg/dL as compared to those with lower levels.[118] In a separate investigation based on the secondary data analyses of over 350,000 medical records, serum urate displayed a U-shaped relation with both all-cause mortality and cardiovascular mortality – the lowest risk was reported for levels between 5 and 6.9 mg/dL.[119,120] In a 2013 meta-analysis of randomized trials in which serum urate was measured at baseline and at the end of follow-up, no significant association was found between changes in serum urate and cardiovascular outcomes.[121] Finally, a 2013 meta-analysis reported estimates on the association between uricemia and cardiovascular mortality or all-causes mortality. For the highest urate level as compared to the lowest, the relative risk for all-causes mortality was 1.23 (1.08 to 1.42) in men, and 1.05 (0.79 to 1.39) in women; for cardiovascular mortality the relative risk was 1.30 (1.07 to 1.59) in men, and 1.35 (1.06 to 1.72) in women.[122]

ii) Stroke – The relation between hyperuricemia and risk of stroke has been summarized in a 2009 meta-analysis including data from a total of 16 prospective studies and over 230,000 individuals. After adjustment for traditional risk factors, the pooled relative risk of stroke was 1.47 (1.19 to 1.76), with results similar in men and women.[123] An increased risk of stroke in individuals with higher urate levels has been confirmed in subsequent studies. In a cohort of 5,700 people in Norway, a 1 SD increase in serum urate (~ 1.5 mg/dL) was associated with a relative risk of 1.22 (1.09 to 1.35).[124] Similarly, a recent population-based study found hyperuricemia to be related to white matter atrophy (assessed by magnetic resonance imaging) and worse cognition, an association that was only partially attenuated by adjustment for several markers of vascular disease.[209]

The results of these studies should be interpreted cautiously, because serum urate positively correlates with several risk factors for cardiovascular disease, including among others, obesity, high plasma triglycerides, high blood pressure, low level of physical activity, metabolic syndrome, and several dietary factors. As expected, therefore, individuals with high levels of serum urate tend to have a higher risk of CHD or stroke, but this risk is strongly attenuated after adjustment for other cardiovascular risk factors. Moreover, serum urate increases with declining renal function, which is also a poor prognostic factor for chronic diseases and survival. Whether hyperuricemia is a contributory cause rather than just a consequence of kidney failure remains uncertain. Adjustment for potential confounders has been incomplete in many studies, because of lack of

information or measurement error in the confounders.[125,126] Conspicuously absent among adjusting factors are physical activity and diet, which are strong determinants of risk of CHD and stroke.[127,128] It remains therefore possible that the positive association between serum urate and cardiovascular risk or overall mortality seen in some studies is artifactual, due simply to confounding risk factors.

In spite of these limitations, an adverse effect of urate on cardiovascular events cannot be excluded. Because many large investigations did not find a significant association, such effects would most likely be modest. The actual number of events attributable to the intervention would depend not only on the relative risk, but also on the underlying incidence of cardiovascular events in the study population. For example, if inosine treatment increased the risk of major cardiovascular events (including nonfatal stroke, nonfatal myocardial infarction, or death from any cardiovascular cause) by 20%, the number of expected cases attributable to the treatment would be 1.5 per 1,000 persons per year in a population with a baseline rate of 7.7 per 1,000, the rate based on 2,607 events observed during the 20-year follow-up of nearly 18,000 men aged 40 to 84 at baseline in the Physician Health Study.[129]

It should be emphasized again that there is no convincing evidence that elevating serum urate will increase the risk of cardiovascular events. The number of excess cases provided as an example above is thus hypothetical. However, to try to further minimize risk, individuals with a history of major cardiovascular morbidity or decreased kidney function will be excluded from SURE-PD3, and all subjects will be closely monitored for increases in blood pressure and other early signs of coronary or cerebrovascular events.

5) Serum Urate and Mortality Among Patients with PD

For the purpose of the planned trial, a key question is whether a slowing of PD progression that may result from increasing serum urate would offset the increased risk of kidney stones, gout, and possibly hypertension and other adverse events due to elevated serum urate. Data on serum urate and survival in PD could be analyzed for 768 of the 804 subjects with early PD enrolled in the DATATOP trial, for whom serum urate at baseline and 13-year survival are available.[36] Two hundred and ninety two patients died during the follow-up (211 among men); the hazard ratio for all-cause mortality, adjusted for age, treatment group, pack-years of smoking, did not significantly increase with serum urate levels. The hazard ratio comparing patients in the top quintile of serum urate to those in the bottom was 0.89 (95% CI: 0.53 to 1.50) in men and 1.96 (95% CI: 0.89 to 4.33) in women.[36] These data, like the epidemiological studies discussed above, are of an observational nature, and potentially confounded by other risk factors for cardiovascular disease and renal function.

B) Drug-Induced Urate Alterations and Clinical Consequences

1) Randomized Trials of Drugs that Elevate Serum Urate (Thiazide-Type Diuretics)

An increase in serum urate is a well-known side effect of thiazide diuretics, which are used by millions of individuals in the U.S. and other countries as a first line drug for the treatment for arterial hypertension. In individuals with high blood pressure, treatment with thiazides clearly reduces morbidity and mortality in spite of modest increases in serum urate levels.

Detailed analyses on the effects of serum urate at baseline and following treatment with a thiazide diuretic (primarily clorthalidone, at 50 mg/day) were conducted among 3,693 participants in the Hypertension Detection and Follow-up Program (HDFP).[130] Half of the participants in the HDFP were randomly assigned to receive intensive antihypertensive treatment given in special clinics (stepped care), and the other half were referred back to their usual sources of care. Step-1 of the

HDFP drug protocol was chlorthalidone, 50mg/d; reserpine, methyldopa, or hydralazine hydrochloride were added as needed. After 4-months of follow-up, 1,230 (59.5%) of the 2,068 men were being treated with a thiazide diuretic. Among these men, 506 (41.1%) had serum urate concentrations ≥ 8.0 mg/dL. Among women, 905 (55.7%) of 1,625 were treated with thiazide diuretics, among whom serum urate concentrations were ≥ 8.0 mg/dL in 140 (15.5%). The HDFP protocol required temporary discontinuation of thiazides if symptoms of gout appeared. Only 15 of the 3,693 individuals at risk had their thiazide diuretic therapy suspended for this reason.

The one-year change in serum urate among men treated with thiazides was 1.3 mg/dL in men in the lowest quartile of serum urate at baseline (mean at baseline 4.9 mg/dL) and 0.85 among men in the highest quartile at baseline (mean at baseline 7.7 mg/dL). Similar increases in serum urate following thiazide treatment were observed in women, although baseline levels were lower. Overall, no significant association was found between serum urate and changes in creatinine levels (as a marker of renal damage) in either men or women. Among men, no significant association was found between serum urate at baseline and overall survival, but a positive association was found in women. As for the studies above, however, this association was more likely due to confounding by hyperlipidemia and other risk factors. Conclusions of the authors was: "…in the HDFP, analysis of data regarding the elevation of uric acid associated with the administration of thiazide diuretics in the treatment of hypertension provides little evidence of an adverse effect for the patient without renal damage or history of gout".

2) Randomized Trials of Drugs that Reduce Serum Urate (Allopurinol, Oxypurinol, Probenecid, Urate Oxidase)

Because of the association between serum urate and hypertension discussed above, it has been hypothesized that drugs that reduce serum urate either by inhibiting its synthesis (e.g., allopurinol), catalyzing its degradation (urate oxidase), or increasing its renal excretion (e.g., probenecid) could contribute to treatment or prevention of hypertension. However, despite their consistent ability to lower urate, these agents have shown variable effects on blood pressure/vascular function in controlled trials;[131-134] new studies of allopurinol are ongoing.[79] Similarly, a recent study of another xanthine oxidase inhibitor oxypurinol in congestive heart failure[135] found no evidence of hypothesized health benefits over 6 months despite a reduction in serum urate levels by 2 mg/dL. Possible beneficial effects of allopurinol have been reported in some observational studies. In a retrospective study in Canada, use of allopurinol in patients with heart failure gout was associated with lower risk of readmission for heart failure or death.[136] In a recent case-control study, the odds ratio for a first myocardial infarction associated with allopurinol use was 0.80 (0.59 to 1.09); this inverse association reached borderline significance when using less stringent matching criteria.[137] It should be noted, however, that these studies do not directly test the hypothesis that urate itself is a cause of hypertension or cardiac dysfunction, because inhibition of xanthine oxidase may also directly reduce the generation of oxygen radicals and thereby affecting endothelial and cardiovascular function.[131]

2.2.3 Experience with Inosine to Elevate Urate in PD (Phase 2 Development)

In preparation for the planned clinical efficacy trial of inosine we conducted a phase 2 study to assess its safety, tolerability and urate-elevating capability in early PD, and to inform the design of the phase 3 trial. The Safety of URate Elevation in PD (SURE-PD)[102] study was a randomized, double-blind, placebo-controlled, dose-ranging trial of inosine. Seventy-five adults (mean age 62; 55% women) with early PD not yet requiring symptomatic treatment and a serum urate concentration below 6 mg/dL (the approximate population median) were enrolled for up to 25 months at 17 credentialed clinical study sites of the Parkinson Study Group (PSG). Subjects were randomized to one of three treatment arms: placebo or

inosine titrated to produce mild (6.1-7.0 mg/dL) or moderate (7.1-8.0 mg/dL) serum urate elevation using 500 mg capsules taken orally up to two thrice daily. They were followed for up to 24 months (median 18) on study drug plus 1 wash-out month. The pre-specified primary outcomes were absence of unacceptable serious adverse events (safety), continued treatment without adverse event requiring dose reduction (tolerability), and elevation of urate assessed serially in serum and once (at 3 months) in cerebrospinal fluid (CSF). The trial was registered at ClinicalTrials.gov under NCT00833690.[79]

The SURE-PD study has yielded extensive data that support and inform an advance to phase 3 development:

A) Safety and Tolerability Outcomes Serious adverse events (17), including infrequent cardiovascular events, occurred at the same or lower rates in inosine groups relative to placebo (Table 2). No subject developed gout and three receiving inosine developed symptomatic urolithiasis (Table 3). These stones were reported only in women after more than 4 months of receiving the study drug[102] and may have been dose dependent (0, 1, and 2 events in the placebo, mild, and moderate groups, respectively; Table 2). Need for alkalinization was rare because urine pH was unaffected by inosine.[102] Urine collected at each visit was also assessed for the presence of various crystals, and their potential use in monitoring inosine-induced urolithiasis risk was investigated. Although no crystal type was predictive of urolithiasis, uric acid crystals were observed in urine from 10 subjects with a dose-dependent distribution (0 placebo, 3 mild, and 7 moderate).[102] The 1 subject who developed a documented symptomatic uric acid stone (after 14 months of inosine in the moderate urate elevation arm) had tested positive for uric acid crystalluria and had relatively low urine pH hovering at 5.5 (just above the trigger for alkalinization). Stones in 2 other subjects were likely not uric acid because the composition of one was documented as "65% calcium oxalate dihydrate + 35% carbonate apatite," and the other though not analyzed was from a subject whose urine pH was ~6.5, which is usually incompatible with uric acid stone formation.

Secondary safety outcomes, including those associated with hyperuricemia,[122,126]

Table 2. Serious Adverse Events (AEs) in SURE-PD

	No. (%)[b]			
Serious AEs[a]	Placebo	Ino→ Mild	Ino→ Mod	Overall
Cardiac	1 (4)	0	1 (4)	2 (3)
Acute coronary syndrome	1 (4)	0	0	1 (1)
Coronary artery disease	0	0	1 (4)	1 (1)
Hepatobiliary				
Cholecystitis	0	0	1 (4)	1 (1)
Infections and infestations	2 (8)	1 (4)	0	3 (4)
Human ehrlichiosis	0	1 (4)	0	1 (1)
Pneumonia	1 (4)	0	0	1 (1)
Urosepsis	1 (4)	0	0	1 (1)
Injury				
Cervical fracture	0	1 (4)	0	1 (1)
Musculoskeletal	4 (16)	0	0	4 (5)
Arthritis	3 (12)	0	0	3 (4)
Synovial cyst	1 (4)	0	0	1 (1)
Nervous system	2 (8)	0	0	2 (3)
Cerebrovascular accident	1 (4)	0	0	1 (1)
Radiculopathy	1 (4)	0	0	1 (1)
Psychiatric	1 (4)	0	1 (4)	2 (3)
Depression	1 (4)	0	0	1 (1)
Suicide ideation	0	0	1 (4)	1 (1)
Renal				
Nephrolithiasis	0	0	1 (4)	1 (1)
Respiratory				
Pulmonary fibrosis	1 (4)	0	0	1 (1)
Overall	11 (36)	2 (8)	4 (15)	17 (20)

Table 3. AEs of Special Concern in SURE-PD

	No. (%)[b]			
AEs[a] of Special Concern	Placebo	Ino→ Mild	Ino→ Mod	Overall
Cardiovascular	5 (16)	0	1 (4)	6 (7)
Acute coronary syndrome	1 (4)	0	0	1 (1)
Atrial fibrillation	1 (4)	0	0	1 (1)
Cerebrovascular accident	1 (4)	0	0	1 (1)
Coronary artery disease	0	0	1 (4)	1 (1)
Palpitations	1 (4)	0	0	1 (1)
Tachycardia	1 (4)	0	0	1 (1)
Goutlike symptoms	1 (4)	3 (8)	2 (7)	6 (7)
Arthralgia of toe(s)[c]	1 (4)	2 (4)	1 (4)	4 (5)
Swelling of toe(s)[d]	0	1 (4)	1 (4)	2 (3)
Urolithiasis or its symptoms	0	2 (2)	2 (2)	4 (5)
Hematuria	0	1 (4)	0	1 (1)
Nephrolithiasis	0	1 (4)	2 (7)	3 (4)
Overall	6 (20)	5 (17)	5 (19)	16 (19)

[a] Medical Dictionary for Regulatory Activities system organ class and preferred terms.
[b] Values show total number of events (% of participants).
[c] Arthralgia of toes combines the following preferred terms in instances where the verbatim complaints mentioned feet or toes: arthralgia and pain in extremity.
[d] Swelling of toes combines the following preferred terms in instances where the verbatim complaints mentioned feet or toes: joint swelling and local swelling.
Abbreviations: Ino→Mild, inosine dosed to mildly elevate urate; Ino→Mod, inosine dosed to moderately elevate urate. [Adapted from Ref. 102.]

did not differ between treatment groups. For example, serial vital signs, serum assays, and electrocardiograms (ECGs) showed no effect of inosine on blood pressure (or its orthostatic changes), body mass index, serum glucose and cholesterol levels, or ECG parameters.[102] And despite the increased frequency of urolithiasis while receiving inosine, there were no other renal SAEs and renal function as assessed by serum creatinine and estimated glomerular filtration rate remained unchanged from baseline in all groups.

Treatment was tolerated by 95% of subjects at 6 months (Fig. 5A), and no subject withdrew due to an adverse event. Similarly, retention and compliance were excellent, with 99% completing the study[102] and 100% of placebo subjects maintaining a mean serum urate within the baseline range (<6.0 mg/dL), consistent with no surreptitious use of OTC inosine. Thus a high proportion tolerated and maintained the assigned treatment, and overall safety results were reassuring. Nevertheless, urolithiasis remains a

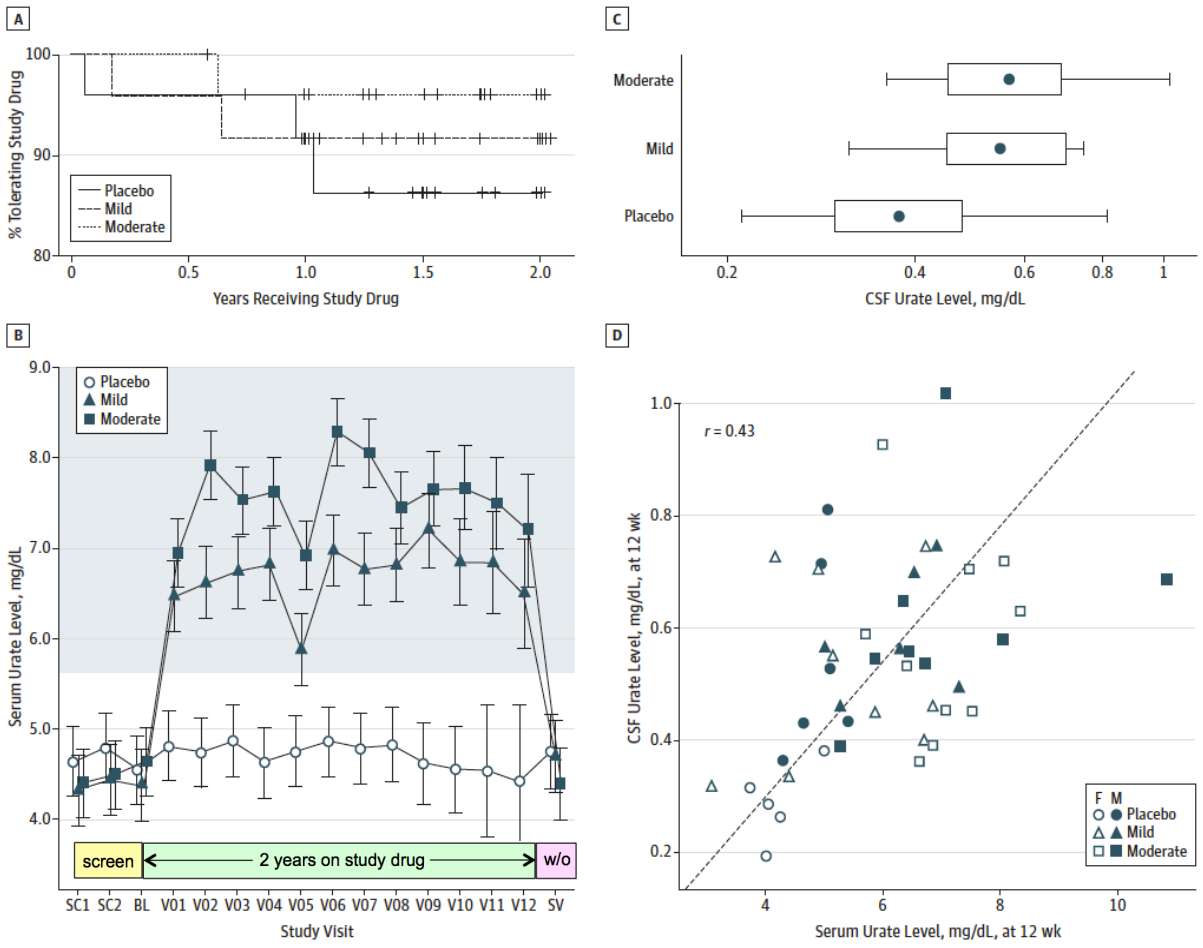


Figure 5. Tolerability of Inosine and Its Effects on Serum and CSF Urate Levels.
A, Tolerability of the study drug from baseline to drug discontinuation displayed as Kaplan-Meier survival curves over the maximum 2-year period for participants taking placebo or inosine dosed to mildly or moderately raise serum urate. Tick marks indicate censored events (see the Methods section). **B**, Estimated time course of serum urate levels across study visits with the study drug initiated at the baseline (BL) visit and continued for as long as 24 months (V12) until 1 month before the final (safety) visit (SV). Means and 95% confidence intervals from a mixed model are displayed. For visits V1 to V12, serum was collected after morning study drug intake, except for the "trough" sample at week 12 (V05). The shaded range of serum urate concentrations represents exclusionary values at the screening visits (SC1 and SC2). **C**, The CSF urate concentrations and ranges (bars, with boxes and dots representing the interquartile and median values, respectively) after 12 weeks of receiving the study drug. $P < .001$ for the mild and moderate inosine groups compared with placebo. **D**, Correlation between CSF and serum urate levels at the 12-week visit for individuals identified by their treatment groups and sex. F indicates female and M, male. [Adapted from Fig. 2 of ref. 102.]

substantial risk that warrants consideration of options for further mitigation (see below, Sec. 3.2.4).

B) 'Target Engagement' Efficacy of Inosine

1) **Urate Elevation in Serum** (Fig. 5B)

A lesson learned from earlier failures of phase 3 trials for disease modification in PD is the importance of demonstrating target engagement at the doses tested for a candidate neurotherapeutic, to the extent feasible. For example, in retrospect a weakness of the PRECEPT trial was the uncertainty over whether the candidate protectant, a mixed lineage kinase (MLK) inhibitor, actually entered the CNS and/or inhibited MLK activity[30] – even in the periphery where it may have been readily measured. Current efforts to identify the most promising neuroprotectants for clinical development in neurodegenerative disease consider evidence that a candidate agent penetrates the relevant compartment (e.g., CNS) and that it engages its putative target in some manner to be key criteria[224,225].

Inosine differs from most other candidate disease-modifying drugs in that its development is uniquely driven by robust epidemiological and clinical biomarker data. Based on the reproducible linkage between serum or CSF urate and favorable PD outcomes, the elevation of urate itself rather than any of its possible mechanistic actions is arguably the most relevant target of inosine treatment to demonstrate, short of disease modification. Indeed, although the promise of urate-elevating inosine treatment is bolstered by consistent laboratory evidence of urate's neuroprotective actions,[12-19] whether they rely on its direct antioxidant,[2] metal chelator,[4] Nrf2 activator[217,218] or other properties is uncertain and therefore may be unnecessary (even if reassuring) to demonstrate in patients.

Participants randomized to mild or moderate elevation treatment arms were titrated to an average inosine dose of 1.18 or 1.51 gm/day, and achieved average increases in serum urate of 2.3 and 3.0 mg/dL, respectively ($p<0.001$).[102] Serum urate levels were significantly elevated above placebo as soon as the 2-week visit (V01, Fig. 5B). They were relatively constant starting at the 2-week visit among those in the mild elevation group and continued to rise during titration until the 4-week visit (V02) among those in the moderate elevation group. The 12-week visit (V05) was the only one for which participants were asked not to take their study drug beforehand, accounting for the apparent dip in serum urate at the time of this trough measurement. Serum urate had fully reverted to baseline levels by the time of the safety visit, one month after discontinuation of study drug.[102]

2) **Urate Elevation in CSF** (Fig. 5C, 5D)

CSF urate levels were measured once (at the 12-week visit) in 44 (59%) of the participants.[102] For the others consent to lumbar puncture was not given (29%) or lumbar punctures were contraindicated (e.g., participants on warfarin; 4%) or were attempted but failed (7%). Among those measured, levels were 40% and 50% higher in mild and moderate elevation treatment groups, respectively, relative to placebo participants ($p=0.006$ and $p<0.001$, respectively; Fig. 5C). There was evidence of a difference by gender. CSF urate levels were lower among female than male placebo participants, and were significantly elevated in the active arms relative to placebo only among female participants ($p<0.001$ for females in both inosine arms; $p=0.6$ and 0.4 for males in the lower and higher inosine arms). Twelve-week serum and CSF urate levels in women and men were modestly correlated ($r = 0.43$, Fig. 5D).

3) **Proof-of-Principle for Potential Antioxidant Mechanism** (Fig. 6) Inosine had a robust effect on plasma ferric reducing antioxidant power (FRAP)[199], as expected given the major contribution that urate makes to plasma total antioxidant capacity in humans.[3] Mild and moderate inosine elevated FRAP levels by ~35% and 50% at 6 months compared to baseline values and the elevations persisted until the third and final FRAP measurement between 12 and 24 months,[256] indicating that the putative benefit is not attenuated by compensatory mechanisms. By contrast, neither CSF FRAP nor the urinary excretion of 8-hydroxy-deoxyguanosine (8-OHdG), a DNA repair product that has been proposed as a marker of DNA oxidative damage,[200] was detectably affected by inosine.[256]

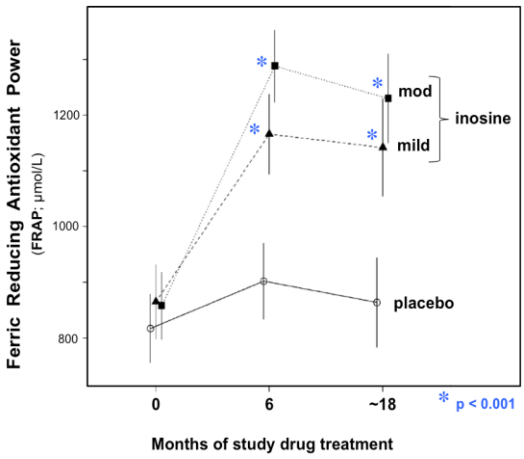


Figure 6. Inosine dose-dependently and chronically elevates plasma antioxidant capacity (measured as FRAP). *p<0.001 compared to baseline value; p=0.025 mod. vs mild Inosine group values at 6 months.[256]

C) Preliminary Clinical Efficacy Data Analyses

1) **Acute efficacy** (measured by change in total UPDRS score over 1-3 months):
No symptomatic effect was apparent during initiation of inosine treatment (p value=0.45 for change in slope of total UPDRS from baseline to 3 months for mild versus placebo, and p=0.23 for moderate versus placebo) or during the 1-month wash-out period (p=0.71 and p=0.30).[102]

2) **Long-Term Efficacy**:
Although not powered to determine the effects of inosine on long-term changes in clinical outcome measures, preliminary data were collected. Time to need for dopaminergic therapy, which was the primary endpoint in DATATOP and PRECEPT trials, was reached in 63% of the randomized participants over an average of 18 months and did not differ significantly among the treatment groups (Fig. 7A).

Plausible efficacy of serum urate elevation to delay symptomatic progression was assessed using a futility analysis approach equivalent to that employed for the primary analysis in the National Institutes of Health Exploratory Trials in PD (NET-PD) program[201,202] except that the active groups were compared to our own relatively small placebo group rather than to historical controls, a more conservative comparison relative to practice in NET-PD.[202] The two active groups were compared to a futility boundary (FB) calculated as 70% of the estimated progression among our placebo participants over 12 months. Both mild and moderate elevation treatments were non-futile based on this comparison for six parkinsonism (sub)scales including total UPDRS scores (Fig. 7B).

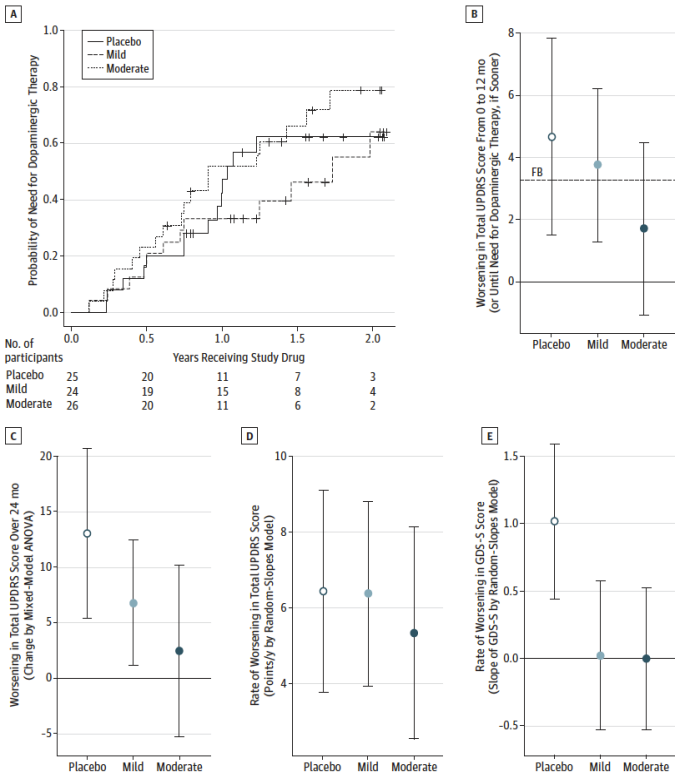


Figure 7. Secondary Analyses of Clinical Outcomes in the SURE-PD Study.

A, Kaplan-Meier curves showing time to disability warranting dopaminergic therapy for up to 2 years of follow-up for each of the 3 treatment groups. **B**, Futility analysis of the change in total Unified Parkinson's Disease Rating Scale (UPDRS) scores over 12 months or until need for dopaminergic therapy, based on National Institutes of Health Exploratory Trials in PD (NET-PD) methods. Much or most of the 95% confidence interval for the mild or moderate inosine treatment groups, respectively, falls below the futility boundary (FB), defined as 70% of the placebo group's mean rate of change. **C**, The 24-month change of total UDPRS score estimated from a mixed-model analysis of variance (ANOVA) allowing unstructured profiles over time suggests a trend of decreasing rate with increasing inosine dose. **D**, A weaker trend is observed when using a complementary random-slopes model incorporating sex-specific effects and assuming linearity in change over time. **E**, Rates of mood change during the study as assessed by differences in Geriatric Depression Scale short form (GDS-S)[189] scores over an average of 18 months' follow-up. Receiving either dose of inosine, the rate appears slower (comparison-wise *P* < .001) compared with placebo. [Adapted from Fig. 3 in ref. 102.]

To reduce the bias introduced by carrying forward the last UPDRS score for participants who develop need for dopaminergic treatment before the end of the observation period, we also employed two random-slopes models with follow-up truncated at the time of dopaminergic therapy initiation: one with no treatment x gender interaction but allowing unstructured profiles over time (i.e., separate treatment x visit estimates; Fig. 7C) and one including gender-specific effects of treatment but assuming linear trends in symptom scores over time (Fig. 7D). Like the futility analysis, these complementary approaches suggested attenuated clinical progression with increasing inosine doses, although the treatment differences were not significant.

Note however that these analyses are substantially underpowered to determine an effect of inosine on rates of clinical progression over 2 years, with a sample over 7-fold smaller than would be required for 80% power. Whereas each inosine or placebo arm in SURE-PD included ~25 early PD subjects, we have estimated that each arm in the SURE-PD3 trial will require an n of 135 early PD subjects with demonstrated DAT deficits, corresponding to n=168 un-enriched early PD subjects (see Fig. 11 below). Even this 7-fold (168/25) sample size difference likely underestimates the inadequacy of SURE-PD's sample size for efficacy analysis given that SURE-PD likely under-dosed inosine for the higher urate elevation target (7.1 – 8.0 mg/dL) based on random urate sampling (vs. trough sampling in this trial; see Sec. 3.2.2 below).

In additional exploratory efficacy analyses stratified by gender, the rate of deterioration as measured by change in total UPDRS scores appeared to decrease significantly with increasing serum urate in women, but not in men (Fig. 8). Whether this difference is related to the greater CSF urate elevation demonstrated in women (as above, Fig. 5D), an unexpectedly greater biological response to urate among women, or the small sample size (e.g., contributing to an anomalous cluster of men on placebo with no UPDRS change (Fig. 8, right panel) is not clear. This preliminary suggestion of greater efficacy of urate-elevating inosine in women than in men runs counter to the stronger epidemiological associations between urate and favorable PD risk or progression in men. The seeming paradox may be explained by naturally lower urate levels in women and the potential benefit of urate only at higher urate concentrations (contributing to significantly greater absolute increases in serum and CSF urate in women in SURE-PD[102]). Given this divergence of findings, no clear conclusions can be drawn from available data regarding gender-dependent differences in the effect of elevated urate levels. They do not warrant focusing an efficacy trial exclusively on either women or men. We believe that an equal potential remains for a protective effect of elevated urate for both women and men.

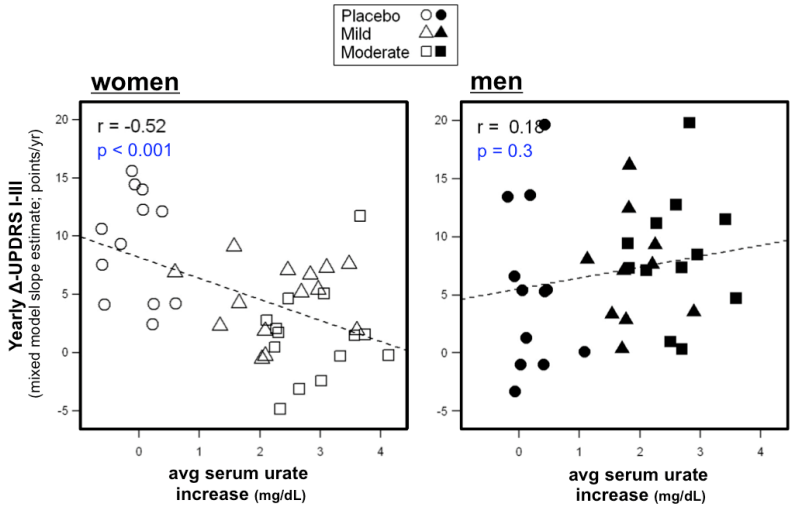


Figure 8. Exploratory long-term outcome: Rate of deterioration (change in total UPDRS) diminishes as urate increases in women, but not men in SURE-PD.[102] For each subject the increase in serum urate from the baseline to the average of on-treatment values was plotted against the individual's change in total UPDRS score using the conservative mixed model slope estimate shown in Fig. 7D. The placebo group data (circles) appear on the left of each plot given the expected lack of any urate change. Although a clustering of men in the placebo group showed no decline during the trial (filled circles in lower left) contributing to an overall trend toward worsening with increasing urate among men, it was not significant.

There was no evidence of an effect of active treatment on cognitive function as assessed by MoCA Rasch scores[203] although only non-demented individuals were enrolled and the placebo group showed no cognitive decline during the study. Mood as assessed on the GDS-S declined during the trial only among placebo participants, suggesting perhaps a preventative effect on depression of urate-elevating inosine (Fig. 7E; comparison-wise p <0.001 for each inosine group versus placebo). A possible prophylactic effect on the development of depression would be convergent with recent data from laboratory, epidemiological and PD biomarker studies. Inosine was recently found to produce beneficial effects in different animal models of depression.[226] Additionally, lower urate has been linked to depression in a cross-sectional study.[227] Also, as noted above, higher serum urate has been reported to predict a reduced risk of non-motor symptoms in PD, with lower urate levels among those PD patients who subsequently developed anxiety/depression domain deficits.[219]

Based on the results of phase 2 study, inosine was found to be generally safe, tolerable, and effective in raising serum and CSF urate levels in early PD. The findings support advancing to more definitive development of inosine as a potential disease-modifying therapy for PD.

3. STUDY DESIGN

3.1 Overall Study Design and Plan

A multicenter, randomized, double-blind, placebo-controlled trial of urate-elevating inosine to slow clinical decline over two years will be conducted on ~270 subjects with early PD. Clinical decline is assessed as change in the primary outcome variable of the Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS),[138] a composite scale comprising patient- and clinician-reported parkinsonian outcomes. Secondary outcome variables include disability warranting initiation of dopaminergic therapy, specific non-motor measures of cognitive, mood and autonomic function, and quality of life and disability measures.

During the enrollment period, approximately 667 subjects will be screened from approximately 60 Parkinson Study Group (PSG) clinical centers in the US (Fig. 9). Approximately 270 of these subjects with early PD and serum urate ≤5.7 mg/dL will be randomly assigned in a 1:1 ratio to oral placebo:inosine capsules. Inosine will be dosed by titration to achieve an elevation of serum urate to trough levels of 7.1 to 8.0 mg/dL, and placebo will be dosed to match the capsule titrations of the inosine group. After screening and randomization at baseline visit, subjects will undergo follow-up evaluations in clinic on treatment at Weeks 3, 6, 12 and Months 6, 9, 12, 15, 18, 21, and 24. A 3-month wash-out period off treatment will include two monthly telephone evaluations and a final in-person evaluation at Month 27. (See timeline in Fig. 10.)

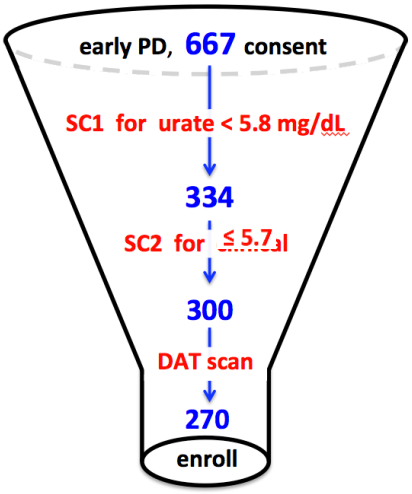


Figure 9. Major screening steps and impact. To achieve enrollment of 270 eligible subjects it is anticipated that ~667 consented subjects will be required, with screening visit 1 (SC1) excluding 50% based on higher serum urate, SC2 excluding 10% more based on the general clinical features (including safety lab data), and a subsequent dopamine transporter (DAT) brain scan excluding an additional 10%.

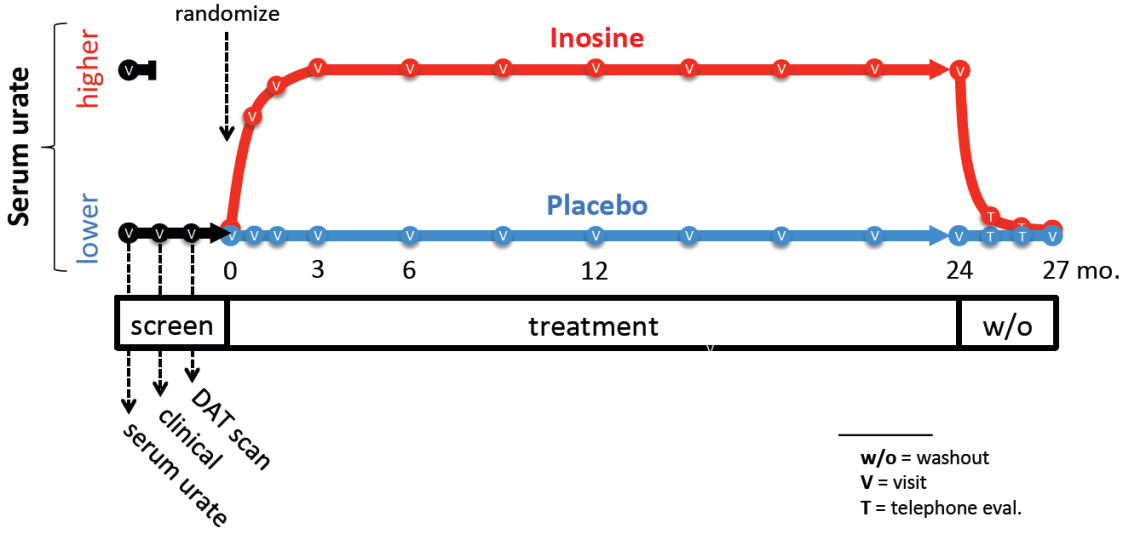


Figure 10. Diagram and Timeline of Treatment

3.2 Rationale for Key/Novel Design Features

The rationale for the planned phase 3 trial of inosine for slowing clinical decline in PD builds on earlier advances in preclinical to phase 2 clinical development, as detailed above (Sec. 2.2.3):

- The rationale for **elevating urate in PD** is based on a convergence of laboratory, epidemiological and clinical data supporting its neuroprotective potential.
- The rationale for **selecting inosine as the means to elevate urate** in PD is based upon knowledge of purine metabolism (Fig. 1), pharmacological data, non-clinical human experience, and clinical studies of non-parkinsonian subjects.
- The rationale for **advancing from a phase 2 to 3 clinical study** *is based on the positive results of the former (i.e., the SURE-PD trial) in supporting inosine's safety, tolerability and validity as a urate-elevating strategy.* In addition, the planned phase 3 design has benefited substantially from the successful design optimization features of SURE-PD. Specifically, phase 2 results have indicated that the higher inosine dosing regimen investigated in that trial is superior to the lower target range tested (based on greater serum urate increase without reduced tolerabililty). In addition to dose-selection, SURE-PD data have guided an improved strategy for prevention of uric acid urolithiasis and other protocol refinements.

3.2.1 Target Population – Four Defining Features

3.2.1.1 Early PD

People with early PD, and specifically those who have not yet developed sufficient motor symptom disability to require treatment with major antiparkinsonian agents, have been targeted in phase 3 as well as phase 2 development of inosine for several reasons:

Generically,

- Earlier disease implies a greater therapeutic impact for a putative neuroprotective strategy (i.e., more neurons to save).
- Earlier, *untreated* disease affords the opportunity to employ validated metrics (e.g., the MDS-UPDRS) of clinical disease burden and progression, which can be greatly confounded later in the disease by substantial and variable effects of antiparkinsonian medications like levodopa.

Specific to inosine development,

- Epidemiological evidence linking higher urate to reduced risk of developing PD are reflective of influences on disease pathophysiology early in the degenerative process, which may differ from that underlying clinical progression late in the disease.
- Clinical evidence linking higher serum and CSF urate in early, untreated PD to favorable rates of clinical or radiographic progression argue further and more directly for targeting this early stage of clinical decline.

Accordingly, as a general principle of guiding the design of this study we are seeking to replicate the features of DATATOP and PRECEPT studies – such as the early, untreated PD subpopulation that both targeted -- as faithfully as possible. If the slower rate of progression among their subjects with higher urate represents a causal relationship then the best way to test this hypothesis is to recapitulate the features of those trials except that we will control rather than observe the concentration of urate, allowing us to determine its effects rather than simply its associations.

3.2.1.2 Lower Serum Urate

A unique additional defining feature of the targeted PD population is its lower serum urate levels (≤5.7 mg/dL on initial screening). This subset of early PD patients is rationally selected for urate elevation for several reasons.

- First, for safety we considered elevating serum urate among those already in the upper range of normal at 7-8 mg/dL to clearly hyperuricemic levels above 8 mg/dL to pose an unnecessary risk to those subjects, especially because we are relying epidemiological and clinical studies with very few data for subjects above 8 mg/dL.
- Second, those with lower serum urate are enriched for faster progressors (by definition of the relation between urate and progression), and thus may have more to gain from putative disease modifying benefit.
- Third, the non-linear shape of the curve describing the urate-progression (see Fig. 3A above)[35,36] and the urate-PD risk[20,21,23,139] relationships further supports the rationale for enrolling all those below the 5.8 mg/dL (near the boundary between the 3rd and 4th quintiles in Fig. 3A). Because the risk of progression remains relatively high and unchanging (flat) across the lowest 3 quintiles for serum urate[20,21,23,35,36,139] before dropping down more sharply to lower risks in the two highest quintiles (with the highest corresponding to ~7-8 mg/dL) it suggests that all in the selected subpopulation could gain a similarly full benefit of urate elevation to 7-8 mg/dL -- regardless of whether one starts at 3.5 mg/dL (lowest quintile) or 5.5 mg/dL (middle quintile).

3.2.1.3 Safety Exclusions

Despite reassuring phase 2 safety data for urate-elevating inosine treatment and FDA guidance prompting liberalization of prior safety exclusions, several key risk factors for potential urate-related AEs are retained among the current eligibility criteria. These include a history of gout or uric acid kidney stones, and low urine pH, which is a major risk factor for uric acid kidney stones. In addition, a history of major cardiovascular events or renal dysfunction remains exclusionary.

3.2.1.4 Enrichment for PD by Neuroimaging

Despite the clinical expertise of the movement disorders specialists who have enrolled subjects in some of the largest trials of de novo PD, a substantial proportion (~10%) have been found to have dopamine transporter (DAT) scans without evidence of dopaminergic deficit (SWEDDs).[30,140-144] These subjects are unlikely to have PD as reflected in their lack of clinical progression over years and measured by various clinical scales including the Experiences of Daily Living components of the MDS-UPDRS,[141,145-147] which will be employed in the current trial. More recently, in the large observational biomarker cohort known as PPMI (Parkinson's Progression Marker Initiative) 15% of subjects identified as probable early, untreated PD subjects were found to have SWEDDs[148]. As these subjects do not generally decline further after enrollment, their inclusion in disease modification trials undermines the power to test the ability of a putative neuroprotectant to slow clinical progression (Fig. 11).

Specifically, for the current trial design we estimated that the ability to exclude 10% of otherwise eligible subjects based on a SWEDD would significantly reduce the number of subjects required to detect a minimal clinically important change (MCIC) of 6.3 total MDS-UPDRS points produced by urate-elevating inosine treatment after 2 years. The power achieved with the planned enrollment of ~270 subjects with DAT scan-documented evidence of a striatal dopamine deficit would have required 66 more subjects to achieve in the absence of this screening method (Fig. 11). Accordingly the SURE-PD3 trial design entails an additional screening evaluation at a local neuroimaging center to measure DAT ligand binding in the striatum as an anatomical marker of residual dopamine.

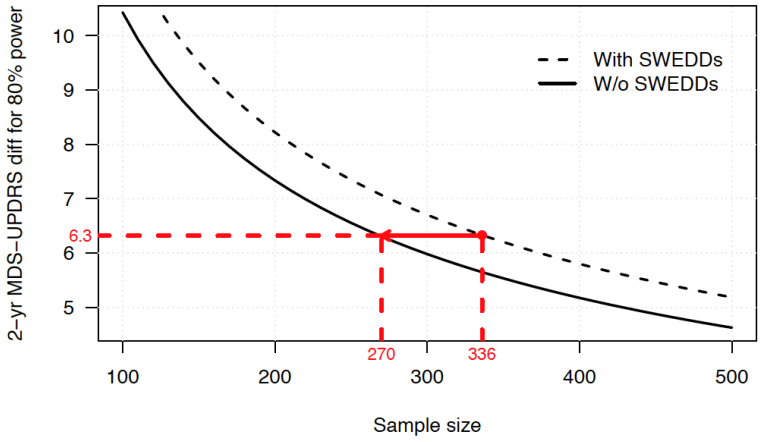


Figure 11. Sample size reduction afforded by exclusion of SWEDD subjects. The treatment effect size (difference in the change of MDS-UPDRS over 2 years) that can be detected with 80% power decreases with increasing study sample size. The curve depicting this relationship under standard de novo subject screening protocols (dashed line) can be shifted to the left by excluding subjects who have DAT scans without evidence of a dopaminergic deficit (SWEDDs; solid line). For the targeted effect size of 6.3 points (see Sec. 10) the use of DAT scans to exclude an estimated 10% with SWEDDs would reduce the sample size by 20% from 336 to 270.

Conversely, the novelty of implementing DAT imaging for diagnostic enrichment in PD intervention trials also reflects prior concerns for drawbacks to its inclusion. We have considered these and found that in the context of the current trial they are mitigated or do not apply:

- Reduced relevance of a DAT-scan enriched population to 'real world' PD, in which DAT scans (appropriately) are not routinely used or indicated for the diagnosis of PD. On the contrary, we contend that incorporating DAT scans for screening in de novo PD trials corrects an artifact of these trials rather than distorting the composition of early PD in clinical practice. Despite the considerable clinical expertise of Site Investigators who have conducted major clinical trials targeting de novo PD subjects (for the reasons noted above in 3.2.1a), these studies have consistently enrolled a substantial proportion (estimated 8-15%) who turn out to be unlikely to have PD.[30,141-144,148] For example in the PRECEPT trial 10% of subjects had DAT scans without evidence of dopaminergic deficit (SWEDDs) at baseline[30] and on follow-up imaging years later[148]. Recruitment pressure to diagnose early PD for such research protocols may encourage premature diagnosis of probable PD that would not normally be made at this point, at least not without assessment for responsiveness to dopaminergic drugs. This practical standard of diagnostic evaluation cannot typically be pursued in such de novo trials, for which these drugs are exclusionary. Thus in the artificial clinical context of disease modification trials for de novo PD, DAT scan imaging may compensate for the absence of clinical testing of dopamine replacement therapy and for unnatural pressures to enroll newly diagnosed early PD.
- Restricted availability of DAT neuroimaging. Earlier DAT imaging protocols relying on single photon emission computerized tomography (SPECT) of radioligands for DAT (like β-CIT uptake scans in the PRECEPT trial) required specialized centralized imaging centers that necessitated long-distance travel by subject and their caregivers. However, in recent years standardized SPECT imaging scanners and protocols have become widely available throughout the US. For example, 80% of the sites selected for participation in SURE-PD3

reported having a DAT neuroimaging center at their institution, and the remaining 20% reported having access to a certified center an average of 11 miles away.

- Untenably high cost of DAT neuroimaging. DAT scans at an estimated $4.5k in direct costs for each (~$2k radionuclide + ~$1.6 local neuroimaging center fee + ~0.9k central neuroimaging core) in a study of 300 subjects constitute a major budgetary component. However these cost would be fully offset and yield a net savings in project costs in the current study. Overall economy can result from improved statistical power or sample size reduction (by 66 subjects or 20% in this study, as above), with associated reduction in per-subject-fee payments, central management, vendor fees and project timeline. In addition the limitation of DAT neuroimaging to a single scan per subject as in this project, reduces costs substantially further compared to studies where DAT imaging is employed serially (a use that may offer ancillary value but falls outside the scope of this protocol). Lastly, the potential contribution of the radionuclide component by one of several commercial suppliers may reduce the costs further. Thus these contained costs may be significantly less than the savings due to a substantially reduced sample size and its resulting efficiencies.

3.2.2 Inosine Dosing Selection

Uniquely for a drug trial in PD, subjects randomized to active drug (inosine) receive a variable dose titrated to a concentration range of serum urate rather than a set number of capsules or milligrams of the study drug. The dosing strategy is driven by evidence (reviewed above) that both safety and putative clinical efficacy follow serum urate values much more closely than the amount of inosine administered. The SURE-PD study pursued a secondary dose-finding goal (see Sec. 2.2.3 above) by comparing the safety and urate-elevating actions of two incremental dosing regimens that titrate the number of inosine capsule to elevate serum urate into target ranges of either 6.1 to 7.0 mg/dL, or 7.1 - 8.0 mg/dL.[102] SURE-PD data support excellent safety and tolerability for both mild and moderate urate elevation, whereas the higher dosing of inosine was confirmed to produce a greater increase in serum urate approaching levels that were associated with the lowest risk of disease progression in the PRECEPT and DATATOP cohorts.[35,36] Accordingly, **moderate elevation of serum urate (into the 7.1-8.0 mg/dL range) is selected as the target for study drug dosing** among those randomized to inosine treatment in phase 3.[102]

Several additional dosing refinements are incorporated into the phase 3 design based on knowledge gained from phase 2 development:

- **Tailored wash-in titration** – In SURE-PD a conservatively slow titration schedule was employed to accommodate uncertainty over the variation and volatility of urate elevation upon initiation of inosine dosing, which began with a fixed low starting dose (500 mg bid) and ceiling doses mandated for all subjects during the wash-in phase. Analysis of SURE-PD provided evidence that baseline characteristics could be used to help predict an individual's dosing requirement to achieve a targeted serum urate elevation. Specifically, baseline serum urate levels, gender and diuretic use (but not weight, measured 24-hour urine urate output, estimated glomerular filtration rate, and triglycerides) were found to meaningfully inform an individual's future dosing requirements. Accordingly, the current protocol incorporates an algorithm to tailor initial inosine dosing based on these or other baseline characteristics of each subject, and thereby enhance the efficiency and safety of inosine wash-in. An identical algorithm will be implemented for subjects in the placebo arm.
- **Fewer initial visits** – In SURE-PD a cautious study drug wash-in strategy included frequent blood draws and evaluations totaling 5 visits in the first 3 months after the baseline visit. However steady-state levels were rapidly achieved in the moderate urate elevation target range of 7.1-8.0 mg/dL (by the second visit on study drug; see Fig. 5B, Sec. 2.2.3). Accordingly, the initial

density of evaluations has been reduced to 3 visits with blood draws in the first 3 months (see Sec. 7.7) on treatment in the phase 3 protocol.

- **Trough urate sampling** – In the SURE-PD (phase 2) trial inosine was titrated to serum urate levels measured in blood drawn at the time of their visits, irrespective of when subjects had taken their morning study drug capsule(s). For improved safety and potentially efficacy, trough urate sampling (i.e. just before the first daily inosine dose) will be adopted in phase 3 and is expected to reduce under- and over- shooting the serum urate target range, and therefore to reduce fluctuation in dosage (capsules/day). Because in practice blood collection for trough urate levels may occur a few hours after the first daily dose is normally taken at home (but delayed on study visit days) this value obtained may slightly underestimate the trough value with regular inosine scheduling, but the small difference is not materially relevant.

  Note that the change from *ad hoc* sampling in SURE-PD (phase 2) to trough sampling in this phase 3 trial will also lead to a greater actual increase in serum urate despite the target range being nominally unchanged at "7.1-8.0 mg/dL". The difference can be appreciated by noting the ~0.7 mg/dL drop in serum urate at the week 12 visit (V05 in Fig. 5B, Sec. 2.2.3), the one visit in SURE-PD at which serum urate was measured at trough (i.e., when subjects were instructed to hold off taking their medications that day until after the blood draw, due to a pharmacokinetic [PK] series being conducted at this visit). Because the *ad hoc* sampling of urate in SURE-PD while highly variable in its timing actually averaged ~3 hr after the last study drug dose and given the PK demonstration of a time-to-peak serum urate concentration of ~3 hr after inosine dosing (unpublished data, SURE-PD Investigators), it is likely that the true average daily serum levels in these subjects were closer to the trough-sampled values at V05. Thus it is expected that **adoption of trough sampling will produce ~0.7 mg/dL higher serum urate levels compared to those achieved in the phase 2 study**. Consequently the targeted trough level range of 7.1 – 8.0 mg/dL should now more closely match the uppermost quintile in the PRECEPT study (median value 7.5 mg/dL), in which the lowest rates of clinical and radiographic progression were observed.[35]

  Importantly, the resultant greater urate elevation in SURE-PD3 would increase the risks of AEs compared to those assessed in SURE-PD. However the small increase in risk expected with trough dosing was deemed acceptable upon FDA review of the SURE-PD safety data assuming adequate justification for the threshold for above-range serum urate levels (>9.0 mg/dL) that trigger dose reductions (Sec. 5.1.2) in terms of estimated Cmax (peak concentration) after inosine doses. We fit a linear regression of Cmax estimates against trough serum urate levels from the timed PK series in SURE-PD. For a trough serum urate of 9.0 mg/dL, the estimated mean Cmax and standard error were 10.6 mg/dL and 0.20 mg/dL. The root mean square error was 0.633 mg/dL, yielding a total variance for the prediction interval of 0.44. By those estimates, the probability of a Cmax ≥13 mg/dL, the alert level employed by the National Health and Nutrition Examination Survey (NHANES) program of the Centers for Disease Control and Prevention (CDC),[215] is 0.00016, or 1 in 6400. This may slightly underestimate the risk of Cmax levels ≥13 mg/dL due to measurement error in the trough serum urate levels used in the regression, possible underestimation of Cmax levels from the time series, and possible asymmetry in the among-person distribution of Cmax levels conditional on a given trough, but having observed no serum urate levels greater than 11.6 mg/dL in SURE-PD, suspending treatment for any serum urate value >9.0 mg/dL appears more than adequate to keep the proportion of participants in the current study with transient (Cmax) peak levels above 13 mg/dL below 1 or 2 percent.

3.2.3 Primary Outcome and Analysis

- **Justification of primary outcome variable: MDS-UPDRS** – Although the original UPDRS was employed in our phase 2 study of urate elevation[102] as well as the key observational studies

(DATATOP and PRECEPT) linking urate to slower PD progression, features of the revised MDS version warrant its use in place of the original as our primary variable of clinical PD status:

- The MDS-UPDRS was developed to improve upon the original UPDRS by better assessing the more recently appreciated breadth of non-motor features. (See Sec. 6.2.1 for scale overview.)
- Responsive to NIH guidance and to increased appreciation of the value of incorporating patient-reported outcomes (PROs) to enhance the relevance and validity of study results, approximately 40% of its items constitute PROs[150] (vs. none in the original UPDRS).
- Importantly for our phase 3 trial, although the MDS-UPDRS is closely modeled on and highly correlated[138,149] with the original UPDRS, it appears to have greater sensitivity for monitoring clinical progression compared to original UPDRS.[145]
- The MDS-UPDRS has been validated across its multiple motor and non-motor domains, and across cultures in independent studies.[138,151,152]
- Availability of standardized training and certification [153] facilitates quality and consistency of its administration. Training resources are accessible for both in–person and online training/reinforcement.
- Increasingly adopted for use in PD clinical research, including as primary endpoint for trials designed to evaluate long-term disease-modification as well as for symptomatic effects.[154] The MDS-UPDRS is now recommended[155] (along with the original UPDRS) as a core instrument among NINDS common data elements (CDEs) for PD studies.

- **Justification of primary analysis: difference in change in MDS-UPDRS over 2 years --** Neuroprotective candidates with dopaminergic properties, even those with only mildly symptomatic antiparkinsonian effects (e.g., selegeline, rasagiline), have proven challenging to assess for their disease-modifying potential. As a result more complex (e.g., delayed-start) study designs have been pursued in attempts to tease apart symptomatic and neuroprotective effects.[156-159] However, the complexities of these designs then created their own challenges of interpretation.[160] By contrast, for candidate protectants lacking symptomatic efficacy the most rational phase 3 efficacy designs may rely on a simpler structure of parallel groups assessed using a 'change in a rating scale over time'.[154] Thus while decidedly problematic in the study of a symptomatic agents for their influence on long-term clinical course, this parallel group design remains the historically best studied and most common 'neuroprotection' trial design in PD.[156,157] It is well suited to putative protectants like inosine that are unlikely to acutely impact dopaminergic neurotransmission or parkinsonian symptoms.

  Accordingly, changes in scores on an appropriate clinical rating scale – the MDS-UPDRS as justified above—will be compared between early PD subjects randomized to placebo or urate-elevating inosine treatment over a 24 month period for the primary efficacy analysis of the trial. The design and primary analysis are further supported by:

  - Evidence of non-futility of inosine (and possible efficacy in subgroup analyses) using change in UPDRS scores from the preceding phase 2 experience in the SURE-PD study[102]. (See Sec. 2.2.3). A recently proposed 'road map' to clinical development of candidate disease-modifying agents[158] highlights the efficiency of sequential investigations and the value of advancing to fuller efficacy trials with an outcome analysis similar to that used in demonstrating non-futility.
  - Observational data[35,36] from the DATATOP and PRECEPT trial cohorts linking higher urate with favorable rates of progression assessed as difference in UPDRS change over two years, approximating the planned primary efficacy analysis. Although higher urate

was also a significant predictor of delayed disability (warranting dopaminergic medication) in these cohorts, comparing MDS-UPDRS change has advantages as our primary outcome variable. Change in a continuous variable over time can offer greater statistical power over that achieved with a dichotomous endpoint like disability warranting dopaminergic therapy.[159,161] Differences in proportions reaching this disability milestone will included among secondary analyses of the trial.

- Recent/current use of this parallel group design with change in UPDRS over time in the primary efficacy analysis in the QE3, FS-ZONE and STEADY-PD III trials of non-dopaminergic agents (coenzyme Q10, pioglitazone and isradipine) posited to have disease-modifying properties.[162]
- Wash-out period option for confirming lack of symptomatic efficacy, though not part of the primary analysis, may strengthen the evidence against an acute antiparkinsonian action of inosine. In the SURE-PD trial no effect of urate-elevating inosine treatment was apparent during the 3-month treatment initiation period or conversely during the 1-month wash-out.[102] If a lack of symptomatic efficacy were replicated using MDS-UPDRS with the greater sample size of the phase 3 trial, then a positive result of divergent rates of change over two years in the primary analysis would be more suggestive of a disease-modifying benefit of urate-elevating inosine treatment. However, even under this scenario in the absence of a commensurate effect on an associated meaningful biomarker of PD progression the results would not suffice to prove slowing of the disease. An indication for disease modification would thus likely require additional clinical investigation.

3.2.4 Additional Key Safety Features

3.2.4.1 Pre-Study Determinations of Food and Drug Interaction

In order to further reduce the variability in inosine bioavailability and urate elevation, and the associated AE risk, standard drug-drug and drug-food interaction studies (per FDA guidance[163]) will be completed in advance of the trial. Evidence for substantial interactions will prompt modified dosing instructions limiting concomitant dosing with specified medication and/or requiring subjects to take study drug on an empty stomach (or consistently with or without food).

3.2.4.2 Modification of Uric Acid Stone Risk Factor Management Plan

The likelihood of gouty arthritis and uric acid urolithiasis – the two major known side effects of hyperuricemia –increase with age.[64,89,164-166] Accordingly, these are the main inosine-induced AEs anticipated in this study of early PD patients, who are expected to be over 60 years of age on average.[30,31] To help avoid them, any history of gout or uric acid urolithiasis will remain an eligibility exclusion criterion, as will a screening urine test result showing low pH (≤5.0), a major risk factor for uric acid urolithiasis. [29, 64, 87,88]

Although no cases of gouty arthritis developed during SURE-PD, three subjects developed urolithiasis on inosine treatment and uric acid composition was documented in one.[102] Because urine pH can be readily identified and raised with common oral alkalinization treatments[64,89] the phase 3 trial will employ additional measures to monitor for uric acid urolithiasis risk and a lower threshold for initiating standard alkalinization therapy. Based on SURE-PD data linking the presence of uric acid crystalluria (UAC) and low urine pH to the subsequent development of uric acid stones, urine samples provided at each visit will be processed centrally for blinded UAC determinations as well as pH measurements. These data will be followed and alkalinization will be triggered by persistently acidic urine (PAU) with or without the presence of UAC as follows (detailed in Sec. 8.4):

- Urine pH ≤5.0 in 2 consecutive samples
- Urine pH ≤5.5 in ~3 consecutive samples (or fewer in the placebo group as needed to maintain a similar likelihood of alkalinization in the placebo and inosine groups; see Sec. 8.4.2)
- Uric acid crystalluria + urine pH ≤5.5 in 1 of the last 2 samples

Alkalinization therapy will be continued, advanced or reduced based on an algorithm that considers urine pH and crystalluria responses. Subjects on alkalinization therapy will also be carefully monitored for any adverse effects of alkalinization. Although asymptomatic renal stones can be detected by ultrasonography, its sensitivity is relatively low[91] and is considered an appropriate monitoring test only when urine pH is below 5.5 in those who are otherwise at high risk of uric acid urolithiasis.[64] Computerized tomography scans have the greatest sensitivity for uric acid renal stones (which are not radioopaque on plain films),[91,167] but drawbacks including the serial low level radiation exposure that would be required for monitoring offset any diagnostic benefit in the present study. All subjects will also be counseled on avoiding dehydration, an avoidable and remediable risk factor for both gout and urolithiasis.[29]

By reducing the number of UA urolithiasis AEs, these bolstered prevention measures will help minimize unblinding. Similarly, knowledge gained from experience with inosine in phase 2 will reduce unblinding as we have observed that stones can form on placebo,[26] do not necessarily contain UA,[102] and would normally be managed without disclosure of treatment assignment (see Sec. 8.8).

3.2.4.3 Reducing Risk of Possible Cardiovascular Adverse Effects

Also of particular relevance to the PD population is the risk of cardiovascular disease (CVD), which is an important even if uncertain risk of higher urate levels, as detailed above. Individuals with higher serum urate have a significantly increased risk of hypertension, coronary heart disease, and stroke.[168,169] Although these associations may in part be confounded by obesity and other risk factors,[98,105] potential for a long-term neuroprotective effect of urate or its precursors must be weighed against potential adverse cardiovascular effects. It is worth noting that amongst PD patients in developed countries CVD is common and indeed is a leading cause of death.[170-172] Moreover, CVD and its major sequelae of myocardial and cerebral infarction are far less reversible than urate-related crystal disease. On the other hand, recent data from a small study of urate-elevating inosine in MS[138] and from the SURE-PD trial showed no evidence of blood pressure elevation compared to placebo despite months to years of substantial elevation of serum urate levels.

The main approach to reducing possible CVD risk during the SURE-PD trial was through the exclusion of those with a significant history (Sec. 4.2), particularly prior myocardial infarction or stroke and heart failure with an ejection fraction <45%. The lack of evidence for inosine-associated CVD events in the SURE-PD trial reflects a sample size and follow-up period that are too small to offer substantial reassurance. Accordingly, these exclusion criteria will remain unaltered in the phase 3 trial. Assessments for development of CVD during the trial will include frequent review of all AEs, and regular blood pressure and electrocardiographic monitoring.

Although serum urate has also been linked to metabolic syndrome and diabetes,[173,174] which is itself a major risk factor for CVD, SURE-PD data[102] showed no effect of long-term urate-elevating inosine treatment on body weight or blood sugar. In any event, risk of type II diabetes-related CVD will also be reduced through exclusion of those with evidence of CVD.

3.3 Study Duration and Periods

The total time of active participation in the study (including screening, treatment and wash-out periods) is 2.25 years for each subject completing the study (see Schedule of Activities; Sec. 7.7), and includes:

- **Screening** of up to 60 days from Screening Visit 1 (SC1) to enrollment at Baseline Visit. Because of the substantial (~50%) chance that the results of serum urate screening test will be exclusionary (Fig. 9, Sec. 3.1), the screening process at PSG sites is split into two visits with testing on a brief initial visit (SC1) primarily limited to the serum urate measurement. For those subjects who pass SC1, most of the remaining screening procedures are conducted on a longer second screening visit (SC2) and if still eligible then a subsequent DAT brain scan at a local neuroimaging center.
- **Treatment (Period 1)** of 720 ± 7 days (~24 months) approximates the 2-year observation periods of the DATATOP[36], PRECEPT[35] and SURE-PD[102] studies, on which the present trial is modeled. Extending follow-up beyond 24 months would provide diminishing returns as the primary outcome variable of MDS-UPDRS score is censored from the primary analysis after initiation of symptomatic antiparkinsonian therapy, which is expected to have occurred in the majority (two thirds) of placebo subjects by that point. Randomized, placebo-controlled, double-blind treatment is initiated at the baseline visit (BL) and gradually advanced and titrated up to a maximum 6 capsules/day or until the target serum urate range of 7.1-8.0 mg/dL is reached in the inosine-treated group. After wash-in/titration visits (V01, V02) at 3 and 6 weeks after BL, scheduled visits (V03 - V10) will occur at 3-month intervals for clinical and safety assessments, including a serum urate measurement for any algorithmic dosage adjustments needed to maintain levels within the 7.1-8.0 mg/dL target range for those in the inosine group. Mirrored adjustment in the number of placebo capsules/day help ensure subject and site staff remain blind to treatment assignment.
- **Wash-out (Period 2)** of 90 ± 7 days, ending ~27 months after enrollment. Three monthly evaluations will be conducted after discontinuation of study drug at V10 (after 24 months of treatment), two by phone (TE1 and TE2 at months 25 and 26, respectively) and the final visit (SV) in person at month 27. The multiple visits will allow for serial assessments of the need for dopaminergic therapy during and after the reversal of any short-term effect of inosine or its withdrawal, as well as for any late adverse effects of the treatment or its withdrawal.

4 SELECTION AND ENROLLMENT OF SUBJECTS

After screening approximately 667 consented subjects, ~270 women and men (age 30 or older) with early idiopathic PD are expected to enroll at approximately 60 PSG study sites (see Fig. 9 in Sec. 3). At the time subjects undergo randomization they will have not yet developed sufficient disability to require treatment with dopaminergic agents other than MAO-B inhibitors, and they will not appear likely to develop it imminently (i.e., within 3 months).

Subjects withdrawn from the study will not be replaced.

4.1 Inclusion Criteria

Study subjects meeting all of the following criteria will be allowed to enroll in the study:

1. Willingness and ability to give written informed consent and to comply with trial procedures.
2. Fulfillment of diagnostic criteria for idiopathic PD with at least two of the cardinal signs of PD (resting tremor, bradykinesia, rigidity) present at 2nd screening and baseline evaluations, as assessed by the Site Investigator.
3. Absence of current or imminent (within 90 days of enrollment) PD disability requiring dopaminergic therapy, as assessed by the Site Investigator.
4. Modified Hoehn and Yahr Scale Stage 1 to 2.5 inclusive.

5. Age 30 or older at the time of PD diagnosis.
6. Diagnosis of PD made within 3 years prior to 1st Screening Visit.
7. Non-fasting serum urate ≤ 5.7 mg/dL at 1st Screening Visit (SC1).
8. If the subject is female, then:
    a) Being surgically sterile (hysterectomy or tubal ligation), or
    b) Being postmenopausal (last menstruation was two years or more prior to 2nd Screening Visit), or
    c) For those of childbearing potential

        • Using a reliable form of contraception for 60 days or more prior to Baseline Visit and agreeing to continue such use for 30 days post last dose of study drug. Reliable forms of contraception include: abstinence; implanted, injected or oral contraceptives (birth control pills), intrauterine device in place for at least 3 months prior to Baseline Visit, vaginal ring with spermicide, barrier with spermicide such as male or female condom, diaphragm or cervical cap, transdermal patch; male partner with vasectomy.

        • And having a negative pregnancy test at the 2nd Screening Visit. [Note that a urine pregnancy test will be performed at screening on all women who are not at least two years postmenopausal or surgically sterile.]

4.2 Exclusion Criteria

Study subjects meeting any of the following criteria during screening evaluations will be excluded from entry into the study:

1. Atypical parkinsonism, including that due to drugs, metabolic disorders, encephalitis, cerebrovascular disease, normal pressure hydrocephalus, or other neurodegenerative disease.
2. Dopamine transporter brain scan without evidence of dopamine deficit.
3. History of gout.
4. History of uric acid or urate urolithiasis, or recurrent urolithiasis all of unknown type.
5. A screening test positive for uric acid crystalluria, urine pH ≤ 5.0, or an estimated glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2.
6. History of myocardial infarction or stroke.
7. Symptomatic congestive heart failure with a documented ejection fraction below 45%.
8. History of severe chronic obstructive pulmonary disease.
9. History of nephrectomy.
10. Mini Mental State Exam score < 25; i.e., a score of 0 to 24.
11. Use of any anti-parkinsonian medication (including levodopa, dopamine agonists, amantadine, entacapone and the anticholinergic agents trihexyphenidyl and benztropine) other than monoamine oxidase-B inhibitors within 60 days of Baseline, or in excess of 90 days.
12. Change in the dosage of (or initiation of) a monoamine oxidase-B inhibitor within 90 days prior to Baseline, i.e., entry on a MAO-B inhibitor requires a stable dosage for the 90 days prior to Baseline.

13. Use of the following within 30 days prior to the Baseline Visit: inosine, allopurinol, febuxostat, probenecid, more than 50 IU of vitamin E daily, or more than 300 mg of vitamin C daily (though a daily standard multivitamin such as Bayer One-A-Day® or Centrum® is permissible), reserpine, methylphenidate, amphetamines, cinnarizine, monoamine oxidase-A inhibitors, tetrabenazine, neuroleptics or other dopamine blocking drugs.
14. Use of the following within 90 days prior to the DAT neuroimaging screening evaluation: modafinil, armodafinil, metoclopramide, alpha-methyldopa, methylphenidate, reserpine, or amphetamine derivative.
15. Unstable dosing of a thiazide -- such as hydrochlorothiazide (e.g., Esidrex), chlorothiazide (e.g., Diuril), chlorthalidone (e.g., Hygroton), indapamide (e.g., Lozol), metolazone (e.g., Zaroxolyn), which are permissible as long as the subject is on a stable dose from 1 week prior to the 1st Screening Visit through the Baseline Visit.
16. Known unstable medical or psychiatric condition that may compromise participation in the study. (Note that difficulty swallowing large capsules might preclude participation due to the size of the study drug capsules.)
17. Clinically serious abnormality in the screening visit laboratory studies or ECG, as determined by the Site Investigator.
18. Participation in another investigational treatment study within 30 days prior to the Baseline Visit.
19. Known hypersensitivity or intolerability to inosine.
20. Known hypersensitivity to DaTscan™ (either to the active substance of ^{123}I-ioflupane or any of the excipients).

4.3 Treatment Assignment Procedures

Each subject who meets all eligibility criteria and who has signed and continues to give informed consent will be randomized to receive either oral inosine or matching placebo for approximately 24 months of treatment.

4.3.1 Identification, Recruitment and Retention of Candidates

Subjects will be recruited to enroll at approximately 60 PSG clinical sites in the US. Each site employs a PSG-credentialed Site Investigator and a study coordinator, and has been selected by the Steering Committee for this trial from ~120 US PSG sites based on recruitment record from prior trials as well as compliance with prior study protocols and regulations, higher proportion of underrepresented ethnic and racial minorities among the patient population, and availability of necessary resources (e.g., local DAT scan accessibility). As credentialed PSG investigators all Site Investigators are well trained in the diagnosis and management of PD, are experienced in the conduct of clinical research, and will be certified in the administration of the MDS-UPDRS[138].

Providing 60 sites an average enrollment period of 18 months yields realistic average site rates of recruitment (7 consented/site/year) and enrollment (3 randomized/site/year) despite the narrow eligibility criteria, which account for the expected exclusion of 60% of consented subjects during screening. The projection of 3.0 enrollments/site/year was calculated as 270 enrolled/60 sites/1.5 years, and is realistic as it closely matches the enrollment achieved under the final protocol of our preceding SURE-PD study. Note that the need to mobilize and motivate a large group of high quality PD clinical sites to serve as both backup and active sites is a particular challenge to which the PSG clinical trial network is well suited based on the track record and commitment of its member investigators and coordinators, who author all PSG trial publications.

Based on our prior experience with clinical trials in PD, including SURE-PD, we anticipate that most subjects will be recruited locally for this trial through the site's center and institutional network. Training and recruitment materials will be developed based on our experience with SURE-PD and informed by the experience of collaborating recruitment specialists of the NINDS Office of Clinical Research, Michael J. Fox Foundation, Parkinson's Disease Foundation (PDF), NCRI and the trial Steering Committee. IRB-approved materials will be tailored to target both potential subjects (e.g., waiting room brochures, PowerPoint slides) and clinicians at the study sites and within their referral networks (e.g., bullet point cards highlighting key points of the trial's purpose, eligibility criteria and contact info).

- **Local outreach** will be conducted by site staff and partnered patient support groups and advocates (through PSG collaboration with the PDF and its PAIR advocacy program[175]) with the encouragement and support of the Clinical Coordinating Center (CCC). Activities will range from letters to local movement disorder physicians and general neurologists, clinical site institutional webpage postings, area clinician symposia highlighting the study, local media engagement, and PD support group meetings.

- **Informatics- and network-based candidate identification** initiatives will build on novel strategies explored during the SURE-PD trial. Outlined below are two innovative methods for accelerating candidate referral – one relying on advances in large healthcare database searches, and one on a national neuroimaging network with early access to de novo PD subjects.

- A healthcare network database and its user interface originally developed for digital 'chart review' research at MGH and known as the Research Patient Data Registry (RPDR)[228,229] was employed late in the SURE-PD trial under a 2011 IRB protocol amendment at MGH to boost the rate of subject enrollment there. The approach effectively identified early untreated PD patients who may have been recently diagnosed by a primary care physician or general neurologist, who was then contacted to encourage referral to the trial as appropriate. Such de novo patients may be interested in participating but unaware of their candidacy, and often may not be referred to a movement disorders trial site until months or years later, when their narrow window of candidacy may have closed due to clinical progression and initiation of antiparkinsonian medications.

  Depending on the size of a healthcare database/network its accessibility could increase a site's subject accrual several fold. In the last few years the software platform for RPDR (now known as i2b2 for 'Integrated informatics from bench to bedside'[230,231]) has been adapted by Dr. Shawn Murphy and colleagues at MGH for distribution to other healthcare network databases and installed at institutions worldwide,[232] including 66 in the US (~32 of which are PSG sites participating in this phase 3 trial). I2b2 fully complies[233] with patient privacy standards of the Health Insurance Portability and Accountability Act (HIPAA), and has successfully identified recruitable cohorts with cancer, depression and epilepsy.[234,235]

  After obtaining local IRB approval each participating site that adopts this optional method would conduct automated ~monthly screens of their broader healthcare network's database with a query structure including several basic features:

  - + PD diagnosis code within past 3 years,
  - No PD diagnosis code > 3 years ago,
  - No exclusionary PD medication (e.g., levodopa, dopaminergic agonists),
  - No exclusionary medical condition (e.g., gout, myocardial infarction).

  The diagnosing physician of each identified candidate will be contacted by site staff with a personalized, template-based email or phone call explaining the subject's potential eligibility and options for referral, if the provider concurs, to the local trial site.

  Of note, this recruitment strategy may provide a novel solution to the persistent challenge of improving enrollment rates among underrepresented minority populations. A repeatedly

identified impediment is the lower rate of referral to specialist care in general[236,237] and PD trials in particular among African-American and Hispanic patients.[238,239] Despite increasing attention to this challenge it has proven resistant to bolstered efforts and interventions designed to enhance minority enrollment in PD research studies.[240,241] Hence, a near real-time, informatics-based method of recruitment from across a site's broader healthcare network offers an innovative alternative strategy, as it may accelerate or engender minority referrals, which otherwise might not have occurred in time or at all.

Lastly, the efficiency of this informatics-based referral process, which entails numerous clinical sites may be enhanced through a centralized program linking multiple i2b2-enabled sites, and known as SHRINE (for Shared Healthcare Information Network).[242] SHRINE was recently established by Dr. Murphy's team at MGH together with the CTSA-supported Harvard Catalyst program, which now provides this service to investigators as a means to aggregate queries and responses from multiple i2b2 databases across multiple institutions. Pilot programs successfully demonstrated the feasibility of linking i2b2-enabled sites nationally for identifying disease-specific cohorts ranging from atrial fibrillation to autism to epilepsy patients[243,244] and suitable for subject recruitment.

- Additional strategies for subject identification may be tailored to this phase 3 inosine trial based on its unique eligibility criteria. A dopaminergic deficit on DAT neuroimaging is a particularly discrete criterion that will generally be fulfilled as the final step in the study screening procedure prior to enrollment for eligible subjects. However, DAT scans are also obtained as part of routine clinical care in the setting of diagnostic uncertainty over early PD and related conditions. A positive result (low levels of DAT ligand uptake into the striatum) can indicate or confirm a clinical diagnosis of probable PD (e.g., when the scan is ordered to distinguish between PD versus essential tremor, and other forms of parkinsonism are less likely). Neuroimaging centers within 60 miles of an active trial site (~180 = ~3/site x 60 sites) will be invited to provide an IRB-approved digital trial flyer (with trial description, eligibility criteria and contact info) with each positive DAT scans report sent to the ordering clinician. S/he can then determine whether the trial may be appropriate and of value for the patient and how to inform the patient.

  Neuroimaging centers in the vicinity of participating clinical sites may be contacted individually by site staff. Alternatively, a more efficient centralized approach may be employed through collaboration with a commercial radiotracer provider, which would provide a digital/paper flyer to the neuroimaging center along with each delivered dose of DAT radionuclide. The provider would identify imaging centers for this program based on knowledge of the overlap between the national network of DAT scan-certified neuroimaging centers and the trial's roster of activated clinical sites. Privacy of potential subjects identified in this manner would be preserved because their identities and test results would not be provided by the neuroimaging center to any trial staff or to the radiotracer company.

- **National/centralized outreach** will include:
  - announcements on NIH's clinical trials registration website, the PSG website (which is also linked from other PD advocacy organization's websites), FoxTrialFinder.org[176] and in PD newsletters and web postings.
  - a dedicated trial website will be developed and serve as the digital hub featuring layered trial information and site contact to facilitate both lay and clinician referrals of candidates. A toll-free '800#' will be routed to NCRI staff.
- social media outreach program will direct traffic to the trial website or 800#. A core media element will be a brief, professionally produced informational/promotional video on the trial, and will reside on MGH's Youtube account as well as on the trial website. It will expand on our experience developing a prior video posted late in SURE-PD recruitment (https://www.youtube.com/watch?v=MN7wHPGv8Lc), and will feature perspectives of PD

advocate as well as investigator presenters. Partnering individuals and institutions (those of the 60 active clinical sites as well as foundations; e.g., see PDF President's letter) will post content to their Twitter, Facebook, Vine, Flickr, etc. accounts, linking to trial website and/or the Youtube video (and in turn the website).

- well-publicized safety guidance on the risks of self-treating with inosine outside of a carefully conducted clinical study (which can rigorously avoid, monitor and manage AE risk factors); it may help preserve eligibility of the de novo PD population while fostering public safety. SURE-PD-related press releases and educational postings (including a series developed by the Michael J. Fox Foundation as grantor) explicitly tempered enthusiasm with caution and highlighted safety priorities. This public information stance is an important responsibility because regular, knowledgeable monitoring of serum urate and urine pH is required to avoid SAEs of inosine taken to meaningfully elevate urate.

  However, despite the availability of inosine as an over-the-counter (OTC) nutritional supplement its widespread use among de novo subjects is unlikely to occur or pose a significant challenge to study recruitment. Given the complexities and inherent risks of self-treating with inosine to elevate urate, together with our continued emphasis on disseminating balanced scientific information on the topic, it is expected that few PD patients will be ineligible due to OTC inosine use. Although little information is available on trends in nutritional supplement consumption, a review of the publicly accessible database of medication and supplement usage by 9,052 people with PD who participate in the self-selected 'PatientsLikeMe' PD community[250] found that 0 listed use of inosine (ten months after publication of SURE-PD results). By comparison, the same PD cohort included 21 on isradipine, 80 on vitamin E, 382 on coenzyme Q10, and 909 on rasagiline. The lack of reported inosine usage in PD likely reflects truly low usage, given that by contrast, inosine was listed as taken (up to 2,000 mg/day) by 10 of 6,883 in the 'PatientsLikeMe' ALS community on the same website.[250] The apparent rarity of PD patients taking inosine may relate to the above safety guidance, possibly in combination with the cautious (reduced risk-taking) personality features often ascribed to people with PD[251] – especially those not taking dopaminergic agonists such as de novo patients.[252] By contrast, greater usage in the ALS population may reflect its relatively dire prognosis, which lowers the threshold for accepting AE risks of unproven therapies.

  Lastly, because serum urate levels will be routinely screened and monitored, any surreptitious use of OTC inosine may be readily detected at screening and during enrollment (when SURE-PD data showed no evidence of OTC usage; see Sec. 2.2.3A above). Thus 'drop-in' non-compliance is unlikely to be a significant problem, but if it were it would be readily detected and then addressed through 'as-treated' secondary statistical analyses (Sec. 10.1.3 and 10.5.2).

Systematic **retention** measures will be similarly deployed. Key strategies that contributed to excellent retention (>98%) in SURE-PD will be replicated and additional approaches employed given that larger trials can be more susceptible to attrition:

- Our core, critical retention strategy of ensuring a highly motivated and informed site staff was successful in SURE-PD and will again be prioritized.
- In addition, a debit card-based, HIPAA-compliant subject reimbursement system tailored to clinical trials (Payoneer®**;** New York, NY) was implemented to enhance retention as well as recruitment in SURE-PD, and was universally appreciated by study subjects based on site staff feedback. Similarly, site staff endorsed its operational efficiency and their minimal burden due to its central administration (with weekly reloading of debit cards by coordination center staff using anonymized subject ID numbers).
- Additional retention plan elements emphasized based on experience with recent early stage PD trials include:
  - tailoring communications with subjects (study drug dose adjustments, visit reminders, etc.) to their preferences; e.g., via phone, email or text,

- ensuring that the Investigator devotes time to meet with the subject at each visit,
- efficiently managing visit time – avoid rushing subjects and minimize their wait times,
- providing flexibility in accommodating patients' schedules ; e.g., with after-hours visits for patients working 9-5 jobs,

…essentially all courtesies that convey respect for the subjects and their major contributions to the research.

4.3.2 Monitoring and Adaptation of Recruitment & Retention

Upon obtaining IRB approval, each site will begin tracking all identified potential study subjects. Candidates who are asked to participate in this trial will be recorded on a Confidential Participant Log, which is kept by the site study staff in a secure location. In addition de-identified information regarding how subjects learned about the trial, referral sources, reasons for ineligibility and reasons for non-participation by eligible subjects will be tracked on the Screening/Demographics form in the eClinical system for all subjects who have signed a consent and are screened for the study. These secure reporting forms will be updated regularly by site staff with current numbers of identified potential candidates, consented, and at each stage of screening (SC1, SC2 and DAT scan 1 [DS1]) prior to enrollment. They will be reviewed and incorporated into monthly study-wide tabulations of recruitment and enrollment statistics for distributions to site staff, coordination center staff, trial Steering Committee (SC), Data and Safety Monitoring Board (DSMB) and NINDS.

A recruitment and retention committee (RRC), which as a subcommittee of the SC will report directly to it, will meet regularly prior to and during the active enrollment and follow-up periods (specifically, at least every other month until full enrollment) in order to optimize recruitment and retention. The RRC will comprise relevant members of the SC (trial PI, prior SURE-PD project manager and patient research advocate), key trial staff (coordination center project managers and representative site investigators and coordinators) and collaborators (recruitment and retention specialists from the Michael J. Fox Foundation and NINDS). In addition to ensuring the design and implementation of planned recruitment strategies, the RRC will continually review recruitment and enrollment activity statistics and also any changes in the broader recruitment landscape (e.g., shifts in prescribing patterns that impact eligibility criteria, or the advent of competing trials, opportunities for media engagement). As warranted it will make recommendations for modification of recruitment and retention practices to coordination center staff (e.g., to facilitate creative publicity strategies) and the SC (e.g., to consider altering eligibility criteria or activating backup sites).

In addition to frequent central review of recruitment and retention status, regularly scheduled conference calls will be held for all Investigators and Coordinators to discuss retention strategies and share successes/failures with each other and coordination center staff. These exchanges will include a particular focus on effectiveness of strategies for enhanced recruitment and retention of participants in minority populations. In parallel, project managers will routinely monitor each site's subject, consent, enrollment and premature withdrawal rates, reporting outliers directly to the RCC and PI. For a site with a problem in any category, a rapid assessment of the cause and action plan will be reviewed with the site.

4.3.3 Consent Procedures

During or before a screening visit, the subject will be thoroughly informed of all aspects of the study, including all scheduled visits and activities, and will be able to ask questions. The subject will be requested to sign and date the informed consent form *prior* to undergoing any study-specific procedures. The original signed and dated informed consent form must be retained by the Investigator in the subject's file and a copy must be provided to the subject. Subjects may also consent to optional substudies: a) blood collection for future biomarker research (see Section 16.2.14), with consent obtained at SC1 at the time of parent trial consent, b) smartphone-based outcomes research (see Section 16.2.15), with consent obtained at SC2 or at any subsequent visit up to and including V09 using an informed consent form separate from

that used for the parent study, and/or c) second DAT neuroimaging to assess change from pre-baseline imaging (see Section 16.2.11.2), with consent obtained at or prior to visit V10.

4.3.4 Identification Number and Intervention Group Assignment Procedure

Subjects will be given three types of identification numbers:

1. 9-digit CTCC Unique ID
   - If agreeable, at the time the consent form is signed the subject will be assigned a unique 9-digit ID number that can be used to connect the subject's SURE-PD3 research data to other studies conducted by the University of Rochester's Clinical Trials Coordinating Center (CTCC) in which s/he may participate.
2. 4- digit Subject ID Number
   - At the time the consent form is signed, all subjects will be assigned a 4-digit Subject ID Number by the site, from a list generated by the DCC and provided to sites by the CCC. This number will be used on all data forms.
3. 5-digit Enrollment/Randomization Number
   - Study drug will be pre-coded by the DCC with Enrollment ID/randomization kit numbers (based on the randomization plan generated by the University of Rochester Biostatistics Department). Pre-assigned drug kits will be supplied to the site Investigator.
   - The treatment for each subject will be assigned by a randomized code. A blocked randomization scheme will be used to ensure approximately even distribution of subjects in treatment groups at each site.
   - Once the subject qualifies for the randomized phase of the study, the site Investigator or Study Coordinator will enter data into the eClinical system and the subject will be assigned a unique 5 digit Enrollment ID, which will match a study drug kit. These numbers are assigned in a randomized order, rather than sequentially.
   - The randomization algorithm and subject enrollment process will be implemented through the Internet accessible Electronic Data Capture (EDC) system using authenticated, password-protected accounts for each study site. The EDC system will automatically validate inclusion/exclusion criteria and generate visit date windows.
   - Once the online enrollment process is completed, the site will print an Enrollment Verification Report that verifies the subject has been randomized. The report will note the Enrollment ID Number that was assigned that corresponds to the drug kit number and the upcoming study visit windows. If a site's EDC system is not operating, the site may alternatively call the DCC for subject enrollment during designated working hours.
   - Once a subject has been allocated an Enrollment ID Number this number cannot be assigned to another subject.

5. STUDY INTERVENTIONS

5.1 Study Interventions, Administration and Duration

5.1.1 Intervention (study drug)

Study drug will be supplied as identical appearing capsules for oral administration.

- **Active study drug** capsules will contain: **500 mg inosine** and 10 mg magnesium stearate.
- **Placebo** will contain **500 mg lactose** and 10 mg magnesium stearate.

5.1.2 Administration and dosing of study drug

Subjects will be randomly assigned to receive one of the two following regimens (1:1 ratio) for taking study drug, administered in doses of one or two 500 mg capsules:

- **Inosine dosed to elevate serum urate to 7.1 – 8.0 mg/dL**
  1-6 capsules (0.5 to 3.0 gm of inosine) per day in 3 divided doses (0-2 caps *t.i.d.*); dosing is titrated to serum urate levels

- **Placebo**
  1-6 capsules (0.5 to 3.0 gm of lactose) per day in 3 divided doses (0-2 caps *t.i.d.*); dosing is algorithm-driven to mimic that of inosine regimens

5.1.2.1 Initiation of Dosing at Baseline Visit

- First capsule will be taken with fluids during the Baseline Visit under the guidance of site staff.
- At that time, staff will provide subject education on study drug administration, explaining:
  - That each dose of 1-2 capsules is to be taken with a glass of water or other unsweetened, non-alcoholic beverage.
  - Inosine may be taken with or without food, but because it may cause mild stomach upset in some it will be recommended to at least start by taking it with meals (unless otherwise indicated by the results of inosine-food interaction studies). It will be stressed that – whether taken with meals or not – every dose of 1-2 capsules should be taken with fluids as above for adequate hydration to reduce the risk of kidney stones and gout.[177,178]
  - If warranted, tips for overcoming difficulty swallowing the relatively large-sized study capsules.
  - Compliance and study drug storage requirements.
  - Return of study bottles at each visit (whether or not empty).
  - Need for immediate reporting of any AEs to the Site Investigator or Coordinator.
  - Importance of not taking study drug on study visit days until immediately after the visit blood draw (with any further daily doses distributed over the remainder of day following the visit).
  - Titration schedule of study medication, as below.

5.1.2.2 Initial Dosing Following Baseline Visit

Following administration of the first dose, each subject will begin taking an initial daily dosage (# of capsules per day) that is individualized. These subject-specific initial dosages are derived from several factors that were identified (upon analysis of SURE-PD data) as predictors of the daily dosage required to achieve serum urate elevation into a specified range. Factors associated with larger increases in serum urate for a given dose were a) higher screening serum urate levels, b) female gender, and c) diuretic use. Upon randomization of a subject the DCC will automatically send site staff an initial daily dosage plan individualized to the subject (along with an enrollment number and study drug kit assignment). The initial daily dosage plan will be one of five (labeled by letters A to E in Table 4) with two stages. The first stage will employ a relatively low starting dosage for 1 week (through day 6 after BL; BL = day 0), before increasing the daily dosage one week after the BL visit (per schedule in Table 4), except that subjects who are on Plan A will stay on 1 capsule/day after day 6.

Table 4: Initial daily dosage (capsules/day)			
Plan	**Day 0 (Baseline)**	**Day 1 - 6**	**Day 7+**
A	1 at visit only	1	1 (no change)
B	1 at visit only	1	2
C	1 at visit + 1 at dinner time	2	3
D	1 at visit + 1 at dinner time	2	4
E	1 at visit + 1 at dinner time	3	5

During this initial dosing period (starting on Day 1) and throughout the study the daily dosing schedule will be determined by total daily capsule (cap) dosage as described in Table 5. Following the initial dosing period subjects will remain on the same daily dosage, as tolerated, until a dose adjustment is triggered in response to serum urate measured in blood drawn at subsequent visits (see below).

Table 5: Daily dosing schedule based on total daily capsule count			
caps/day	**Breakfast time**	**Lunch time**	**Dinner time**
1	1	-	-
2	1	-	1
3	1	1	1
4	1	1	2
5	2	1	2
6	2	2	2

Unless otherwise indicated by the results of inosine-food interaction studies, doses will be organized by approximate mealtimes as subjects are instructed to initially take doses with meals to reduce any risk of dyspepsia. Dose times may vary but consecutive doses should be maintained at least 4 hours apart.

At study visits on treatment (V01-V09) subjects will hold their dose(s) until after the blood draw for trough serum urate sampling, when they will promptly take their first dose of the day. Any subsequent doses for that day will be distributed across the remainder of the day (i.e., with all the day's doses delayed rather than skipped; e.g., for a subject taking at least 3 capsules/day, and therefore on a t.i.d. schedule, who undergoes a visit blood draw at 11 AM s/he will then take the first dose of the day [with fluids during the visit], then the second dose at ~dinner time, and then the third/final dose before bed). A normal dosing schedule will be resumed the following day.

At the discretion of the Investigator, the administration of any two-capsule dose of study drug (i.e., for subjects taking 4 or more caps/day) may be split if it were associated with a minor AE that does not warrant dose reduction. If a two-capsule dose is split then the 2nd capsule may be taken after any delay as long as it is taken at least 4 hours ahead of the next dose.

5.1.2.3 Study Drug Dosage Titration Algorithms

A titration algorithm will be employed to lower inosine dosing for over-range levels (>8.0 mg/dL) as well as to increase inosine dosing for those that are under 7.1 mg/dL. A parallel titration algorithm for dosing of subjects on placebo will be used to match their daily capsule dosage to that of subjects on active drug. The resultant adjustments in study drug dosage will be made after each on-treatment visit via person-to-person phone call (or receipt-confirmed forms of email, text message, voice message, etc) from staff to subject within 3 days of receiving a dose adjustment notification from the DCC. At the visits subjects will be instructed to continue their current dosing unless they receive a call (or are otherwise contacted if they mutually agree to an alternative means of communication). When staff contact a subject electronically or otherwise leave a message, the staff must receive from subjects an acknowledgement of receipt of the message and a plan to initiate the change at a specific time and date. Contact will normally only be made when a dose adjustment is warranted, or if a test result warrants a dose change/suspension or other intervention (such as a urine alkalinization).

The Site Investigator and Coordinator will be contacted with instructions by the DCC (by automated email) that will indicate what the study drug dosing should now be and whether this is a change. When subjects are then contacted they should be instructed of the/any dose change and that it should be put into effect immediately (at their next dose). All post-visit contacts, their timing and outcome should be logged in the subject's source documents and the Dose Management Log electronic Case Report Form (eCRF) updated appropriately if a dose adjustment is ordered.

Specific dose adjustments will be made in response to serum urate results at on-treatment visits V01 to V09 (and at any unscheduled on treatment) as follows:

<u>Subjects randomized to active drug</u>:

- Serum urate < 67% increase from baseline level (calculated as average of SC1, SC2 and BL visit values) to lower limit of target range (7.1 mg/dL) ==> increase by 2 caps/day, except:
  - If subject is already on 5 caps/day, then increase by 1 cap/day up to 6 caps/day.
  - If subject is already on 6 caps/day (possible only after V01) then continue at this dose.
- Serum urate 67-99% increase from baseline level to lower limit of target range ==> increase by 1 cap/day, except:
  - If subject is already at 6 caps/day then continue at this dose.
- Serum urate within target range ==> no change.
- Serum urate >upper limit of target range (8.0 mg/dL) and ≤ 9.0 mg/dL ==> reduce by 1 cap/day the dose being taken at the most recent visit (unless the subject was already down to 1 cap/day in which case s/he will continue at 1 cap/day).
- Serum urate > 9.0 mg/dL ==> hold treatment (i.e., take 0 caps/day) x 6 days, and then resume treatment at 1 less cap/day than the prior dose (i.e., at the most recent visit), except:
  - If that reduction results in no caps being taken, then continue the hold (i.e., 0 caps/day) x 6 more days, then resume treatment at 1 cap/day and return for an unscheduled visit (e.g., U01) in 4 weeks, employing the same titration study drug adjustment algorithm as presented here (Sec. 5.1.2.3) after the unscheduled visit labs are received.
  - If a subject returns 3 consecutive serum urate levels > 9.0 mg/dL on 1 cap/day, then the study drug will be permanently discontinued due to "excess sensitivity to study drug" (not an AE).

Subjects randomized to placebo:

To preserve blinding to treatment assignment study drug titration will be according to the same titration schedule used for active subjects, but those titrations will be based on a hypothetical serum urate (SU) level calculated as an adjustment from the placebo subject's observed SU level at any given time point. The adjustment will initially be a fixed increment calculated as a function of the subject's current placebo dose. After accumulating some experience with how actual inosine doses affect both mean SU levels and among-subject variation in SU levels, then the increment calculation may be adjusted. Thus the system is designed with the additional flexibility to include some degree of variation among subjects and a non-constant effect of inosine over time. The following algorithm will be used:

1. For placebo subject i at visit j: hypothetical SU[ij] = observed SU[ij] + (U[i] + v[j] + w[ij]) * # of placebo capsules in current dose.
2. U[i] ~ Normal(mean S, variance T); S will be a function of gender, baseline SU, and diuretic use or other participant characteristics; initially T = 0, later T may be greater than 0 based on accumulating data in the trial.
3. Initially v[j] = 0 and w[ij] = 0; later v[j] or w[ij] may differ from 0 in order to best match placebo pill counts to those required for titration of participants in the active arm.

5.1.2.4 Missed Doses

The subject should not take a double dose to make up for a missed dose. If a subject forgets to take a particular dose, it should not be made up with the next scheduled dose. Subjects may take a forgotten dose as long as the delayed dose is taken at least 1 hour before the next regularly scheduled dose.

5.1.2.5 Dose Reductions/Suspensions

A. Dose Reductions

In addition to serum urate-triggered algorithmic dose reductions (See 5.1.2.3), placebo and active drug may also be reduced during the study if a subject develops a persistent AE that the Enrolling Investigator believes warrants a study drug reduction. Non-Investigator-approved dose reductions may be retroactively assessed and determined to be appropriate by the Site Investigator, for example a dose reduction initiated by another physician upon the subject's hospitalization for an AE.

The duration of the decrement should be the minimum period required to ensure subject safety and comfort. Whether or not the Investigator or another clinician initiates an AE-triggered dose reduction, the Site Investigator should follow the subject as closely as reasonable during the dose reduction period to ensure a prompt re-challenge when appropriate (see Section 5.1.2.6). If an AE-triggered dose reduction or suspension (see 5.1.2.5.B1) is sufficiently long, study drug will be deemed "intolerable" as part of the key trial assessment of tolerability (see Section 10.2.2.2).

The extent of the decrement is at the discretion of the Investigator, and can range from reducing a specific dose (e.g., halving or eliminating the last dose of the day) to eliminating all doses (effectively 'dose suspension'; see 5.1.2.5.B1).

Clinician-initiated dose reductions and their duration are to be recorded on the Dose Management Log and reported to the CCC within 24 hours of initiation. In the event that an AE-triggered dose reduction of any type lasts longer than 72 hours, then the Site Coordinator or Investigator is to contact the Medical Safety Monitor or CCC Project Manager to discuss the situation and review management options.

B. Dose Suspensions – may be AE-triggered, precautionary or logistical as follows:

1. AE-triggered suspensions – As for AE-triggered dose reductions, AE-triggered suspensions may contribute to a determination that the study drug is intolerable (see Section 12.4.2).
   a. *Automatic*:
      - Myocardial or cerebral infarct (If confirmed, drug will be permanently discontinued. See Sections 7.5.1 and 9.0)
      - Gout attack (suspend at least until acute symptoms resolve)
      - Uric acid or urate urolithiasis (suspend at least until any acute symptoms resolve)

   b. *Investigator-initiated*: as above in 5.1.2.5.A.
2. Precautionary suspensions – Investigators may approve a brief non-AE-triggered suspension of study drug employed as a medical precaution (e.g., as in a peri-operative suspension of study drug required by the subject's surgeon for an elective procedure, during a day of potential dehydration as in religious fasting, etc).
3. Logistical suspensions – Brief interruptions in study drug use may be incurred due to unavailability (e.g., as in inadvertent destruction, or loss during travel). Re-supply and resumption should occur as soon as possible.

All dose suspensions are to be recorded on the Dose Management Log and reported to the CCC within 24 hours of initiation. In the event that an AE-triggered suspension of any type lasts longer than 72 hours, then the Site Coordinator or Investigator is to contact the Medical Safety Monitor or CCC Project Manager to discuss the situation and review management options.

5.1.2.6 Dose Re-challenge and Resumption

Subjects whose dose has been suspended for precautionary or logistical purposes (see Section 5.1.2.5B2 and 5.1.2.5B3) should resume drug as soon as the precautionary or logistical concerns have been adequately addressed. They should resume drug at the level being taken at the time of the suspension.

Subjects whose dose has been reduced or suspended due to an adverse effect (see Sections 5.1.2.5A and 5.1.2.5B1) must tolerate the reduced or suspended drug level for two or more full days (i.e., equal to or greater than 48 hours) before being re-challenged. After this time the re-challenge may begin with a partially or fully resumed dose of the study drug (i.e., the dose being taken at the time of reduction or suspension). Whether the re-challenge is immediately at the full dosage (i.e., resumption) or gradually escalating is at the discretion of the Site Investigator. Re-challenge and resumption should be pursued with expedience while ensuring the subject's likely safety and comfort. If the AE recurs as the dosage is increased, the subject should continue on the maximally tolerated dosage up to the full dose.

All dose re-challenges and resumptions are to be recorded on the Dose Management Log and reported to the CCC within 24 hours of initiation. Having to back down on the dosage during a re-challenge is considered another dose reduction and should be reported to the CCC as such and recorded on the Dose Management Log.

Note that persistent dosing below the full study drug regimen (as dictated by serum urate-titrated dosing plan; Section 5.1.2.3) would lead to a determination that the intended dose was "intolerable" as per Section 10.2.2.2.

5.1.2.7 Question of Need for Downward Titration

There is no downward titration necessary when study drug is reduced, suspended temporarily, or permanently discontinued.

5.1.3 Potential Adverse Effects (AEs) of Study Drug

5.1.3.1 Potential AEs of Active Drug (Inosine)

Anticipated AEs of inosine are expected to be primarily mediated by urate, to which it is rapidly metabolized. Established AEs of elevating urate are disorders of crystallization, as reviewed above. The two main disorders are gout (a form of arthritis triggered by urate crystals forming in a joint) and uric acid urolithiasis (uric acid crystalizing at low urine pH to form stones). Although gout is often a progressive degenerative arthritis, any inosine-induced episodes of acute gouty arthritis would be expected to be fully reversible upon discontinuation of inosine and thus would not require additional medication. Similarly urolithiasis is often a recurrent nephropathic disease, whereas uric acid stones induced by inosine would not be expected to recur if inosine were discontinued. Nevertheless the study incorporates numerous safeguards against these conditions, particularly uric acid urolithiasis given our phase 2 data indicating that a small but significant portion of the study cohort may develop stones. (See Sec. 2.2.3.)

In addition, as surveyed above in Sec. 2, numerous other medical conditions have been linked to higher serum urate and include cardiovascular diseases, including hypertension, coronary artery disease, heart failure, stroke, and renal dysfunction, and metabolic disorders (metabolic syndrome and diabetes mellitus). Although it remains uncertain whether or not urate itself contributes to these conditions and their increased risk with higher urate is generally modest, special attention will be paid to both avoiding and monitoring for these conditions during the trial.

5.1.3.2 Potential AEs of Placebo (Lactose)

No specific AEs of placebo drug are anticipated. Note that symptoms due to lactose intolerance (a theoretically placebo-specific potential AE) are unlikely given that the maximum single dose of 1 gm lactose (two 0.5 gm placebo capsules) is well below the limit of lactose sensitivity (typically >12 gm/serving) in lactose-intolerant people.[179,180] By comparison, 50 gm lactose is considered the standard challenge dose in lactose intolerance tests.

5.2 Handling of Study Medications

Study drug will be manufactured in the same opaque white, hard gelatin capsules for both placebo and active drug formulations. Their indistinguishable appearance will be confirmed initially after manufacturing and at least annually thereafter during the study through a scheduled stability testing program. Study drug will be packaged with 100 capsules per bottle, which will be white high-density polyethylene (HDPE) with a standard lid closure. All study drug formulation, encapsulation and bulk packaging (into bottles) will be conducted by a single contract manufacturer.

Study drug will undergo final packing, labeling and distribution to clinical sites by a central pharmacy. Six study drug bottles of placebo or active drug will be assembled into a kit box and labeled to meet applicable US regulatory requirements. Each kit box is good for at least 3 months of dosing. Each subject

who completes the study without early drug discontinuation will receive from 2 to 8 kit boxes (12 to 48 bottles) in total depending on the dose level maintained.

The central pharmacy will label and supply subject kits for each study center according to the randomization list provided by the project DCC Biostatistics Core. Each kit and its bottles will be identified via a 5-digit Enrollment ID Number as defined by this randomization list to maintain study blindedness. Pre-assigned start-up study drug kits will be supplied to the Site Investigator once Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approval is granted and all regulatory documents are filed and contractual arrangements are in place. Additional start-up kits will be provided by the central pharmacy as site enrollment warrants. The central pharmacy will send additional supplies for a given enrolled subject in advance of each dispensing visit.

In accordance with local regulatory requirements, the Site Investigator or designated site staff must document the amount of investigational product dispensed and/or administered to study subjects, the amount received from the central pharmacy, and the amount destroyed upon completion of the study. The Site Investigator is responsible for ensuring product accountability records are maintained throughout the course of the study. The inventory will include details of *the study drug* and dispensed to subjects, batch, and ID numbers. All unused capsules and bottles must be kept until reconciliation of delivery records with accountability logs by the monitor. Subjects will be asked to maintain a daily drug diary to confirm drug was taken as prescribed. After the monitor has performed accountability, the site will be instructed by the CCC or designee to either destroy the remaining study medication per institutional policy or return it to the central pharmacy. An accounting must be made of any drug deliberately or accidentally destroyed. Discrepancies between the amount of study drug received and dispensed drug must be reconciled.

5.3 Concomitant Medications

5.3.1 Required Therapy

There is no required therapy to take on a regular basis in addition to study drug. Subjects with persistently acidic urine will be prescribed potassium citrate (or an alternative medication) as a urine alkalinization method to reduce the risk of uric acid stone formation. See Sec. 8.4.2.

5.3.2 Prohibited Medications

5.3.2.1 Disallowed Medications Prior to Study Entry

Subjects who have used certain medications as specified under the study exclusion criteria (Sec. 4.2) will not be eligible for participation in this study.

5.3.2.2 Disallowed Medications during the Study

Use of the following medications is not allowed during the study. The use of any disallowed medication must be documented.

- Multivitamins and/or mineral preparations, other than a standard daily multivitamin*.
- Antioxidants such as vitamin E, vitamin C and inosine (other than as may be in study drug, a standard daily multivitamin or a normal diet)*
- Neuroleptics (typical and atypical), metoclopramide
- Methylphenidate, amphetamine, cinnarizine, reserpine, tetrabenazine, MAO-A inhibitors, or appetite suppressants*
- The urate-reducing agents, allopurinol, febuxostat and probenecid.

*Please see Section 18, Concomitant Medications, in the site Manual of Procedures located on the study ePortal section: for further details related to these disallowed medications.

5.3.3 Allowed Concomitant Medications

All concomitant medications that the subject is taking at study initiation must be recorded on the Concomitant Medication Log. The log will include a list of all medications taken during the 30-day period prior to the 2nd Screening Visit. Any changes in concomitant medications or new medications added, including use with an intercurrent illness, must be recorded in the case report forms. All concomitant medications must be used in accordance with approved labeling and as prescribed.

5.3.3.1 Initiation of Antiparkinsonian Medication

Although anti-PD medications other than monoamine oxidase B inhibitors are not permitted at entry, anti-PD medications may be initiated during the study. The decision to initiate antiparkinsonian treatment must be made by the Site Investigator at an in-person visit (regular or unscheduled), and only after a determination that the subject has developed the need for dopaminergic therapy. For this study 'dopaminergic therapy' is defined as symptomatic treatment with an antiparkinsonian drug (levodopa, a dopamine agonist, amantadine or an anticholinergic agent trihexyphenidyl or benztropine), other than a MAO-B inhibitor taken at enrollment. Increasing an entry dosage of a MAO-B inhibitor or initiating a MAO-B inhibitor during the study will be considered as requiring dopaminergic therapy. Once treatment has been initiated, dosage adjustments may be ordered by telephone. Dosing of a monoamine oxidase B inhibitor that was taken at enrollment should remain unaltered until (additional) dopaminergic therapy is initiated or until the subject's final study visit.

5.3.3.2 Initiation of Thiazide Therapy during the Study

Initiation of treatment with a prescription thiazide -- such as hydrochlorothiazide (e.g., Esidrex), chlorothiazide (e.g., Diuril), chlorthalidone (e.g., Hygroton), indapamide (e.g., Lozol), metolazone (e.g., Zaroxolyn) -- will be allowed. However, thiazide therapy can cause an increase in serum urate (usually <1 mg/dL). Accordingly, a regular or unscheduled visit with serum urate measurement by the central lab must occur between 1 and 4 weeks after initiation of any thiazide to afford timely algorithmic adjustment of study drug dosing. Although diets can also influence serum urate,[139] they are generally stable and any changes are expected to prompt titration of study drug dosing based on placebo and inosine dosing algorithms.

5.4 Adherence Assessments

At each study visit, the Site Investigator and/or Study Coordinator will assess the subject's compliance with the study requirements. This will include checks of protocol compliance, concomitant medication use, and use of study drug in order to assess the reliability of subject-generated data. The primary mechanism for assessing compliance with use of study drug will be capsule counts i.e., the number of capsules dispensed and returned at each visit will be documented in the Study Drug Dispensing/Return Log. Subjects are instructed to return all study bottles (whether empty, partially used, or unused) at each visit on study drug (V01-V09) and upon its discontinuation (V10).

Non-compliance will be defined as taking less than 80% or more than 125% of study medication as determined by capsule counts. If a study subject is non-compliant with study medication, the Site Investigator and staff should re-educate and train the subject in administration of study drug. Subjects who fail to comply with the study requirements may be withdrawn from the study. Data indicating non-compliance will be used in the end of study analysis, when they may be related to pharmacodynamic evidence of non-compliance (e.g., lack of increase in serum urate on maximum inosine dosage).

6. CLINICAL AND LABORATORY EVALUATIONS

6.1 Safety Assessments

6.1.1 Clinical Variables

In addition to the data from assessments listed below, subjects will provide information on their demographics, past medical history, including PD, socio-economic history, smoking, alcohol and caffeine usage, as well as concomitant medication usage and compliance with study procedures.

6.1.2 Vital Signs, their Orthostatic Changes, Weight and Height

Blood pressure (supine and standing), heart rate (supine and standing), and body weight will be measured at Screening Visit 2, Baseline Visit, Week 12, Month 12, Month 24 and Month 27. Height will be measured at the Baseline Visit only.

6.1.3 Clinical Laboratory Assessments

The following laboratory tests will be performed at Screening Visit 1 and 2, Baseline before first dose of study drug is administered, at Weeks 3-12, Months 6-24, and Month 27 for safety (as indicated below and on the Schedule of Activities; Sec. 7.7):

- Serum Uric Acid [all visits]
- Complete blood count (CBC) with differential (hematocrit, hemoglobin, platelet count, RBC indices, Total RBC, Total WBC, and WBC & differential) [at baseline and after 3 months and 2 years on treatment; and at all other visits for subjects on potassium citrate alkalinization therapy]
- Basic blood chemistry panel: bicarbonate, blood urea nitrogen (BUN), chloride, creatinine, glucose, potassium, sodium, GFR [at 2nd screening visit, all on-treatment visits, and safety visit]
- Specialty blood chemistry: Liver function tests (LFTs)/Lipids/ thyroid-stimulating hormone (TSH); LFTs: alanine aminotransferase (ALT (SGPT)), aspartate aminotransferase (AST (SGOT)), albumin, alkaline phosphatase, calcium, magnesium, phosphate, total bilirubin and total protein; Lipids comprise cholesterol, HDL, LDL and triglycerides [at baseline and after 3 months and 2 years on treatment]
- Urinalyses: bilirubin, blood, clarity, color, glucose, ketones, nitrate, pH, protein, specific gravity, urobilinogen and WBC screen; plus urine sediment exam for uric acid crystalluria [at 1st screening visit, all on-treatment visits, and safety visit].
    - Subjects may be asked to obtain additional urine samples at home if they begin alkalinization therapy (see section 8.4.2 for futher instruction), or they may be given the option to obtain additional urine samples at home as deemed appropriate by the Site Investigator (for example, to ensure subjects are adequately hydrated as assessed by urine specific gravity). If the reason the subject is being asked to collect a urine sample is to monitor their hydration level, study staff should ask subjects to collect this sample anywhere from one week after a visit to one week before the next visit (further guidance can be found in the Manual of Procedures section 13.4). Subjects will be instructed by sites as to how to obtain and send the at-home urine samples to the central lab.
- 24-Hour urine analysis: chloride, creatinine, pH, potassium, sodium and urate, with total volume [at baseline and 1 year visit]

- Urine pregnancy test for women of childbearing potential (WOCBP) [if warranted at 2nd screening visit and as necessary thereafter at on-treatment visits]

All subjects will have safety laboratory tests at the designated visits outlined in the protocol. These samples will be analyzed at a central laboratory. The Site Investigator may order additional testing, if needed, to further assess an adverse event (AE), or if there were any suspicion that a subject may be pregnant, throughout the course of the study.

6.1.4 12-Lead Electrocardiogram (ECG)

A standard 12-lead ECG will be performed at Screening Visit 2, Month 12 and Month 24 or the Discontinuation of In-Person Follow-up Visit for subjects unwilling to continue in-person visits. Results will be based upon the Site Investigator's interpretation of the standard machine readings and the reading provided by the central cardiology lab service. It is the Site Investigator's decision as to whether the ECG needs to be interpreted by a cardiologist in addition to the one reading by the central laboratory. A copy of the tracings will be kept on site as part of the source documents.

6.1.5 Physical Examination

A physical examination will be performed and recorded at Screening Visit 2. The following systems will be examined: skin, head/neck/lymphatic, eyes, ears/nose/throat, cardiovascular, lungs, abdomen, musculoskeletal, neurological and psychiatric.

6.1.6 Neurological Examination

A neurological examination will be performed and recorded at Screening Visit 2.

6.1.7 Adverse Events

Adverse events (AEs) will be documented at each study visit, starting with the signing of informed consent. Events that occur prior to administration of first dose of study drug will be noted accordingly. Information on adverse effects of study medication and on intercurrent events will be determined at each visit by direct questioning of the subjects, review of concomitant medications, and vital sign results.

6.2 Other Assessments

6.2.1 Movement Disorders Society Unified PD Rating Scale (MDS-UPDRS)

The MDS-UPDRS[138] will serve as the primary outcome variable of the study (as justified in detail in Sec. 3.2.3) and will be conducted at all standard visits beginning with the 2nd screening visit. The MDS-UPDRS was designed by movement disorders experts to address weaknesses of the original UPDRS (e.g., by adding questions on constipation and sialorrhea) while preserving its overall format. The MDS-UPDRS has four parts:

- **Part I (non-motor experiences of daily living)**, comprising
  - Part IA concerning behaviors that are assessed by the Site Investigator with all pertinent information from patients and caregivers
  - Part IB that is completed by the patient with or without the aid of the caregiver, but independently of the Site Investigator.
- **Part II (motor experiences of daily living)**, designed to be a self-administered questionnaire like Part IB, but similarly can be reviewed by the Site Investigator to ensure completeness and clarity.
- **Part III (motor examination)** has instructions for the rater to give or demonstrate to the patient; it is completed by the clinician rater.

- **Part IV (motor complications)** with instructions for the rater and also instructions to be read to the patient. This part integrates patient-derived information with the rater's clinical observations and judgments and is completed by the rater.

Subjects will self-administer Parts IB and II, but will review responses for accuracy and clarity with the Site Investigator or Coordinator. Parts IA, III and IV must be conducted by the Site Investigator. Parts I, II, and III will be conducted at study visits as indicated on the Schedule of Activities (Sec. 7.7). Part IV will be conducted at visits where MDS-UPDRS Parts I-III are conducted/collected but only on subjects who have started on symptomatic therapy after the Baseline visit. Use of MDS-UPDRS is responsive to core instrument recommendations for the Quality of Life subdomain of the NINDS CDEs for PD, and to FDA guidance encouraging use of patient-reported outcomes (PROs) as a substantial portion of the responses are patient-reported. The same Site Investigator should assess all subjects on parts IA and III of the MDS-UPDRS at all study visits.

6.2.2 Modified Schwab & England Activities of Daily Living Scale

The Schwab & England scale[181,182] is a Site Investigator and subject assessment of the subject's level of independence. The subject will be scored on a percentage scale reflective of his/her ability to perform acts of daily living. Printed scores with associated descriptors range from 100% to 0% in increments of 10%, where 100% is "subject has full ability and is completely independent; essentially normal" and 0% is "vegetative functions such as swallowing, bladder and bowel functions are not functioning; bedridden". Scores should be coded in increments of 5, (i.e. 095, 090, 085). This joint subject/Site Investigator assessment will be conducted periodically at the visits indicated in Sec. 7.7.

6.2.3 Parkinson's Disease Questionnaire - 39 item version (PDQ-39) scale

The PDQ-39 asks 39 questions organized over eight domains: mobility (10 items), activities of daily living (6 items), emotional well-being (6 items), stigma (4 items), social support (3 items), cognitions (4 items), communication (3 items), and bodily discomfort (3 items). The PDQ-39 is the most widely used health related-QoL instrument in PD, and is considered to have generally good psychometric properties and content validity.[183,184] Use of PDQ-39 is responsive to core instrument recommendations for the Quality of Life subdomain of the NINDS CDEs for PD, and to FDA guidance encouraging use of PROs. This assessment will be collected from subjects periodically at the visits indicated in Sec. 7.7.

6.2.4 Modified Hoehn and Yahr Scale

The Modified Hoehn and Yahr Scale[181, 185] is an 8-level PD staging instrument. Stage 0 is "no signs of disease" and the highest stage (5) is "wheelchair bound or bedridden unless aided." This Site Investigator assessment will be conducted periodically at the visits indicated in Sec. 7.7.

6.2.5 Assess Need for Dopaminergic Therapy

At each visit beginning with Baseline Visit, the Site Investigator will assess the subject's need for dopaminergic therapy. (See Sec. 5.3.3.1 for definition of dopaminergic therapy.) A questionnaire will be used to facilitate the Site Investigator's decision. As in the DATATOP[31,32] and PRECEPT[30] trials, this will be based on PD disability posing a threat to the subject's current occupational status, current abilities (potential capacities) related to occupational matters, to handle routine personal finances and domestic responsibilities, and activities of daily living.

Subjects who are judged to require dopaminergic therapy at Baseline or are thought likely to need therapy within the 3 months after Baseline, will be excluded from participation in this study. Subjects who are judged to require dopaminergic therapy after starting study drug will continue in the study after anti-parkinsonian therapy is instituted.

6.2.6 Mini-Mental State Examination (MMSE)

The Mini-Mental State Examination[186] is a 30-point scale that is widely used for the evaluation of degenerative dementia in patients with a variety of neurologic and psychiatric disorders, and is designated an NINDS CDE for PD. The MMSE includes evaluations of orientation to time and place, immediate recall, attention, delayed recall, naming, repetition, stage command, reading, copying and writing. The test is referred to as "mini" because it focuses only on the cognitive aspects of mental functions and does not include questions related to mood, abnormal mental experiences, or the form of thinking. The total score ranges from 0 to 30 (highest function).

Subjects will complete the MMSE at the Screening Visit 2. Subjects with an MMSE score less than 25 will be excluded from participation in the study.

6.2.7 Montreal Cognitive Assessment (MoCA)

In early Parkinson's disease, when cognitive deficits occur, they are subtle and mild and the subjects usually perform in the normal range of the widely used Mini-Mental State Exam. The Montreal Cognitive Assessment[187] is a rapid screening instrument like the MMSE, but was developed to be more sensitive to patients presenting with mild cognitive complaints. It is designated an NINDS CDE for PD. Compared to the MMSE the MoCA may be more sensitive to mild cognitive deficits in PD.[188] The MoCA assesses short term and working memory, visual-spatial abilities, executive function, attention, concentration, language and orientation. The total score ranges from 0 to 30 (highest function). The MoCA will be administered at Baseline, Week 12, Month 12, Month 24 and Month 27 or the Discontinuation of In-person Follow-up Visit for subjects unwilling to continue in-person visits.

6.2.8 Quality of Life in Neurological Disorders (Neuro-QOL)

Neuro-QOL is a set of patient-reported outcome (PRO) measures that assess health-related quality of life of people with neurological disorders.[190,191] It facilitates comparisons between diseases and within individual patients over time. Developed through a collaborative NINDS-sponsored research initiative, Neuro-QOL is an NINDS CDE and has been validated in multiple patient populations including PD.[190,192] It comprises item banks and scales (with short form versions covering physical, psychological and social domains) that assess symptoms and concerns that are meaningful across disorders, while others are of particular relevance to specific patient populations. In a PD population, Neuro-QoL measures including its short forms have demonstrated high internal consistency, with acceptable test-retest reliability and support for convergent validity with PD specific measures including PDQ-39 and MDS-UPDRS.[190] An instrument comprising multiple short form domains will be employed before and after the study drug treatment period (at SC2 and SV) while the depression domain will be employed on its own at additional visits during the treatment period, as indicated in Sec. 7.7.

6.2.9 Diagnostic Features Assessment

The Diagnostic Features form is a companion to the Primary Diagnosis form. It is a review by the Site Investigator of factors that do and do not suggest a diagnosis of Parkinson disease. This assessment is completed at Month 27 or the Discontinuation of In-person Follow-up Visit for subjects unwilling to continue in-person visits.

6.2.10 Primary Diagnosis Assessment

The Primary Diagnosis form captures, in the Site Investigator's opinion, a current percentile probability the subject has idiopathic Parkinson disease based on available information. Ranges include: 90-100%; 50-89%, 10-49% and 0-9%. In addition the Site Investigator selects the most likely primary diagnosis from a listing that includes idiopathic PD, many other neurological disorders, and the option of no neurological disorder. To correlate with the MDS-UPDRS, this percentile probability and most likely

diagnosis will be captured at Month 27 or the Discontinuation of In-person Follow-up Visit for subjects unwilling to continue in-person visits.

6.2.11 Dopamine Transporter (DAT) Neuroimaging

The radionuclide-labeled dopamine transporter (DAT) ligand DaTscan™ (^{123}I-ioflupane injection) is approved by the FDA for striatal DAT visualization using single photon emission computed tomography (SPECT) brain imaging to assist in the evaluation of adult patients with suspected parkinsonian syndromes. DaTscan™ is approved to help differentiate essential tremor from tremor due to parkinsonian syndromes (including idiopathic PD, multiple system atrophy and progressive supranuclear palsy) and not for the diagnosis of PD among parkinsonian syndromes. Nevertheless, DAT brain scans (using any of several radioligands) in clinical research have consistently identified a small but substantial (~10%) portion of subjects who are enrolled in clinical trials based on an expert clinician diagnosis of probable early PD but who turn out to be unlikely to have PD.[30,140,143,145-147] In addition, quantitiative changes in striatal DAT bindng sites assessed by serial DaTscan™ imaing studies over years provide a biomarker of progressive nigrostriatal dopaminergic neuron degeneration, which correlated inversely with serum urate levels at baseline in the PRECEPT study.[36]

6.2.11.1 Required Dopamine Transporter (DAT) Neuroimaging for Eligibility Determination

DAT scans will be performed prior to the baseline visit, and will generally be conducted as the final screening evaluation (DS1) at a certified neuroimaging center at or near the clinical site. A determination of whether DaTscan™ imaging supports a diagnosis of PD and therefore study eligibility will be made by the study imaging core. Its experienced nuclear medicine specialists (trained in the visual read method appropriate for DATscan™ imaging) will perform the qualitative eligibility assessment. Each scan will be assessed independently by two readers. If the readers disagree then a third, expert reader will be used to adjudicate and the majority read outcome will determine whether the scan is classified positive or negative. In order to avoid false negative reads, if the readers agree that the result is positive then no further assessment is required; however if they agree it is negative, then a third, expert reader will reassess the image and make a final determination of whether the scan is classified positive or negative.

6.2.11.2 Optional Follow-up DAT Neuroimaging

An additional follow-up DaTscan™ imaging study will be performed one to two months following the final study clinic visit (V10), and before the final safety visit (SV), for active SURE-PD3 subjects who have consented to participate in a serial DAT scan substudy, who have been on study drug through at least the 1-year visit (V06), and who are not expected to use any of the following within 90 days prior to DS2: modafinil, armodafinil, metoclopramide, alpha-methyldopa, methylphenidate, reserpine, or amphetamine derivative. The substudy will quantify changes in DAT binding between the pre-study drug exposure and post-study drug exposure timepoints.

6.2.12 Blindedness Evaluation

At week 6 and either month 24 or an Unscheduled Visit due to study drug discontinuation or a Discontinuation of In-person Follow-up Visit (whichever of the 3 visits comes first), the Site Investigator, Coordinator, and subject will complete a blindedness evaluation in which each is asked to give his/her independent impression of the subject's treatment assignment and the primary and secondary reasons for this opinion. Subjects' responses will not be available to the site Investigator or Coordinator when they make their assessments.

6.2.13 Exploratory Assessments

Three brief, self-administered questionnaires will be included to explore whether readily ascertained historical factors modify or otherwise interact with inosine effects.

- **REM Behavior Disorder (RBD) Question** – A single semi-quantitative RBD question will be asked at SC1, BL, and 3 mo, 12 mo, 24 mo, and 27 mo visits.
- **PD Risk Factors Questionnaire** – A self-administered questionnaire assessing exposures and experiences linked to the risk of PD will be collected at BL. The questionnaire was developed by the PSG and derived from NINDS CDEs.
- **PD - Expectancy Questionnaire** – A self-administered questionnaire assessing expectations of study drug effect will be collected at BL.

6.2.14 Optional Blood Collection for Biomarker Research

The following optional blood samples will be collected, at the visits indicated, from subjects who have 'opted in,' by written consent, to participate in a biomarker research substudy of SURE-PD3.

- Blood (5-10 ml) for DNA [collected at BL]
- Blood (5-10 ml) for plasma biomarkers [collected at BL, 24 mo and 27 mo visits]*

  *If the participant permanently discontinues study drug, for any reason, before the 24 mo visit, the 24 mo blood sample should be taken at an unscheduled study visit on the day they plan to discontinue the study drug (instead of at the 24 mo visit. Please see section 7.5.1 for more about this unscheduled visit).

6.2.15 Optional Smartphone Outcomes Research Participation

Eligible, consenting subjects may participate in a research substudy designed to assess the feasibility of using smartphone metrics as decentralized assessments of motor and non-motor outcomes of clinical progression in early PD[257-259]. Self–administered questionnaires and tests are completed by participants using a smartphone application adapted for the SURE-PD3 trial and known as Smart*4*SURE, as is the substudy. The primary aim of the Smart*4*SURE substudy is:

- to assess the feasibility of incorporating smartphone application-based outcome measures in phase 3 'disease-modifciation' trials for people with PD. If feasibility were supported by the substudy results, they may facilitate adoption of smartphone metrics as secondary or eventually primary outcomes in future neurotherapeutics trials for PD.

Key secondary aims of the substudy are:

- to correlate the cross-sectional assessments and the longitudinal changes documented by smartphone sensors and questionnaires with related elements of SURE-PD3's primary (MDS-UPDRS) and secondary outcome measures.
- to compare rates of change in motor performance and in cognitive and other non-motor functions among substudy participants randomized to inosine versus placebo (i.e., an exploratory test of the parent study's central hypothesis for these novel measures).

without interfering with the parent project's goals. Substudy participation is voluntary. After subject training at the 2nd screening visit (SC2), or if necessary at a subsequent visit, smartphone assessments will be conducted off-site at each participating subject's convenience. Accordingly Smart*4*SURE is not expected to appreciably increase burden to subjects or site staff during regular study visits, nor to compromise the clinical assessments conducted during visits. Serial assessments for Smart*4*SURE consist primarily of:

- Activity Sessions comprising ~15 minutes of motor and cognitive tests (including finger tapping, voice, tremor, gait and memory) to be conducted at least monthly but no more frequently than weekly. At least one activity session should be completed prior to the baseline visit for subjects who consented prior to their baseline visit.
- Surveys comprising ~20 minutes of responding to questions from a) the patient-reported parts (1b and 2) of the MDS-UPDRS[145,150], b) an 8-item PD quality of life (QoL) survey[260,261], and c) an 18-item apathy evaluation[262-267]. The surveys are to be conducted at least every 3 months but no more frequently than monthly. At least one survey should be completed prior to the baseline visit for subjects who consented prior to their baseline visit.

To be eligible the subject should expect to have access to a Smart*4*SURE-compatible smartphone and service plan (neither of which will be provided, replaced or otherwise paid for or supported by the study) and should be interested and able to commit the time needed to conduct serial activity sessions usually at least monthly and to complete the serial surveys usually at least every three months. Smart*4*SURE subjects can miss scheduled smartphone activities and still continue in the substudy. Similarly, substudy participants may withdraw from the Smart*4*SURE substudy at any time without jeopardizing participation in the parent trial.

7. STUDY SCHEDULE

7.1 Pre-Randomization Evaluations

Prior to performing any study activity, the candidate will be thoroughly informed of all aspects of the study, including all scheduled visits, activities and procedures, and will be requested to sign and date the IRB-approved informed consent form. All subjects will be given a photocopy of the signed consent form.

In this study the Site Investigator will be responsible for

- Assuring that the subject has given informed consent;
- Determining subject eligibility;
- Conducting the physical (medical) and neurological examinations;
- Conducting the MDS-UPDRS Parts IA, III and IV;
- Assessing Modified Hoehn and Yahr stage and Modified Schwab and England Activities of Daily Living;
- Assessing the need for dopaminergic therapy; and,
- Assuring subject safety.

After a subject discontinues study drug, the Site Investigator will also assess blindedness to treatment assignment and probability of PD as the accurate diagnosis.

Screening activities for this study will include two screening visits at the clinical site (Visit SC1 and Visit SC2) and one DAT neuroimaging study (DS1), which will take place within 60 days prior to the Baseline Visit. It is anticipated that many subjects will not be eligible to participate due to their serum urate level and/or their history of exclusionary medical conditions. Therefore, the first screening visit is designed as a relatively quick, preliminary screen focused on obtaining a blood sample to measure serum urate, and thus to spare those subjects who are excluded based on this result the unnecessary physical and neuropsychiatric assessments as well as additional laboratory and ECG procedures.

Following these two clinical site screening visits, those subjects who remain eligible will undergo a DAT scan as a third screening activity (DS1). Promptly after receipt of results from each of the three screening activities (SC1, SC2 and DS1), site staff will contact subjects to inform them either of exclusionary results that preclude their enrollment, or of their continued eligibility and in that case, to schedule or confirm their next screening activity or baseline visit.

All the inclusion criteria must be met and none of the exclusion criteria may apply unless the site is given a waiver (exception) for a particular criterion by the Medical Safety Monitor, a physician whose sole role in the study is to ensure participant well-being. **All** results from **both** screening visits and the DAT scan visit must be available before the final determination of a subject's eligibility for the study, which will be made at the baseline visit (BL).

7.1.1 Screening Visit One (Visit SC1)

The following procedures and evaluations are to be performed at Screening Visit One (SC1):

- Request/obtain written informed consent for the study
- Assign 4-digit Subject ID Number
- Obtain 9-digit CTCC Unique ID Number

- Obtain demographic information
- Review assessable inclusion/exclusion criteria
- Obtain blood for serum urate testing
- Obtain urine for routine analysis/pH/sediment
- Provide RBD Question to subject for self-administration
- Schedule time to contact subject after serum urate results have been received
- Tentatively schedule SC2

7.1.2 Post SC1 Telephone Call

A follow-up phone call will take place once the serum urate and urine test results have been received and reviewed. The following will be performed during the call:

- Review the results with the subject
- Inform the subject of his/her eligibility for proceeding to SC2
- Inform or remind the subject – if eligible and interested in participating in the Smart*4*SURE substudy – of the need to bring his or her substudy-compatible (and adequately charged) smartphone to SC2 in order enroll in and train for the substudy at SC2.

7.1.3 Screening Visit Two (SC2)

SC2 must occur after SC1 and before the DS1 visit. Subjects are to proceed to SC2 only if they met preliminary eligibility at SC1, including serum urate level.

The following procedures and evaluations are to be performed at SC2:

- Review assessable inclusion/exclusion criteria
- Obtain medical history
- Review and document concomitant medications
- Assess and document adverse events/experiences (AEs)
- Conduct neurological exam
- Conduct physical exam
- Assess PD features
- Administer MDS-UPDRS Parts IA and III
- Provide MDS-UPDRS Parts IB and II and Neuro-QOL to subject for self-administration
- Assess Modified Hoehn and Yahr Scale
- Administer Mini-Mental State Exam (MMSE)
- Obtain weight and orthostatic vital signs (supine and standing blood pressure and heart rate – refer to operations manual for standardized procedure)
- Conduct 12-Lead Electrocardiogram (ECG)
- Obtain blood for safety lab tests: serum urate and chem 7 panel
- Obtain urine for pregnancy test for all women unless they are at least two years postmenopausal, or surgically sterile
- For an interested, eligible subject obtain written informed consent for the optional Smart*4*SURE substudy
- Assist a consented Smart*4*SURE substudy subject in downloading and registering the Smart*4*SURE app on the subject's smartphone, and conduct an online training session via the app (Please note: If necessary, consent and training for the Smart*4*SURE substudy can take place at an additional visit to the study site after SC2).
- Distribute container for 24-hour urine collection at home and instruct subject in collection/storage procedures

- Schedule time to contact subject after SC2 serum urate and other lab and ECG results have been received
- Tentatively schedule DAT scan and Baseline Visit (with at least 5 business days between them)

7.1.4 Post SC2 Telephone Call

A follow-up phone call will take place once the serum urate, laboratory results and ECG results have been received and reviewed. The following will be performed during the call:

- Review the results with the subject
- Inform the subject of his/her eligibility for proceeding to DS1

7.1.5 DAT scan (DS1)

DS1 must occur after SC2 and before the Baseline visit. Subjects are to proceed to DS1 only if they met preliminary eligibility after the results of the SC2 labs and ECG have been received and reviewed by the Site Investigator.

DAT scan screening will be conducted at a qualified neuroimaging center. Results will be analyzed by the study imaging core to determine whether there is evidence of a dopamine deficit, which is required for eligibility.

7.1.6 Post DS1 Telephone Call between DS1

A follow-up phone call will take place once the DS1 results have been received and reviewed. The following will be performed during the call:

- Review the results with the subject
- Inform the subject of his/her eligibility for proceeding to BL
- Remind the subject (if proceeding to BL) to collect the 24-hour urine on the day before the BL visit, and to bring the container to the visit

7.1.7 Baseline Visit Telephone Call Immediately Prior to Baseline

Two or three days before the Baseline Visit is scheduled to occur, the subject should be contacted to remind him/her to collect the 24-hour urine on the day before the visit and to bring the container to the visit.

7.1.8 Baseline Visit (Visit BL)

The Baseline Visit must be within 60 days after SC1 and may not be conducted until the result of the DS1 scan has been received and reviewed by the Site Investigator.

The following procedures & evaluations are to be performed at the Baseline Visit:

- Review all inclusion/exclusion criteria to ensure subject remains eligible
- Obtain socio-economic information
- Obtain smoking, alcohol & caffeine information
- Review and document concomitant medications
- Assess and document AEs
- Administer MDS-UPDRS Parts IA and III
- Assess Modified Hoehn and Yahr Scale
- Assess Modified Schwab & England Activities of Daily Living
- Assess need for dopaminergic therapy

- Administer Montreal Cognitive Assessment (MoCA)
- Provide MDS-UPDRS Parts IB and II, PDQ-39, depression module of the Neuro-QOL, RBD, PD Risk Factors, and PD-Expectancy questionnaires to subject for self-administration
- Obtain height, weight and orthostatic vital signs (blood pressure, heart rate)
- Obtain blood for safety lab tests: serum urate, chem 7 panel, LFTs, TSH, lipids, CBC with differential
- If the subject has given consent, obtain extra blood samples for (DNA and plasma) banking
- Obtain urine for routine analysis/pH/sediment
- Retrieve 24-hour urine container from subject and process urine sample
- Use electronic enrollment module to randomize to treatment the subject who continues to meet all eligibility requirements per the Site Investigator's final assessment (see Section 4.3.4 for information regarding the enrollment module). The DCC will assign the subject's 5-digit enrollment/randomization number at this time.
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional Smart*4*SURE substudy
- Assist a consented Smart*4*SURE substudy subject in downloading and registering the Smart*4*SURE app on the subject's smartphone, and conduct an online training session via the app if not performed at a prior visit.

Under no circumstances may study drug be pulled from storage and given to a subject unless that drug kit has been assigned to that subject via the enrollment process.

The coordinator should write the subject's initials and Subject ID Number assigned at SC1 on the drug kit when it is taken from storage.

- Dispense sufficient study drug to cover time to next visit
- Observe subject taking one capsule and offer tips[193] if difficulty swallowing is a concern. (Note: This will be the only capsule taken on enrollment day for subjects whose initial dosing is one capsule daily; those whose initial dosing is one capsule *b.i.d.* or *t,i,d,* will take one more capsule after the visit around dinner time.)
- Provide verbal instructions regarding study drug covering:
    - Storage requirements
    - Importance of always taking capsules with a glass of water or other unsweetened, non-alcoholic beverage to ensure adequate hydration that should reduce the risk of kidney stones and gout.[64,177,178]
    - May be taken with or without food, but because it can cause stomach upset in some it is recommended to at least start by taking it with meals.
    - Titration schedule (see section 5.1.2.2)
    - Importance of not taking any study medication on study visit days until after the blood draw.
    - Return of all study bottles at each visit (whether empty, partially used, or unused)
- Distribute written dosing instructions
- Distribute subject reimbursement card and information
- Distribute Study Drug Record sheets and instruct subject how to record doses ordered and taken
- Stress to subject the importance of calling site to report any AEs
- Begin tracking dosing on the Dose Management Log
- Schedule Visit 01 (week 3) for 21 +/- 3 days after the Baseline Visit

7.2 On-Intervention Evaluations

7.2.1 Visit 01 (Week 3)

Visit 01/Week 3 will occur 21 ± 3 days from the Baseline Visit. The following procedures and evaluations are to be performed at Visit 01 (week 3):

- Review and document concomitant medications
- Assess and document AEs
- Obtain blood for safety lab tests: serum urate and chem 7 panel
- Obtain blood for safety lab test CBC with differential if subject treated with potassium citrate alkalinization therapy (at any time) since last visit
- Obtain urine for routine analysis/pH/sediment
- Review drug compliance on Study Drug Record with subject and re-educate as necessary
- Distribute additional Study Drug Record sheets
- Retrieve previously dispensed study drug/bottles and calculate compliance
- Dispense/re-dispense sufficient study drug to cover time to next visit
- Assess need for dopaminergic therapy
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II to subject for self-administration
- Schedule Visit 02 (week 6) for 42 +/-5 days after the Baseline Visit
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional Smart*4*SURE substudy
- Assist a consented Smart*4*SURE substudy subject in downloading and registering the Smart*4*SURE app on the subject's smartphone, and conduct an online training session via the app if not performed at a prior visit.

7.2.2 Post Study Visit Telephone Calls

Beginning with the Baseline Visit, serum urate test results will not be provided to sites. Instead the serum urate result will only be provided to an unblinded DCC Programmer and used in an algorithm to determine if a subject's study drug should be increased, decreased, or remain steady. (See Sec. 5.1.2.3)

If serum urate level warrants altering capsule dosing, the DCC will notify both the Site Investigator and Coordinator by email within 3 days of the visit. The Site Investigator or Coordinator should immediately notify the subject and update the Dose Management Log. Dosage increases, reductions, or holds should be initiated as soon as the subject is notified.

If the most recent urine pH/sediment results trigger the initiation or change of alkalinization therapy, then the Site Investigator or Coordinator should immediately notify the subject on how to begin or modify use of potassium citrate (or an alternative alkalinizing agent) according to the alkalinization algorithm in Sec. 8.4.2.

A follow-up phone call will take place after each study visit once the site is informed of a dosage change. The following will be performed and documented during the call:

- Inform the subject to either increase, reduce or hold their study drug

7.2.3 Visit 02 (Week 6)

Visit 02/Week 6 will occur 42 ± 5 days from the Baseline Visit. The following procedures and evaluations are to be performed at Visit 02 (Week 6):

- Review and document concomitant medications
- Assess and document AEs

- Obtain blood for safety lab tests: serum urate and chem 7 panel
- Obtain blood for safety lab test CBC with differential if subject treated with potassium citrate alkalinization therapy (at any time) since last visit
- Obtain urine for routine analysis/pH/sediment
- Review drug compliance on Study Drug Record with subject and re-educate as necessary
- Distribute additional Study Drug Record sheets
- Review and update Dose Management Log as necessary
- Retrieve previously dispensed study drug/bottles and calculate compliance
- Dispense/re-dispense sufficient study drug to cover time to next visit
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II to subject for self-administration
- Administer MDS-UPDRS Part IV, if dopaminergic therapy was previously initiated during study
- Assess blindedness to treatment assignment (Site Investigator, Coordinator, and subject), unless previously assessed during an Unscheduled Visit due to drug discontinuation.
- Schedule Visit 03 (week 12) for 84 +/-7 days after the Baseline Visit
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional Smart*4*SURE substudy
- Assist a consented Smart*4*SURE substudy subject in downloading and registering the Smart*4*SURE app on the subject's smartphone, and conduct an online training session via the app if not performed at a prior visit.

7.2.4 Visit 03 (Week 12)

Visit 03/Week 12 will occur 84 ± 7 days from the Baseline Visit. The following procedures and evaluations are to be performed at Visit 03 (week 12):

- Review and document concomitant medications
- Assess and document AEs
- Obtain weight and orthostatic vital signs (blood pressure, heart rate)
- Obtain blood for safety lab tests: serum urate, chem 7 panel, LFTs, TSH, lipids, CBC with differential
- Obtain urine for routine analysis/pH/sediment
- Review drug compliance on Study Drug Record with subject and re-educate as necessary
- Distribute additional Study Drug Record sheets
- Review and update Dose Management Log as necessary
- Retrieve previously dispensed study drug/bottles and calculate compliance
- Dispense/re-dispense sufficient study drug to cover time to next visit
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II, RBD, depression module of the Neuro-QOL, and PDQ-39 questionnaires to subject for self-administration
- Administer MDS-UPDRS Part IV, if dopaminergic therapy was previously initiated during study
- Assess Modified Hoehn and Yahr Scale
- Assess Modified Schwab & England Activities of Daily Living
- Administer Montreal Cognitive Assessment (MoCA)
- Schedule Visit 04 (month 6) for 180 +/-7 days after the Baseline Visit
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional Smart*4*SURE substudy

- Assist a consented Smart*4*SURE substudy subject in downloading and registering the Smart*4*SURE app on the subject's smartphone, and conduct an online training session via the app if not performed at a prior visit.

7.2.5 Visit 04 (Month 6)

Visit 04/Month 6 will occur 180 ± 7 days from the Baseline Visit. The following procedures and evaluations are to be performed at Visit 04 (month 6):

- Review and document concomitant medications
- Assess and document AEs
- Obtain blood for safety lab tests: serum urate and chem 7 panel
- Obtain blood for safety lab test CBC with differential if subject treated with potassium citrate alkalinization therapy (at any time) since last visit
- Obtain urine for routine analysis/pH/sediment
- Review drug compliance on Study Drug Record with subject and re-educate as necessary
- Distribute additional Study Drug Record sheets
- Review and update Dose Management Log as necessary
- Retrieve previously dispensed study drug/bottles and calculate compliance
- Dispense/re-dispense sufficient study drug to cover time to next visit
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II to subject for self-administration
- Administer MDS-UPDRS Part IV, if dopaminergic therapy was previously initiated during study
- Schedule Visit 05 (month 9) for 270 +/-7 days after the Baseline Visit
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional Smart*4*SURE substudy
- Assist a consented Smart*4*SURE substudy subject in downloading and registering the Smart*4*SURE app on the subject's smartphone, and conduct an online training session via the app if not performed at a prior visit.

7.2.6 Visit 05 (Month 9)

Visit 05/Month 9 will occur 270 ± 7 days from the Baseline Visit. The following procedures and evaluations are to be performed at Visit 05 (month 9):

- Review and document concomitant medications
- Assess and document AEs
- Obtain blood for safety lab tests: serum urate and chem 7 panel
- Obtain blood for safety lab test CBC with differential if subject treated with potassium citrate alkalinization therapy (at any time) since last visit
- Obtain urine for routine analysis/pH/sediment
- Review drug compliance on Study Drug Record with subject and re-educate as necessary
- Distribute additional Study Drug Record sheets
- Review and update Dose Management Log as necessary
- Retrieve previously dispensed study drug/bottles and calculate compliance
- Dispense/re-dispense sufficient study drug to cover time to next visit
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II to subject for self-administration
- Administer MDS-UPDRS Part IV, if dopaminergic therapy was previously initiated during study

- Distribute container for 24-hour urine collection at home and instruct subject in collection/storage procedures
- Schedule Visit 06 (month 12) for 360 +/-7 days after the Baseline Visit
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional Smart*4*SURE substudy
- Assist a consented Smart*4*SURE substudy subject in downloading and registering the Smart*4*SURE app on the subject's smartphone, and conduct an online training session via the app if not performed at a prior visit.

7.2.7 Pre-V06 Telephone Call

A follow-up phone call will take place 2-3 days BEFORE V06 to remind the subject of the following:

- Remind the subject to collect the 24-hour urine on the day before the V06 visit, and to bring the container to the visit

7.2.8 Visit 06 (Month 12)

Visit 06/Month 12 will occur 360 ± 7 days from the Baseline Visit. The following procedures and evaluations are to be performed at Visit 06 (month 12):

- Review and document concomitant medications
- Assess and document AEs
- Obtain weight and orthostatic vital signs (blood pressure, heart rate)
- Conduct Electrocardiogram (12-lead ECG)
- Obtain blood for safety lab tests: serum urate and chem 7 panel
- Obtain blood for safety lab test CBC with differential if subject treated with potassium citrate alkalinization therapy (at any time) since last visit
- Obtain urine for routine analysis/pH/sediment
- Retrieve 24-hour urine container from subject and process urine sample
- Review drug compliance on Study Drug Record with subject and re-educate as necessary
- Distribute additional Study Drug Record sheets
- Review and update Dose Management Log as necessary
- Retrieve previously dispensed study drug/bottles and calculate compliance
- Dispense/re-dispense sufficient study drug to cover time to next visit
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II, RBD, depression module of the Neuro-QOL, and PDQ-39 questionnaires to subject for self-administration
- Administer MDS-UPDRS Part IV, if dopaminergic therapy was previously initiated during study
- Assess Modified Hoehn and Yahr Scale
- Assess Modified Schwab & England Activities of Daily Living
- Administer Montreal Cognitive Assessment (MoCA)
- Schedule Visit 07 (month 15) for 450 +/-7 days after the Baseline Visit
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional Smart*4*SURE substudy
- Assist a consented Smart*4*SURE substudy subject in downloading and registering the Smart*4*SURE app on the subject's smartphone, and conduct an online training session via the app if not performed at a prior visit.
- For an interested, eligible subject, obtain written informed consent for the optional DAT scan substudy

7.2.9 Visit 07 (Month 15)

Visit 07/Month 15 will occur 450 ± 7 days from the Baseline Visit. The following procedures and evaluations are to be performed at Visit 07 (month 15):

- Review and document concomitant medications
- Assess and document AEs
- Obtain blood for safety lab tests: serum urate and chem 7 panel
- Obtain blood for safety lab test CBC with differential if subject treated with potassium citrate alkalinization therapy (at any time) since last visit
- Obtain urine for routine analysis/pH/sediment
- Review drug compliance on Study Drug Record with subject and re-educate as necessary
- Distribute additional Study Drug Record sheets
- Review and update Dose Management Log as necessary
- Retrieve previously dispensed study drug/bottles and calculate compliance
- Dispense/re-dispense sufficient study drug to cover time to next visit
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II to subject for self-administration
- Administer MDS-UPDRS Part IV, if dopaminergic therapy was previously initiated during study
- Schedule Visit 08 (month 18) for 540 +/-7 days after the Baseline Visit
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional Smart*4*SURE substudy
- Assist a consented Smart*4*SURE substudy subject in downloading and registering the Smart*4*SURE app on the subject's smartphone, and conduct an online training session via the app if not performed at a prior visit.
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional DAT scan substudy

7.2.10 Visit 08 (Month 18)

Visit 08/Month 18 will occur 540 ± 7 days from the Baseline Visit. The following procedures and evaluations are to be performed at Visit 08 (month 18):

- Review and document concomitant medications
- Assess and document AEs
- Obtain blood for safety lab tests: serum urate and chem 7 panel
- Obtain blood for safety lab test CBC with differential if subject treated with potassium citrate alkalinization therapy (at any time) since last visit
- Obtain urine for routine analysis/pH/sediment
- Review drug compliance on Study Drug Record with subject and re-educate as necessary
- Distribute additional Study Drug Record sheets
- Review and update Dose Management Log as necessary
- Retrieve previously dispensed study drug/bottles and calculate compliance
- Dispense/re-dispense sufficient study drug to cover time to next visit
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II to subject for self-administration
- Administer MDS-UPDRS Part IV, if dopaminergic therapy was previously initiated during study
- Schedule Visit 09 (month 21) for 630 +/-7 days after the Baseline Visit

- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional Smart*4*SURE substudy
- Assist a consented Smart*4*SURE substudy subject in downloading and registering the Smart*4*SURE app on the subject's smartphone, and conduct an online training session via the app if not performed at a prior visit.
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional DAT scan substudy

7.2.11 Visit 09 (Month 21)

Visit 09/Month 21 will occur 630 ± 7 days from the Baseline Visit. The following procedures and evaluations are to be performed at Visit 09 (month 21):

- Review and document concomitant medications
- Assess and document AEs
- Obtain blood for safety lab tests: serum urate and chem 7 panel
- Obtain blood for safety lab test CBC with differential if subject treated with potassium citrate alkalinization therapy (at any time) since last visit
- Obtain urine for routine analysis/pH/sediment
- Review drug compliance on Study Drug Record with subject and re-educate as necessary
- Distribute additional Study Drug Record sheets
- Review and update Dose Management Log as necessary
- Retrieve previously dispensed study drug/bottles and calculate compliance
- Dispense/re-dispense sufficient study drug to cover time to next visit
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II to subject for self-administration
- Administer MDS-UPDRS Part IV, if dopaminergic therapy was previously initiated during study
- Schedule Visit 10 (Month 24) for 720 +/-7 days after the Baseline Visit
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional Smart*4*SURE substudy
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the Smart*4*SURE substudy Assist a consented Smart*4*SURE substudy subject in downloading and registering the Smart*4*SURE app on the subject's smartphone, and conduct an online training session via the app if not performed at a prior visit.
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional DAT scan substudy

7.2.12 Visit 10 (Month 24)

Visit 10/Month 24 will occur 720 ± 7 days from the Baseline Visit. The following procedures and evaluations are to be performed at Visit 24 (month 24):

- Review and document concomitant medications
- Assess and document AEs
- Obtain weight and orthostatic vital signs (blood pressure, heart rate)
- Conduct Electrocardiogram (12-lead ECG)
- Obtain blood for safety lab tests: serum urate, chem 7 panel, LFTs, TSH, lipids, CBC with differential
- If the subject has given consent, obtain extra blood sample for (plasma) banking unless one was obtained previously during an Unscheduled Visit due to drug discontinuation
- Obtain urine for routine analysis/pH/sediment

- Retrieve previously dispensed study drug/bottles and calculate compliance (Note: The last dose of study drug will have been taken on the day prior to Visit 10. No study drug is to be taken during or after the visit.)
- Record last dosing on Dose Management Log
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II, RBD, depression module of the Neuro-QOL, and PDQ-39 questionnaires to subject for self-administration
- Administer MDS-UPDRS Part IV, if dopaminergic therapy was previously initiated during study
- Assess Modified Hoehn and Yahr Scale
- Assess Modified Schwab & England Activities of Daily Living
- Assess blindedness to treatment assignment (Site Investigator, Coordinator, and subject) unless previously assessed during an Unscheduled Visit due to drug discontinuation
- Administer Montreal Cognitive Assessment (MoCA)
- For an interested, eligible subject who has not already enrolled, obtain written informed consent for the optional DAT scan substudy

During this visit the following will be scheduled with the subject:

- Telephone Evaluation TE1 (Month 25) for 30 +/-3 days after the current V10 visit
- Telephone Evaluation TE2 (Month 26) for 60 +/-3 days after the current V10 visit
- Safety Visit SV (Month 27) for 90 +/-3 days after the current V10 visit
- Optional DAT scan DS2 (Month 25-26) for 45+/-15 days after the current visit.

7.3 On Study/Off-Intervention Evaluations

7.3.1 Optional DAT scan (DS2; Month 25-26)

For subjects who have consented to the optional DAT scan substudy, who have been on study drug through at least the 1-year visit (V06), and who have not taken any drug disallowed within 90 days prior to DS2 (see Sec. 6.2.11.2), visit DS2 will occur 45+/-15 days after the V10 visit, and before the Safety Visit (SV).

The DAT scan process will be conducted at a qualified neuroimaging center. Results will be analyzed by the study imaging core.

7.3.2 Telephone Evaluation 1 (TE1; Month 25)

Visit TE1/Month 25 will occur 30 +/-3 days after the current V10 visit. The following procedures and evaluations are to be performed via the TE1 Telephone evaluation (month 25):

- Review and document concomitant medications
- Assess and document AEs
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)

7.3.3 Telephone Evaluation 2 (TE2; Month 26)

Visit TE2/Month 26 will occur 60 +/-3 days after the current V10 visit. The following procedures and evaluations are to be performed via the TE2 Telephone evaluation (month 26):

- Review and document concomitant medications
- Assess and document AEs
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)

7.4 Final On-Study Evaluations

7.4.1 Safety Visit (SV; Month 27)

Visit SV/Month 27 will occur 90 +/-3 days after the current V10 visit (810 ± 10 days from the Baseline Visit). The following procedures and evaluations are to be performed at the SV visit (month 27):

- Review and document concomitant medications
- Assess and document AEs
- Obtain weight and orthostatic vital signs (blood pressure, heart rate)
- Obtain blood for safety lab tests: serum urate and chem 7 panel
- If the subject has given consent, obtain extra blood sample for (plasma) banking.
- Obtain urine for routine analysis/pH/sediment
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II, RBD, Neuro-QOL and PDQ-39 questionnaires to subject for self-administration
- Administer MDS-UPDRS Part IV, if dopaminergic therapy was previously initiated during study
- Assess Modified Hoehn and Yahr Scale
- Assess Modified Schwab & England Activities of Daily Living
- Assess diagnostic features and PD diagnosis
- Administer Montreal Cognitive Assessment (MoCA)
- Inform the subject – if participating the Smart*4*SURE substudy – that s/he should complete a final smartphone Activity Session and Survey set within one month of the SV (if s/he has not already completed them in the past month).
- Complete the Conclusion of Study Participation eCRF

7.5 On-Study/Non-Standard Evaluations

7.5.1 Unscheduled Visit (Visit U01, U02, etc.)

An unscheduled visit may be performed at any time during the study at the subject's request or as deemed necessary by the Site Investigator. The date and reason for the unscheduled visit should be recorded in the source documentation.

In most cases, an unscheduled visit will be warranted due to an AE, significantly abnormal lab values, permanent discontinuation of study drug before 24 month visit, initiation of treatment with a thiazide 1-4 weeks earlier, or need for an in-person assessment for possible initiation of dopaminergic therapy.

The following procedures and evaluations at a minimum are to be performed at an Unscheduled Visit:

- Review and document concomitant medications
- Assess and document AEs
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Obtain serum urate for blinded central analysis if subject initiated thiazide treatment 1-4 weeks earlier, or if otherwise warranted based on the Site Investigator's judgment
- Update the Dose Management Log as needed

If the reason for the unscheduled visit is that the participant is permanently discontinuing drug at the visit, whether by choice, or for reasons concerning the health or well being of the subject, the following additional procedures and evaluations should be completed:

- Assess blindedness to treatment assignment (Investigator, Coordinator and subject)

- Retrieve all previously dispensed study drug and calculate compliance (Note: If study drug has not already been discontinued and there is no medical reason to warrant otherwise, the final dose will be taken when the last dose of the day is normally taken on the day prior to the visit . No study drug is to be taken during or after the study visit.)
- Record last dosing on Dose Management Log
- If participant consented to the optional future biomarker study a blood sample will be taken (please see section 6.2.14 for information about altered sample collection schedule)

In addition as warranted by the purpose of the unscheduled visit, the following procedures and evaluations may be performed at the discretion of the Site Investigator:

- Obtain weight and orthostatic vital signs (blood pressure, heart rate)
- Obtain blood and/or urine for additional study chemistry or hematology tests, or other laboratory testing if warranted based on the Site Investigator's judgment

7.5.2 Discontinuation of In-Person Follow-Up Visit (Visit DF)

The Discontinuation of In-person Follow-up (DF) Visit should be conducted at the time the decision to stop in-person follow-up is made or as soon thereafter as possible. The DF Visit (and subsequent Safety Visit) should be conducted whether this decision is made when drug is discontinued or later after some visits off drug have already occurred.

The following procedures and evaluations are to be performed at the Discontinuation of In-person Follow-up Visit:

- Review and document concomitant medications
- Assess and document AEs
- Obtain weight and orthostatic vital signs (blood pressure, heart rate)
- Conduct Electrocardiogram (12-lead ECG)
- Obtain blood for safety lab tests: serum urate, chem 7 panel, LFTs, TSH, lipids, CBC with differential
- Obtain urine for routine analysis/pH/sediment
- Retrieve previously dispensed study drug/bottles and calculate compliance (Note: The last dose of study drug will have been taken on the day prior to visit or earlier. No study drug is to be taken during or after the visit.)
- Record last dosing on Dose Management Log
- Assess need for dopaminergic therapy (if need has not been determined at a previous visit)
- Administer MDS-UPDRS Part IA and III
- Provide MDS-UPDRS Parts IB and II, RBD and PDQ-39 questionnaires to subject for self-administration
- Administer MDS-UPDRS Part IV, if dopaminergic therapy was previously initiated during study
- Assess Modified Hoehn and Yahr Scale
- Assess Modified Schwab & England Activities of Daily Living
- Assess diagnostic features and PD diagnosis
- Assess blindedness to treatment assignment (Site Investigator, Coordinator, and subject)
- Administer Montreal Cognitive Assessment (MoCA)
- Review with the subject the plan for quarterly telephone visits (see Sec. 7.5.3)

Call the CCC within 24 hours to report the discontinuation of in-person follow-up

Note: If a subject who permanently discontinues study participation via in-person visits also declines a Safety Visit and also declines future telephone visits, then the Conclusion of Study Participation eCRF should be completed at the Discontinuation of In-person Follow-up Visit and the premature withdrawal must be reported to the CCC at the same time that the permanent discontinuation of in-person follow-up

is reported. In addition, any AE that remains active at the Discontinuation of In-person Follow-up Visit for such a subject must be followed for 30 days or until resolved or stabilized, whichever occurs first.

7.5.3 Telephone Visits after Permanent Discontinuation of In-Person Follow-Up

For any subject who permanently discontinues in-person follow-up prior to Month 24, study participation will continue via telephone visits. Calls from the Site Investigator or Coordinator will occur during the pre-established study visit windows for all remaining study visits through Month 27.

The purpose of these telephone visits will be to:

1. Review any AEs that have occurred since the last visit or phone call
2. Review and document concomitant medications used during this time period
3. Assess need for dopaminergic therapy (if need has not been determined at a previous visit)

7.6 Premature Study Withdrawal

The following events will be considered a premature study withdrawal, and the CCC must be notified of such within 24 hours of the Site Investigator's awareness of the following:

- A subject who has completed 24 months on study drug does not complete the Month 27 Safety Visit.
- A subject who is participating in the study via in-person visits discontinues those visits prior to his/her Month 24 visit and declines to continue study participation via telephone visits.
- A subject who is participating in the study via telephone visits discontinues prior to his/her Month 27 call.

The following will NOT be considered a premature study withdrawal:

- A subject permanently discontinues study drug but does continue participation in the study via in-person visits.
- A subject permanently discontinues study drug and does not complete a Discontinuation of In-Person Follow-up Visit and/or a Safety Visit, but does continue participation in the study via telephone visits.

Each of these cases will be considered a reportable drug discontinuation but not a reportable premature withdrawal. The non-conducted Discontinuation of In-Person Follow-up, and/or Safety visit(s) will be recorded as missed visit(s).

7.7 Schedule of Activities

ACTIVITY	SCREEN			TREATMENT (Period 1)											WASHOUT (Period 2)				UNPLANNED VISITS			
				Wash-in			Maintenance															
Day Interval	- 60 to - 13	- 58 to - 11	- 52 to - 5	00	Week 3 21 ± 3	Week 6 42 ± 5	Week 12 84 ± 7	Month 6 180 ± 7	Month 9 270 ± 7	Month 12 360 ± 7	Month 15 450 ± 7	Month 18 540 ± 7	Month 21 630 ± 7	Month 24 720 ± 7	Month 25 V10 + 30 ± 3	Month 25-26 V10 + 45 ± 15	Month 26 V10 + 60 ± 3	Month 27 V10 + 90 ± 3	n/a	n/a	n/a	n/a
Visit#/code	SC1	SC2	DS1[6]	BL[6]	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	TE1	DS2[7]	TE2	SV	U01, U02, etc.	DF	T(vst #) e.g.,T06	PW
Written Informed Consent	x																					
Consent for Optional Blood Sample Collection/Storage/Future Use[1]	±																					
Smart eSURE Consent, Registration, & Training[1]		±	±	±	±	±	±	±	±	±	±	±	±									
Consent for 2nd DAT Scan[1]										±	±	±	±	±								
Assign Subject ID Number	x																					
Screening/Demographics	x																					
Socio-Economics				x																		
PD Risk Factors				x																		
Smoking, Alcohol and Caffeine Status				x																		
Inclusion/Exclusion	x	x		x																		
Medical History General		x																				
Vital Signs (orthostatics & weight)		x		x			x			x				x				x	±	x		
Height				x																		
Electrocardiogram		x								x				x						x		
General Neuro & Medical Exams		x																				
Blood Draw ---> Serum Urate[2]	x	x		x	x	x	x	x	x	x	x	x	x	x				x	±	x		
Blood Draw ---> Chem 7 Panel[2]		x		x	x	x	x	x	x	x	x	x	x	x				x	±	x		
Blood Draw ---> LFTs, TSH and Lipids[2]				x			x							x					±	x		
Blood Draw ---> CBC[2]				x	±	±	x	±	±	±	±	±	±	x					±	x		
Urine Collection ---> Analysis/Sediment[2]	x			x	x	x	x	x	x	x	x	x	x	x				x	±	x		
Blood Draw ---> Biomarker Study[1]				±										±				±	±			
Most Revent Food Intake, Study Drug and PD Medication				x	x	x	x	x	x	x	x	x	x	x								
1st DAT Scan[1]			±																			
2nd DAT Scan[1]																±						
24 Hr Urine Collection: Process/Ship				x						x												
Pregnancy Form[3]		x																				
Mini-Mental State Exam		x																				
PD Features		x																				
Diagnostic Features - PD																		x		x		
Primary Diagnosis																		x		x		
MDS-UPDRS Parts I-III		x		x	x	x	x	x	x	x	x	x	x	x				x		x		
MDS-UPDRS Part IV (as warranted)						±	±	±	±	±	±	±	±	±				x		x		
Modified Hoehn & Yahr		x		x			x			x				x				x		x		
Modified Schwab & England ADL and PDQ-39				x			x			x				x				x		x		✓
NeuroQOL		x																x				
Depression module of NeuroQOL				x			x			x				x								
MoCA (cogntion test)				x			x			x				x				x		x		
RBD Question	x			x			x			x				x				x		x		
Assess Need for Dopaminergic Therapy[4]				x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	
Concomitant Medication Log		x		x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	
Adverse Event Log[5]		x		x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	
Randomization to Med Assign				x																		
Dose Management Log				x	x	x	x	x	x	x	x	x	x	x					x	x		
Study Drug Dispensing/Return Log				x	x	x	x	x	x	x	x	x	x	x						x		
PD-EQ				x																		
Blindedness Questionnaire						x								x					±	x		
Post Visit Telephone Call Regarding Eligibility and DAT Scan	x	x	x																			
Post Visit Telephone Call to Adjust Dose					x	x	x	x	x	x	x	x	x									
Conclusion of Study Participation																		x				x

[1] Optional (opt-in) study

[2] Safety lab samples sent to central laboratory

[3] For women of child-bearing potential; repeated at other times if clinically indicated

[4] Once need for dopaminergic therapy has been determined, no longer complete the form at future visits

[5] For subjects that discontinue study participant prior to Month 27, AEs active at the time the subject discontinues participation should be followed for 30 days post discontinuation or until resolved or stablized (whichever comes first) as long as the subject agrees to the follow-up.

[6] DAT scan may not be conducted until SC lab results are reviewed; and Baseline visit may not be conducted until SC lab results and DAT scan results are reviewed

[7] End of study DAT scan will only be conducted with subjects who have consented to the additional DAT scan.

± = Optional as specified in protocol

8. MANAGEMENT OF ADVERSE EXPERIENCE

Chronic oral inosine treatment poses small but likely increased risks of gout and urolithiasis because inosine here is intended and expected to elevate serum urate levels significantly. A less substantiated but important possible risk of elevating urate is cardiovascular disease (CVD), based on an association in some studies between urate levels and the risk of CVD. Accordingly, precautions at the levels of subject selection, monitoring, prophylaxis, and/or treatment have been included to mitigate the risk of potential adverse effects.

8.1 Adverse Event / Experience (AE) Definition

An adverse event or experience is any symptom, sign, illness, or event that develops or worsens during the course of the study, whether or not the experience is considered related to study drug. Some examples of AEs are:

- Acute gout, uric acid urolithiasis or any other medical problem to which higher urate levels are known to contribute
- A myocardial or cerebral infarction, or any other medical problem with which higher urate levels have been associated without clear evidence of causality
- A change, excluding minor fluctuations, in the nature, severity, frequency, or duration of a pre-existing condition
- Development of an intercurrent illness during the study
- Development of symptoms that may or may not be related to the use of a concomitant medication or study drug
- Appearance of abnormal laboratory results, or substantial shifts of lab values within the reference ranges that the Site Investigator considers clinically important.

Worsening of a PD symptom is to be recorded as an AE only when there is a change in nature, severity, or frequency of that Parkinson's disease symptom beyond what the Site Investigator expects is within the normal range of fluctuation for that subject.

8.2 Serious Adverse Events / Experiences (SAEs)

A serious adverse drug experience or SAE is defined as any adverse event or experience that occurs at any dose that results in any of the following outcomes:

- Death;
- A life-threatening adverse event or experience;
- Inpatient hospitalization or prolongation of existing hospitalization;
- A persistent or significant disability/incapacity; or
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include (but are not limited to) allergic bronchospasm requiring intensive treatment in an emergency room or at home, convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

This category also includes any event the Site Investigator or the Medical Safety Monitor judges to be serious or that would suggest a significant hazard, contraindication, side effect or precaution. It can also involve the withdrawal of a subject from study drug due to abnormal lab values, excluding Screening Visit lab results.

8.3 Recording of Adverse Events / Experiences

The site study staff will assess AEs at each subject visit by recording all voluntary complaints of the subject and by assessment of clinical features. At each study visit, the subject should be questioned directly regarding the occurrence of any AE since their last visit.

All AEs whether observed by the Site Investigator and/or Coordinator, elicited from or volunteered by the subject, and whether ascribed to the study drug or not, should be recorded on the eCRF AE Log and in the subject's clinical chart. The following information is gathered on the Log: event term, onset date, resolution date if any, whether serious or not, severity level, action taken with study drug, outcome, and the Site Investigator's opinion of the possible relationship between the AE and the study drug or participation in the study. If a subject is withdrawn from drug or the study because of an AE, it must be recorded on the eCRF as such.

All serious adverse events/experiences, as defined above, must be reported to the Medical Safety Monitor and the CCC within 24 hours of the site's awareness, whether or not the Site Investigator feels that the experience is related to study drug, or was expected. Within that 24 hour period the Site Investigator must also complete a SURE-PD3 SAE Report Form and e-mail or fax it to the CCC.

For AE or significant abnormal result that is identified during a visit, telephone call or other study activity, the site staff should ensure that the information is available to the subject and his or her primary care physician. As appropriate and permitted by the subject, the primary care physician should be notified by the research staff via written correspondence for further follow-up. Should the result affect the subject's immediate safety (e.g., severe depressed mood, significant out of range laboratory value), appropriate, immediate action should be taken by the Site Investigator to ensure the subject's well-being.

8.3.1 Adverse Event/Experience Causality Definitions

Table 6: Causality Definitions for Adverse Events/Experiences in SURE-PD3

TERM	DEFINITION	EXPLANATION
Unrelated	No possible causation	The temporal relationship between drug exposure and the adverse experience (AE) onset/course is unreasonable or incompatible, or a causal relationship is implausible.
Unlikely	Not reasonably related, although a causal relationship cannot be ruled out	Although the temporal relationship between drug exposure and the onset/course of the AE does not preclude causality, there is a clear alternate cause that is more likely to have caused the adverse event than the study drug.
Possibly	Causal relationship uncertain	The temporal relationship between drug exposure and the onset or course of the AE is reasonable or unknown, dechallenge or rechallenge information is either unknown or equivocal, and although other potential causes may not exist, a causal relationship to the study drug does not appear probable.
Probably	High degree of certainty for causal relationship	The temporal relationship between drug exposure and the onset or course of the AE is reasonable. There is a clinically compatible response to dechallenge (rechallenge is not required), and other causes have been eliminated or are unlikely.
Definite	Causal relationship certain	The temporal relationship between drug exposure and the onset or course of the AE is reasonable, there is a clinically compatible response to dechallenge, other causes have been eliminated, and the event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

For each adverse event or experience, the relationship to the study drug must be recorded on the AE Log as one of the defined in Table 6.

8.3.2 Adverse Event/Experience Severity Definitions

The severity of each adverse event or experience must be recorded as one of the following on the AE Log:

Mild	No limitation of usual activities
Moderate	Some limitation of usual activities
Severe	Inability to carry out usual activities

8.4 Known Adverse Event / Experience Prophylaxis Plan

8.4.1 Monitoring of Urine pH and Uric Acid Crystalluria

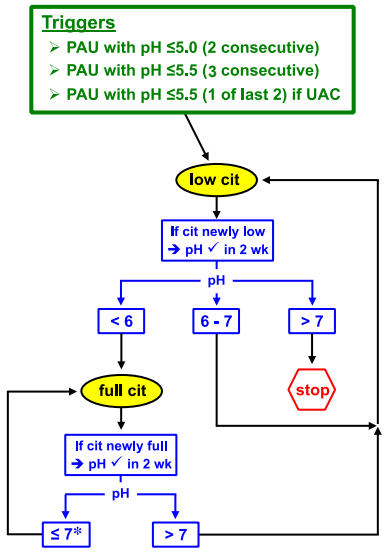
Acidic urine is a major, modifiable risk factor for uric acid urolithiasis, which form from uric acid crystals. Thus microscopic uric acid crystals in the urine (uric acid crystalluria; UAC) are an intuitive additional risk factor for these stones. Our experience in SURE-PD provides empiric evidence that identification of uric acid crystalluria by a central laboratory in the setting of low urine pH can in fact provide a useful further indication of increased risk of uric acid urolithiasis. Accordingly, urine samples will be collected at all on-treatment site visits for central laboratory urinalysis to determine pH and the presence of UAC, in a blinded manner. (See Schedule of Activities, Sec. 7.7.) Both risk factors will be integrated into the following algorithm for triggering urine alkalinization therapy for uric acid stone prophylaxis.

8.4.2 Algorithm for Triggering Urine Alkalinization Method

Based on the premise that repeated measures of urinary risk factors for stone formation will be more reliable than a single measure,[194,195] persistently acidic urine (PAU) will trigger alkalinization treatment with potassium citrate (or an alternative means of urine alkalinization were potassium citrate [51] to be contra-indicated) according to the schematized algorithm of Fig. 12. The threshold for alkalinization in response to PAU is lowered by greater urine acidity (i.e., the lowest pH values), by greater persistence of lower values, and by the presence of UAC.

Figure 12.

Urine Alkalinization Flow Chart



PAU = persistently acidic urine
UAC = uric acid crystals present
pH ✓ in 2 wk (using home urine sample shipped to central lab) and at next visit (when serum electrolytes, BUN, creatinine and CBC will also be checked as safety labs on cit).

low cit = lower-dose potassium citrate**
= 10 mEq tid (<70 kg) or qid (≥ 70 kg)
full cit = fuller-dose potassium citrate
= 20 mEq tid (<70 kg) or qid (≥ 70 kg)
stop = discontinue potassium citrate

* If pH <6 consecutively on **full cit** then reassess compliance and consult with Medical Monitor to consider increase up to 100 mEq/day potassium citrate in divided doses (tid or qid), or trial of an alternative alkalinizing agent such as sodium bicarbonate if cit not tolerated.

** Alternative, non-potassium alkalinizing agents such as sodium bicarbonate should be used in lieu of potassium citrate in those who have a history of peptic ulcer disease, are taking an anticholinergic agent (e.g., trihexyphenidyl), have another contraindication to potassium citrate, or do not tolerate it.

- **Alkalinization Triggers**
  - PAU with lowest urine pH -- Although the lowest urine pH value of ≤5.0 on the initial screening visit (SC1) is an enrollment exclusion criterion, it may occur at the baseline visit or as a finding in any subsequent urine sample collected throughout the study. If it occurs in any two consecutive urine samples (first possible at the 3-week visit V01) it will trigger alkalinization per the algorithm outlined in Fig. 12.
  - PAU with sustained lower urine pH – Three consecutive study urine pH values of ≤5.5 will trigger alkalinization (as in Fig. 12), unless the DSMB or SC determine that fewer (two) or more (four) consecutive study urine pH values of ≤5.5 will trigger alkalinization to better balance the benefits and risks of alkalinization in the trial.

- PAU with lower urine pH and UAC – If UAC is identified in the sediment of a urine sample, than a urine pH value of ≤5.5 either in the same sample or in the sample from the prior urine sample will trigger alkalinization (as in Fig. 12).

- **Alkalinization Impact on Blinding**

  Subjects and site staff will be blinded to the specific urine pH and crystalluria test results to minimize unblinding to treatment assignment. Because urate-elevating inosine treatment does not have an appreciable effect on urine pH as demonstrated in SURE-PD, the rate of alkalinization due to low urine pH will be similar in placebo- and inosine-assigned subjects, also mitigating any unblinding effect. However, a difference in alkalinization rates may occur, because UAC is expected to occur primarily in inosine-treated subjects and would further lower the threshold for alkalinization (as above). Accordingly, if alkalinization is initiated in more inosine- than placebo-assigned participants then the threshold for initiating alkalinization would be altered in placebo participants in order to maintain similar frequencies of alkalinization across treatments. For example, if the number of participants in the inosine group who have initiated alkalinization exceeds that in the placebo group by 2 or >2, then the placecbo group alkalinization trigger for consecutive urine pH values ≤5.5 even in the absence of UAC would be 2 or 1, respectively (rather than 3 as in Fig. 12). Further, the DSMB will monitor rates of alkalinization, any alkalinization AEs and any urolithiasis AEs (in aggregate and by treatment assignment) and will periodically assess whether any change in the alkalinization protocol is warranted during the study.

- **Alkalinization Protocol**

  Site staff will receive automated notification from the DCC of the need to initiate alkalinization within a few days of the urinalysis that triggers the protocol by the above criteria. The following steps should then be taken:

  - Site staff will prescribe (via a central pharmacy) "lower-dose" potassium citrate, which depends on subject weight (as recorded most recently on a study visit):
    - Less than 70 kg: 10 mEq, in tablet form, po tid (with meals)
    - 70 kg or greater: 10 mEq, in tablet form, po qid (with meals and at bedtime)

    The potassium citrate prescription may be written for a one month supply of tablets that will cover dosing at either the lower or higher dose -- for efficiency given the possibility that the subject may double the dose (from lower to higher) within two weeks of initiation. Accordingly, recommended prescriptions depending on subject weight are:
    - [Less than 70 kg] 10 mEq tabs; # 180; sig: 1-2 po tid (with meals); refills: 5.
    - [70 kg or greater] 10 mEq tabs; # 240; sig: 1-2 po qid (with meals and at bedtime); refills: 5.

  - Staff will phone the subject to review the purpose, administration, potential adverse effects of potassium citrate, and plan rechecking urine pH in two weeks. In addition, staff will review recommendations for adequate hydration. The prescriber should review with the subject:
    - Any contraindications to potassium citrate, including a history of peptic ulcer disease, current use of an anticholinergic agent (e.g., trihexyphenidyl), hyperkalemia or renal insufficiency per most recent electrolyte measurements, or an active urinary tract infection.
      - If potassium citrate were deemed contraindicated (or later found insufficient) then site staff may work with the Medical Safety Monitor to employ an

alternative dosing schedule or an alternative alkalinizing agent such as sodium bicarbonate.

- Potential adverse effects of potassium citrate, including minor gastrointestinal complaints, such as abdominal discomfort, vomiting, diarrhea, loose bowel movements or nausea, and rarely gastrointestinal bleeding. The subject should be instructed to check with site staff or another doctor at once if tarry stools or other evidence of gastrointestinal bleeding is noticed.
- The possibility of abnormal results of blood lab test that will be monitored regularly on subsequent visits, and if this is the first prescription of potassium citrate at either a 10 mEq or the 20 mEq dose then in 5-10 days at an unscheduled visit.
- The importance of taking each dose faithfully,[91] and without crushing, chewing or sucking the tablet, and to check with physician if there is trouble swallowing tablets or if the tablet seems to stick in the throat. Subjects should not change dosing alkalinization medication until instructed to do so based on follow-up urinalysis results.
- Instructions for obtaining and mailing to the central lab a urine sample 2 weeks following initiating the alkalinization mediation (using a kit and mailer to be received by courier mail).

- Site staff will receive automated notification from the DCC within a few days of any home (and visit) urine sample analysis for a subject on alkalinization therapy. The notification will include the assumptions of what the subject is currently taking for alkalinization therapy (e.g., low or higher dose potassium citriate or the equivalent) and recommendation to continue on this dosing, to reduce/discontinue it, or to increase it (according to algorithm outlined in Fig. 12).
  - Staff will phone the subject to review the results, confirm the assumptions of the subject's dosing when the urine sample was collected, and make the indicated recommendation to continue on this dosing, to reduce/discontinue it, or to increase it.
    - For subjects on "lower-dose" potassium citrate who are instructed to increase it to "higher-dose" potassium citrate, they will be instructed to double their current dose. Depending on their weight:
      - less than 70 kg: 20 mEq, in tablet form, po tid (with meals)
      - 70 kg or greater: 20 mEq, in tablet form, po qid (with meals and at bedtime)
  - For any deviations from compliance with the medication site staff will contact the CCC who may engage the Medical Safety Monitor to obtain guidance.
  - Staff will review (as detailed above) the purpose, administration, potential adverse effects of potassium citrate, and plan for rechecking urine pH at home in two weeks if the subject is to increase or to reduce the alkalinization dose. Home recheck will not be performed if the subject is instructed to continue the current alkalinization dose or to discontinue it, or if the subject is scheduled for a site visit in the next two weeks.
- Subjects will be instructed to follow this algorithm for alkalinization for the duration of the treatment period of the trial. Any alkalinization therapy ongoing at the time of 24 month visit will be discontinued immediately after that visit (or at the time of study drug discontinuation if occurring earlier) irrespective of urine pH or sediment results at the visit.

8.4.3 Hyperkalemia and Other Laboratory Abnormalities

Current full prescribing information for potassium citrate[196] indicates that laboratory tests should be checked every 4 months for:

- Electrolytes (to monitor for potassium elevation)
- Creatinine (to monitor for renal disease, a risk factor for hyperkalemic complications)
- CBC (to monitor for anemia as an indicator of gastrointestinal bleeding due to mucosal lesions, which have been observed rarely on solid potassium treatments – at an estimated frequency of approximately 1 lesion per 100,000 patient-years).

Under the study protocol electrolytes and creatinine will be monitored even more frequently via routine laboratory testing no more than 3 months apart. A CBC will be added to the central lab tests in subjects treated with potassium citrate at any time since their last study visit.

8.4.3.1 Hyperkalemia

If hyperkalemia develops while a subject is taking potassium citrate, it should be suspended. Potassium testing should be repeated within 7 days of the suspension.

If serum potassium falls back into the normal range, then the potassium citrate will be resumed at a reduced dosage in an effort to find a lower dosage that does not produce hyperkalemia but on which urine pH remains at 5.5 or above.

If hyperkalemia remains, the potassium citrate will be discontinued. An alternative, non-potassium-based alkalinization measure[64] may be tried if the Medical Safety Monitor and Site Investigator believe such a measure is warranted. If a non-potassium-based alkalinization measure is not warranted, then the subject should discontinue study drug.

If urine pH remains <5.5 on potassium citrate at the maximum tolerated dosage (i.e., not producing hyperkalemia, and up to 100 mEq/day), or on a non-potassium-based-alkalinization measure, then the subject will discontinue study drug due to 'unmitigated PAU.'

8.4.3.2 Rise in Serum Creatinine or Fall in Hematocrit or Hemoglobin

If a significant rise in serum creatinine or a significant fall in blood hematocrit or hemoglobin develops while a subject is taking potassium citrate, it should be suspended and the source of the abnormality determined.

If these abnormalities are attributable to irreversible renal disease or to gastrointestinal bleeding, then the potassium citrate should not be resumed. Consideration may be given to an alternative, non-potassium-based alkalinization measure if the Medical Safety Monitor and Site Investigator believe such a measure is warranted.

If urine alkalinization cannot be resumed or an alternative cannot be instituted, then a urine sample will be sent for central laboratory pH testing within 2 weeks. If urine pH is found to be <5.5 then the subject will discontinue study drug due to 'unmitigated PAU'.

8.5 Known Adverse Event / Experience Management Plan

8.5.1 Urolithiasis

If urolithiasis occurs with symptoms of suggestive pain, hematuria or painless urolithiasis observed or if incidentally noted on imaging, the following steps should be undertaken:

- Seek prompt medical attention to diagnose and manage elimination of any stone(s)
- Medical evaluation and treatment (preferably at the time of symptom onset) should include assessment of
  - Serum urate measurement
  - Urinalysis for pH; and initiation of alkalinization therapy[64,91] as warranted
  - Urine sediment examination for crystalluria (and type of any crystals)
  - State of hydration assessment (by history and exam); and rehydration,[177, 178] as warranted
- Submit stone, if available, for chemical analysis
- Study drug plan:
  - Suspend study drug until medical condition is stabilized (including resolution of symptoms that may be due to urolithiasis)
  - Resume dosing if the analysis of the stone or associated sediment crystalluria indicates no uric acid or urate composition, or the subject is found not to have urolithiasis.
  - If the analysis of the stone or associated sediment crystalluria indicates uric acid or urate composition, or is indeterminant, then:
    - Resume dosing if a remediable contributor (e.g., unduly elevated serum urate, low urine pH and/or dehydration) can be reasonably expected to be better controlled.
    - Discontinue study drug if there is no evidence of remediable source or precipitant of urolithiasis (e.g., no serum urate level above targeted range, no urine pH <6.0, no dehydration)
    - Discontinue study drug if there is evidence of remediable source or precipitant of urolithiasis but subject prefers not to continue on drug.

8.5.2 Acute Gouty Arthritis

If gout-like acute arthritis develops, the following steps should be undertaken:

- Seek prompt medical attention to diagnose and treat
- Medical evaluation should include prompt serum urate level checks
- Study drug plan:
  - Suspend study drug during evaluation period
  - Resume drug if the diagnosis of gout is associated with a remediable above-range serum urate level that resolves within 4 weeks off study drug. Drug resumption must be accompanied by continuing close monitoring of serum urate.
  - Discontinue study drug if the diagnosis of gout is confirmed without a remediable above-range serum urate level.

8.6 Responsibilities of the Site Investigator for Reporting SAEs

- The Site Investigator or Coordinator should record all SAEs that occur during the study period on the AE Log and in the appropriate source documents.
- For the purposes of reporting SAEs, the study period is defined as the time when the subject signs the informed consent (1st Screening Visit) through Month 27 or in the event of a premature withdrawal, for 30 days following the premature withdrawal (if subject is willing).
- The Site Investigator or Coordinator should notify the CCC by telephone within 24 hours of becoming aware of the occurrence of an SAE. If calling after hours or on the weekend, a message will instruct the caller on how to reach the physician on-call. Upon completion of the telephone report, the CCC will enter the appropriate subject information into the Incident Module. Upon completion of

entry of the incident, an immediate notification will be disseminated by email to the Steering Committee (including PI and Co-PI), DCC PI and project staff, Medical Safety Monitor, site monitoring staff, CCC project staff, CCC Biostatistics staff, and DSMB if desired.

- The following information should be supplied if available, and as applicable, at the time of the telephone call: site number, subject ID number, subject age and gender, date of randomization, study drug start date, whether study drug dosage has been reduced or discontinued, date of last study drug dose, date of onset of event, event description, whether event required treatment, death and autopsy report, an identification of which criteria for an SAE have been met, the Site Investigator's current opinion of the relationship between the event and the study drug and/or study participation.
- The Site Investigator and Coordinator will complete the SURE-PD3 SAE Report Form and update the AE, concomitant medications and dose management eCRFs and e-mail or fax the SAE Report Form to the CCC Project Manager within 24 hours of the site's becoming aware of the SAE. A SURE-PD3 SAE Report Form must be completed for all SAEs regardless of causality or expectedness.
- The Medical Safety Monitor will review the SAE incident report and SURE-PD3 SAE Report Form and related database entries and confer with the Principal Investigator and/or the Site Investigator and/or Coordinator as necessary.
- The Principal Investigator or designee will report the SAE to regulatory authorities and the grantor (NINDS program staff) as applicable.
- The Site Investigator must also comply with the site's local Institutional Review Board (IRB) regulations regarding the reporting of AEs.
- If follow-up information is required, e.g., hospital discharge report, the Site Investigator and/or Coordinator will provide it to the CCC in a timely manner.

8.7 Follow-Up of Unresolved AEs and SAEs

- All AEs on-going at the time the subject discontinues study drug use must be followed until the Month 27 Visit or until resolved or stabilized, whichever occurs first. In the event a subject prematurely withdraws from the study, AEs should be followed for 30 day post study withdrawal (if subject is willing). SAEs may need to be followed beyond the 30 day period as requested by the Principal Investigator or Medical Safety Monitor.
- Follow-up may involve the Site Investigator's or Coordinator's contacting other clinicians responsible for the subject's care to obtain information on diagnoses, investigations performed and treatment given.

8.8 Emergency Actions

Inosine is widely available as a dietary supplement. It appears to be very well tolerated in the short-term,[48-50] and generally well tolerated in the long term when the only consistently reported adverse effects have been kidney stones (apparently uric acid urolithiasis).[41,45,46,102] These have occurred at inosine doses (up to 4 gm/day) and over time periods (up to two years) that are comparable to those planned in the current study. However, most human studies of inosine at such doses have targeted healthy volunteers or athletes (with acute or sub-acute administration), or subjects with multiple sclerosis (with chronic administration). The preceding phase 2 SURE-PD trial and the present trial are the first to our knowledge to systematically target an older subject population. Accordingly the mitigation and assessment of AEs will remain a priority.

There are no known reports of acute overdose with inosine. Maximal doses in the current study (up to 1.0 gm/dose and up to 3.0 gm/day) are 3-5 times lower than the maximal doses (5.0-6.0 gm/dose and up to 10.0 gm/day for over a week) administered to a small number of healthy volunteers or athletes, and who reportedly experienced no AEs.[48,50]

In the event of accidental or intentional overdose exceeding the study maximum daily dose of 6 capsules (which for active drug constitutes >3.0 gm inosine) in a 24 hour period, then additional fluids (the equivalent of at least three 12 oz unsweetened, non-alcoholic beverages in addition to subject's normal fluid intake during the study) should be taken to reduce the risk of any acute hyperuricemic or hyperuricosuric AE.

Any overdose exceeding 20 capsules (which for active drug constitutes >10.0 gm inosine) in a 24 hour period (i.e., the limits of published human experience) should be considered an adverse effect potentially warranting emergency medical care and monitoring. Although inosine itself is a natural component of human intermediary metabolism, rapid conversion to urate, the end product of the inosine pathway, could theoretically lead to transient pathogenic elevation of serum and urinary urate beyond the limits of solubility, with attendant arthropathic (gout-like) and nephropathic (acute renal failure; as in 'tumor lysis syndrome') consequences. Site Investigator/staff should facilitate emergency medical services in the event of high level overdose by providing this background information, and encouraging consideration of supplemental intravenous hydration and close monitoring of serum urate levels. Site staff should be prepared to break the blind for the subject at the discretion of the treating emergency service physician, although it is expected that empiric monitoring of serum urate would be sufficient for overdose management.

After any acute concerns for the subject's safety have been addressed, the site staff should work with the subject to determine the basis for an overdose if it were not already apparent. If an overdose were deemed accidental, then steps should be taken to avoid any further accidental overdose. If an overdose were deemed intentional, then consideration should be given for permanent study drug discontinuation (e.g., administratively for non-compliance, medically for major psychiatric AE, etc).

For subjects continuing in the study after an overdose that did not prompt medical attention with serum urate monitoring, the regular dosing schedule should be resumed after skipping half the number of doses that were taken in excess. (E.g., for a subject taking 4 caps/day who inadvertently took 12 caps one day, regular dosing could resume after one day's hiatus.) For subjects continuing in the study after an overdose that prompted medical attention with serum urate monitoring, any resumption of study dosing should be determined by the Site Investigator after discussion with the Medical Safety Monitor.

There are no known drug interactions with inosine. Any results indicating otherwise from formal drug-drug interaction studies conducted on GCP conditions will be incorporated into guidance for subjects. Theoretical interactions should be considered with any drug that affects urate levels. Although urate-lowering drugs will not be allowed, thiazide diuretics can mildly elevate serum urate levels and are allowed at enrollment and during the study. Because urate levels are continually titrated to serum urate levels, the effects of mildly hyper- or hypo-uricemic agents on serum urate will be compensated.

Neither drug discontinuation, premature withdrawal from the study, nor most clinical emergencies necessitate disclosure of treatment assignment. Most emergency situations can be handled by withdrawing study drug without disclosure of treatment assignment. However, in rare circumstances under which knowledge of the drug assignment is necessary for the treatment of an SAE, Site Investigators are encouraged to discuss the situation with the Medical Safety Monitor before deciding whether to disclose treatment assignment. Disclosure of individual treatment assignment must be made by the Site Investigator responsible for the care of the involved subject (or by the Coordinator or other physician as designated by the Site Investigator). Assigned drug treatment must not be revealed to other study staff, CCC or DCC staff or to individuals who are not involved directly in the clinical care of the subject unless disclosure is critical to the care of the subject.

8.9 Analysis of Adverse Events / Experiences

On a regular basis the Medical Safety Monitor will evaluate AE and SAE data to determine if there is any indication of toxicity associated with inosine.

The Data and Safety Monitoring Board (DSMB) will periodically review blinded, and if necessary, unblinded medical event data. SAEs, drug suspensions/rechallenges, drug discontinuations, and premature withdrawals will be tracked in real time, and the DSMB alerted if any imbalances arise between treatment groups. If there is a significantly ($P< 0.05$) greater incidence of a major (severe) adverse event in the inosine-treated group, the study may be modified after discussion with the DSMB.

9. CRITERIA FOR DRUG DISCONTINUATION AND SUBJECT WITHDRAWAL

Subjects will be advised in the written informed consent forms that they have the right to permanently discontinue study drug and/or withdraw from the study at any time without prejudice, and may be withdrawn from drug and/or the study at the discretion of the Site Investigator, CCC Director and/or Study Principal Investigator at any time. Subjects who prematurely discontinue study drug will be asked to continue study participation via in-person visits if willing, and otherwise via quarterly telephone visits. Some premature drug discontinuations and/or study withdrawals may be unavoidable, but the Site Coordinator and Site Investigator will work closely with the study CCC, Medical Safety Monitor and Study Principal Investigator to attempt to retain subjects, if appropriate. See Sections 7.5 to 7.6 for further discussion of non-standard visits for drug or visit discontinuation and premature withdrawal.

Every effort should be made to obtain the subject's final laboratory tests and evaluations of clinical status. Reasonable effort should be made to contact any subject lost to follow-up during the course of the study in order to complete assessments and retrieve any outstanding data, drugs or clinical supplies. In this case, the Site Investigator must use reasonable effort to contact the subject in order to finalize study-related procedures. Following unsuccessful telephone contact, an effort to contact the subject by mail using a method that provides proof of receipt, should be attempted. Such efforts should be documented in the source documents.

A subject may discontinue study drug and/or withdraw from the study and/or be withdrawn from drug and/or the study for the following reasons:

<u>Administrative</u>

1. Withdrawal of subject consent
2. Request of Site Investigator or Study Principal Investigator
3. Request of primary care physician
4. Non-compliance
5. Pregnancy (Study drug will be discontinued in the event of a pregnancy, and every reasonable effort should be made to assess the health of the mother/fetus during the pregnancy and its outcome.)
6. Protocol deviation
7. Premature termination of the study

<u>Adverse Event/Experience</u>

1. Worsening of pre-existing disease (other than disease under study)
2. Intercurrent illness
3. Death

4. Major/clinically significant alteration in laboratory values after beginning study drug
5. Other AE
6. Other reasons concerning the subject's health or well-being

Persistently Acidic Urine
Study drug will be discontinued for subjects who have persistently acidic urine (PAU) unresponsive to alkalinization. However, neither PAU nor 'PAU unresponsive to alkalinization' is considered an AE (which is defined for this study in Sec. 8.1.)

All premature study drug discontinuations, study withdrawals, and dropouts (lost to follow-up) must be reported to the CCC within 24 hours of the Site Investigator's decision or his/her becoming aware of the subject's decision.

A Screening/Demographics eCRF must be completed for all individuals who sign the study consent form. This includes participants who fail screening or do not proceed to study drug initiation for some other reason, as well as those subjects who complete the study or withdraw prematurely. If a subject discontinues study drug or withdraws from the study due to an AE, the AE must be documented on the eCRF AE Log.

Subjects withdrawn from the study before completion will not be replaced.

10. STATISTICAL CONSIDERATIONS

10.1 General Design Issues

10.1.1 Trial Design

The trial is a phase 3, randomized, two-arm, parallel-group, placebo-controlled, two-period, multicenter clinical trial of oral inosine titrated to elevate trough serum urate to 7.1 to 8.0 mg/dL over 24 months with a 3-month wash-out among de novo PD patients exclusive of SWEDDs. The primary aim is to test efficacy of serum urate elevation based on rate of change in MDS-UPDRS I-III total score over 24 months. Randomizations will be stratified by site to avoid chance confounding between site characteristics and treatment.

The randomized, two-arm, parallel-group design will provide an unbiased estimate of effectiveness of inosine dosed to elevate serum urate in slowing or delaying PD progression over 24 months during period 1. Tracking symptoms during randomized wash-in and during the non-randomized 3-month wash-out of period 2 will allow estimation of symptomatic effects of serum urate elevation and thereby better evaluate whether any observed effects on PD progression reflect a disease-modifying effect. Note that although suspension of study drug during the wash-out will be unmasked, subjects and staff will remain blinded as to whether this represents a transition off of active treatment or of placebo treatment, and thus treatment comparison of changes during the 3-month wash-out should remain unbiased. The difference in the proportion of subjects requiring dopaminergic therapy after the 3-month wash-out among all those randomized will provide an estimate of disease-modification by serum urate elevation free of any symptomatic effects of study drug.

10.1.2 Primary Outcome

Use of rate of change in MDS-UPDRS I-III total score as our primary efficacy measure is based on an interest in an outcome that can be evaluated within 2 years and an interest in a patient-reported outcome. Moreover, our preliminary data from the SURE-PD trial showed dose-dependent efficacy of serum urate

elevation for 24-month change in UPDRS I-III total score. Anticipating that recruitment will require 18 months, evaluations of the primary outcome needs to be completed in 2 to 2.5 years in order to launch, implement, and publish results from the trial within a 5-year grant period.

Assessing change in MDS-UPDRS or other measures of motor symptoms among initially de novo PD patients becomes more complex as subjects begin initiating dopaminergic therapy, with roughly two-thirds expected to have initiated dopaminergic therapy by 2 years in the placebo group. We feel that assessing motor symptoms when not on dopaminergic therapy best reflects the efficacy of the intervention under study, whereas evaluation in the OFF condition after dopaminergic therapy has already been initiated is burdensome to subjects and unreliable due to incomplete and variable wash-out. Evaluating motor symptoms in the ON condition is prone to bias due to adjustment of dopaminergic therapy to achieve a pre-targeted level of symptoms. We propose instead to analyze MDS-UPDRS measurements made prior to initiating dopaminergic therapy, considering use of such treatments a censoring event that precludes observation of future untreated MDS-UPDRS scores. While delaying progression over only 2 years provides less benefit to patients than we would ultimately wish to offer, we feel that a demonstration of delay over 2 years in the absence of symptomatic effects is an achievable result, one that could provide evidence of disease modification early in the clinical course, and may motivate a longer-term trial focused on functional and quality of life endpoints.

10.1.3 Analysis Samples

A modified intention-to-treat (mITT) sample will include all randomized subjects who received at least one dose of study drug, each classified according to their randomized treatment assignment without regard to compliance with their assigned treatment. An as-treated (AT) sample will include all subjects who received at least one dose of study drug classified according to the actual treatment received and reclassifying subjects randomized to inosine who report not taking inosine and reclassifying subjects randomized to placebo who report taking inosine or whose serum urate during treatment averages 6.5 mg/dL or greater (note: no such subjects out of 25 randomized to placebo were observed in SURE-PD). Separate analysis will also look only at intervals on study drug, censoring follow-up after study drug discontinuation, and at serum urate elevation as achieved, distinct from either randomized or self-selected treatment as administered.

The primary efficacy analyses will use the mITT sample to best estimate the expected effectiveness of urate-elevating oral inosine supplementation in clinical practice, recognizing that compliance in clinical use may differ from compliance in the clinical trial. Analyses of tolerance will also use the mITT sample. Analyses of safety will use the AT sample to avoid downwardly biased estimates of adverse outcomes associated with inosine exposure due to treatment non-compliance. Secondary analyses of efficacy outcomes will also use the AT sample to perhaps better estimate efficacy of inosine supplementation as administered and serum urate elevation as achieved.

10.1.4 Interim Analysis

In addition to periodic review of accrual and adverse events by an independent DSMB to evaluate feasibility and safety, the trial will utilize an information-based group-sequential design with plans for two non-binding interim analyses for evaluating efficacy and futility. Adequacy of the planned sample size will also be reviewed on an ongoing basis by the Steering Committee based on updated estimates of nuisance parameters using blinded data.

10.2 Outcomes

10.2.1 Primary Outcome

The primary outcome of the trial is rate of change in MDS-UPDRS I-III total score over 24 months estimated from a shared-baseline, random-slopes mixed model, censoring follow-up of subjects after initiation of dopaminergic therapy. Details of the analysis are described in Sec. 10.5.1.

10.2.2 Secondary Outcomes

Secondary outcomes include safety and tolerability of the protocol-specified urate-elevating inosine treatment, symptomatic effects of urate elevation, and a range of secondary efficacy measures, including time to disability warranting dopaminergic therapy, levodopa equivalent dose, cognition, mood, quality of life, and functional disability.

10.2.2.1 Safety

Safety of oral inosine titrated to elevate trough serum urate to 7.1 - 8.0 mg/dL will be evaluated by comparing active vs. placebo treatment with respect to overall AE and SAE rate, time to first SAE, and proportions of subjects experiencing (a) each type of AE, classified by Medical Dictionary for Regulatory Activities (MedDRA) preferred term and system organ class, (b) clinically-significant abnormal labs, and (c) clinically significant abnormal vital signs, including orthostatic hypotension defined as positional dizziness or other clinical symptoms of orthostatic hypotension or a drop in systolic and diastolic blood pressures of 20 mm Hg and 10 mg Hg or more, respectively, when moving from a supine to a standing position.

10.2.2.2 Tolerability

Tolerance of treatment by an individual subject will be defined as remaining on-study and on their assigned treatment without one or more dose reductions lasting more than 4 weeks cumulative due to AEs. Tolerability of a treatment will be defined as a proportion of all subjects in a treatment group who are tolerant of the treatment at 12 weeks (short-term) and 24 months (long-term). A treatment will be declared tolerable if the proportion who are tolerant is significantly greater than 50%.

10.2.2.3 Symptomatic effect

Symptomatic effects will be estimated by changes in motor symptoms during the first 3 months of wash-in at the start of period 1 and during the 3-month wash-out of period 2.

10.2.2.4 Efficacy outcomes

Additional efficacy outcomes will include time to disability warranting dopaminergic therapy, the prescribed levodopa equivalent dose, motor function as measured by MDS-UPDRS ambulation subset and the patient-reported sections (IB and II), cognition as measured by MoCA, quality of life as measured by PDQ-39 and by Neuro-QOL (including its depression module as a measure of mood), and functional disability as measured by Schwab and England.

10.2.2.5 Two-period evaluations

Analysis of the persistence of benefit after the 3-month wash-out of period 2 among subjects randomized to active treatment during period 1 permits an evaluation of disease-modifying effect of serum urate elevation. Not all measures are amenable to two-period evaluation because of the censoring effect of initiation of dopaminergic therapy. In particular, with only one-third of subjects expected to have not initiated dopaminergic therapy at the end of period 1, few data on MDS-UPDRS trajectories during wash-out will be available and subjects contributing that data are a non-random subset of slow progressors. For other measures that are equally evaluable at the end of both periods, a three-part test of significantly

slower worsening during period 1, non-inferior rates of worsening during period 2, and a significant net benefit at the end of period 2 would provide evidence of disease modification if all three evaluations were favorable.

10.3 Sample Size and Accrual

10.3.1 Effect Size

The effect size for determining power is based on estimates of the minimum clinically important difference (MCID) for changes in MDS-UPDRS I-III total scores and on the previously observed association between baseline serum urate levels and the rate of change in UPDRS I-III total scores in the DATATOP, PRECEPT, and SURE-PD trials.

Hauser et al.[197] report that placebo-treated subjects who reported being minimally worse over 26 weeks on a global impression of change assessment experienced an average increase in UPDRS I-III total scores of 4.9 units. Given our expectation that elevated serum urate will delay progression, not improve symptoms, a MCID related to minimal worsening among placebo subjects seems most appropriate in this context. Based on estimates by Goetz and colleagues[145,149,152,198] for PD patients with mild symptoms (Hoehn and Yahr stage I/II), MDS-UPDRS I-III total scores are roughly 30% larger than UPDRS I-III total scores (2.5x for 16 questions in Part 1, 1.1x for 52 questions in Part II, and 1.2x for 108 questions in Part III, yielding a weighted conversion of 1.29x), implying a MCID of 6.3 MDS-UPDRS I-III total score units.

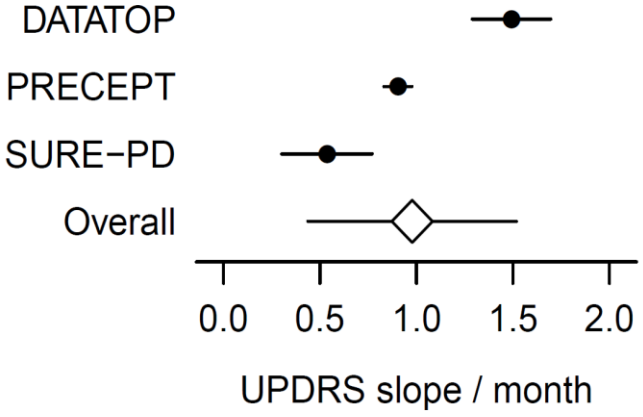


Figure 13. Meta-analysis of the rate of UPDRS change in early PD subjects with lower serum urate. Early, largely untreated PD subjects with a serum urate of <5.9 mg/dL at baseline from DATATOP (n=155; double-placebo only), PRECEPT (n=446) and SURE-PD (n=25; placebo only) were followed for up to two years. The overall UPDRS rate was obtained by weighting each estimate by the inverse of its standard error.

We expect to enroll subjects with mean baseline serum urate of approximately 4.5 mg/dL based on data from SURE-PD and then raise levels in the active arm 3 mg/dL on average at trough sampling. A 3 mg/dL difference in baseline serum urate predicted 8.1 and 2.1 unit per year differences in UPDRS I-III total score slopes in the DATATOP[36] and PRECEPT[35] trials. In the SURE-PD trial[102], subjects randomized to moderate serum urate elevation (7 to 8 mg/dL at random sampling) progressed 1.1 unit / year slower than placebo arm subjects with wide confidence bounds (95% CI 4.8 units / year slower to 2.8 units / year faster). Note that this estimate from SURE-PD (see Fig. 7D) is conservative relative to an alternative model for the effect of serum urate elevation (see Fig. 7C). A random-effects meta-analysis of results from the three studies yields a weighted estimate of 4.5 units slower progression in UPDRS I-III total score over 2 years among patients with higher serum urate levels.

We propose that 6.3 units over two years or a difference in slopes of 3.15 units per year is a reasonable minimum clinically important difference (MCID) in MDS-UPDRS I-III total scores, equal to a MCID of 4.9 units on the scale of UPDRS I-III total scores. This would correspond to a reduction of 20% of the expected placebo rate based on a random effects meta-analysis (Fig. 13) of the mean rates of decline in UPDRS I-III total scores among de novo PD patients with baseline serum urate levels below 5.9 mg/dL in the DATATOP[32,34], PRECEPT[30], and SURE-PD[102] cohorts. Changes in UPDRS I-III total scores were analyzed using random-slope mixed models censoring follow-up when dopaminergic therapy was

initiated. The weighted mean estimate was 0.98 points / month or 23.5 points over 2 years with substantial heterogeneity in the estimate among studies (see Fig. 13).

10.3.2 Sample Size

Power for the primary outcome of rate of change in MDS-UPDRS I-III total score is based on a random slopes model with shared baseline. The model will include fixed effects of time, treatment x time, gender, gender x time, an indicator of baseline MAO-B inhibitor use, and baseline MAO-B inhibitor use x time and random site- and subject-specific intercepts and slopes, each with unstructured covariance. MDS-UPDRS assessments completed after a subject has initiated dopaminergic therapy will be censored.

Based on applying the same primary analysis model to data from SURE-PD, the following variance components were estimated for UPDRS I-III total scores: site-level variance (intercept = 9.75, slope = 0.0123 / month, covariance = -0.346), subject-level variance (intercept = 77.4, slope = 0.230 / month, covariance =2.87), and residual variance = 13.9. We assume that 70% of subjects will initiate dopaminergic therapy based on experience in SURE-PD plus up to 8% additional lost to follow-up prior to initiating dopaminergic therapy. With the planned schedule for MDS-UPDRS assessments (screening, baseline, 3 weeks, 6 weeks, 12 weeks, and then quarterly through 24 months), the variance and censoring estimates above imply an effective standard deviation for UPDRS I-III total score slopes of 0.587 units / month or 0.758 units / month on the scale of MDS-UPDRS I-III total scores.[210]

Given a standard deviation of 0.76 units / month, a final two-sided test at alpha = 0.046 allowing conservatively for two interim analyses at alpha = 0.001 each, the study would have 80% power with n = 270 subjects randomized 1:1 to placebo or urate elevation if the true effect of treatment were to reduce the rate of increase in MDS-UPDRS I-III total score by 6.3 points over 2 years.

This estimate of power is robust to variable gender-specific enrollment rates and treatment efficacy as long as the average effect of treatment across genders in the ratio enrolled is 6.3 points over 2 years (Fig. 14A). If urate elevation reduces the average rate of progression by 6.3 points over 2 years only among male or female participants and the other gender experiences less benefit, power would be lower (Fig. 14B). This is unavoidable if a large proportion of the enrolled population accrues less benefit from the intervention. Conversely, power would be greater than 80% if one gender experiences a benefit greater than 6.3 points over 2 years and the other gender experiences at least that large a benefit.

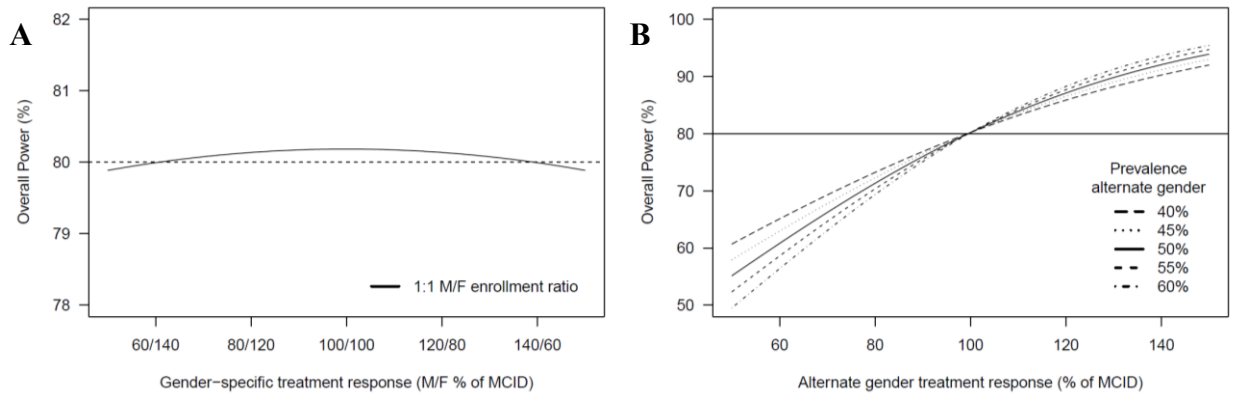


Figure 14. (**A**) Power for the primary aim across a range of gender-specific treatment responses, all cases with the average treatment response across genders equal to the MCID of 6.3 points over 2 years. Note that equal prevalence of male (M) and female (F) participants is plotted, but the power curve for other prevalence ratios are so similar as

to be indistinguishable at this scale. (**B**) Power for the primary aim when the effect of treatment on one gender is equal to the MCID and the effect on the alternate gender varies from 50% to 150% of MCID for a range of different prevalence ratios.

10.3.3 Secondary Outcomes

The trial will have an 80% probability of observing at least one instance of any class of adverse event expected to occur in at least 1.2% of individuals receiving inosine. The trial will have 80% power to detect increased risk of any class of adverse event among subjects receiving inosine if the true risk is two-fold higher and the expected proportion among placebo subjects is at least 14% or if the true risk if four-fold higher and the expected proportion among placebo subjects is at least 3.4%. The trial has 80% probability of declaring serum urate elevation tolerable if the true proportion tolerant under the definition given in Sec. 10.2.2.2 at 12 weeks and 24 months is at least 62%.

The estimated SE for symptomatic effects on UPDRS I-III total scores during wash-in between the moderate elevation and placebo arms in the SURE-PD trial was 0.53 units / month. With n = 25 subjects per treatment group in SURE-PD, the expected SE for estimating symptomatic effects in this trial will be roughly sqrt (25/135) * 0.53 = 0.23 units / month. With that SE, the trial would have 80% power to detect symptomatic effects on the order of a difference in slopes during wash-in or wash-out of 0.65 units/month.

The Kaplan-Meier product-limit estimate from the SURE-PD trial of the proportion of placebo subjects requiring dopaminergic therapy by 24 months was 62%. Assuming constant hazard at the same rate among placebo subjects in this trial during period 1 (0 to 24 months) and 8% loss to follow-up prior to determining need for dopaminergic therapy, the trial will have 80% power to detect a hazard ratio of 0.62. Extrapolating to 27 months, we expect a total of 67% of placebo arm subjects to have disability warranting dopaminergic therapy at the end of period 2 (24 to 27 months). The study would have 80% power to detect a difference in the proportion of subjects with disability warranting dopaminergic therapy if 50% or fewer of active arm subjects have progressed by the end of the trial.

Given preliminary data from SURE-PD on the variance components from random-slope models for MoCA after Rasch score conversion and S&E ADL, the effective standard deviations (in points per month) for these measures given our planned follow-up schedule are 0.038 and 0.31, respectively. Weaver et al.[204] report an among-person standard deviation for PDQ-39 at 24 months after deep brain stimulation of 14.2 to 15.3. Assuming conservatively no within-person covariance, that would imply a standard deviation for 24-month change of approximately 30. Given these estimates, the trial will have 80% power to detect 2-year treatment differences on these outcomes as small as 0.31, 2.5, and 10.3, respectively.

10.4 Data Monitoring

In addition to periodic review of accrual and adverse events by an independent DSMB to evaluate feasibility and safety, the trial will utilize an information-based group-sequential design with plans for two non-binding interim analyses for evaluating efficacy, futility, and sample size requirement.

10.4.1 Data and Safety Monitoring Board

An NINDS-appointed independent Data and Safety Monitoring Board (DSMB) has been established to monitor the trial. The DSMB is responsible for periodic review of data related to feasibility, safety, and efficacy. A DSMB Charter specifying the composition, responsibilities, frequency, and format of DSMB meetings and the content of DSMB reports has been created and is available to sites.

10.4.2 Feasibility

Feasibility of completing the trial and adequately addressing the primary aim will be evaluated by the DSMB at each meeting. While no hard criteria for declaring the trial infeasible are specified, delays in accrual that would preclude adequate enrollment and follow-up within the funding period might be cause for the DSMB to recommend termination of the trial.

10.4.3 Safety

Safety of serum urate elevation will be evaluated by the DSMB at each meeting and at other times as the DSMB may request. Given the diversity of possible adverse event data and the complexities of weighing risks and benefits from treatment, no definitive early stopping criteria for safety are specified. As described in Sec. 5.1.3a, urolithiasis and gouty arthritis are the safety outcomes of greatest concern. If greater than 20% of subjects receiving inosine develop uric acid stones or experience gouty arthritis, then the DSMB might recommend termination of the trial.

10.4.4 Blinded Sample Size Re-Estimation

The Steering Committee will review updated estimates of nuisance parameters required for sample size calculation from blinded interim analyses performed using pseudo-randomizations. Interim analyses for possible sample size re-estimation will be performed at the same time as the first planned interim efficacy and futility analysis, i.e., after 2000 person-months of period 1 follow-up are completed, roughly one-third of total anticipated period 1 follow-up. To maintain blinding, pseudo-randomizations stratified by site will be generated to assign pseudo-treatment labels to each participant. This yields a slightly conservative estimate of variance components given that variance attributable to true treatment assignment will remain in the slope and residual terms.

An information-based approach will be used, comparing the square of the observed standard error (SE) for the pseudo-treatment x time interaction term from analysis of the interim data to the square of the SE expected given the observed distribution of follow-up times and the assumptions used for the initial sample size and power calculations. Additional details are provided in the SAP.

A proposal to increase, but not decrease, the sample size will be considered based on the estimated sample size required to achieve 80% power. To avoid the administrative burden of small increases that negligibly affect power, we would not expect to recommend sample size increases of fewer than 30 participants. And recognizing practical constraints, we would not expect to recommend sample size increases of more than 130 participants. The option to decrease sample size is not needed (and can undermine power) because the trial is likely to be stopped early for efficacy or futility if estimates of the true treatment x time interaction are more precise than anticipated. Note that this is an information-based design updating only the nuisance parameters required for power calculations, not an adaptive design updating the planned effect size.

10.4.5 Efficacy or Futility

Interim analyses for efficacy or futility will be performed by the unblinded statistician after 2000 and 5000 person-months of period 1 follow-up are completed, roughly one-third and three-quarters of total anticipated period 1 follow-up. Non-binding early stopping for efficacy will be proposed if the active treatment group is superior to placebo for the primary efficacy outcome based on a one-sided p-value of 0.001 or less. Other criteria used in deciding whether to stop early for efficacy will include evaluation of safety and secondary efficacy outcomes. We propose stringent early stopping criteria for efficacy in order to ensure that evidence for efficacy is unambiguous if the study is stopped early. Non-binding early stopping for futility will be proposed based on a beta-spending rule quadratic in information time. If there were no benefit from treatment, then the trial would have a 51% probability of early stopping for futility based on this rule. As with the efficacy evaluation, the decision to stop early for futility will also include

evaluation of feasibility, safety, and secondary efficacy outcomes. Because the efficacy and futility stopping rules are non-binding, we conservatively assume that each look is cumulative for alpha and ignore possible findings of futility when calculating the overall type I error.

10.5 Data Analyses

The following plans describe proposed analyses of all outcomes. A definitive statistical analysis plan (SAP) will be finalized and must be approved by the Steering Committee prior to the final lock of the trial data and breaking of the blind. The final approved SAP may differ in detail from analyses described here.

10.5.1 Primary Analysis

The primary mITT analysis of rate of change of MDS-UPDRS I-III total scores during period 1 will use a random slopes model with shared baseline. The model will include fixed effects of time, treatment x time, gender, gender x time, an indicator of baseline MAO-B inhibitor use, and baseline MAO-B inhibitor use x time and random site- and subject-specific intercepts and slopes, each with unstructured covariance. Use of a shared baseline adjusts for baseline MDS-UPDRS score[245,246] in addition to the adjustment for gender and baseline MAO-B inhibitor use. Other baseline covariates, including Smart*4*SURE participation, may be included in the final model based on review of blinded data and any revision in our understanding of predictors of PD progression prior to finalizing the analysis plan and breaking the blind. Measurements made subsequent to initiating dopaminergic therapy will be censored. Inference of benefit from serum urate elevation will be made by testing whether the treatment x time interaction term is significantly less than zero (i.e., slower progression among subjects randomized to the active arm) using a two-sided test at $p < 0.046$ for a cumulative two-sided alpha = 0.05.

Our estimate of the effect of serum urate elevation on MDS-UPDRS will be unbiased if observed trajectories are predictive of MDS-UPDRS assessments that are missing due to loss to follow-up or are censored due to initiation of dopaminergic therapy. Data from the SURE-PD trial suggest good conformance of UPDRS I-III total score trajectories to the model assumptions. No non-linearity in treatment effects on observed UPDRS scores was found and models that included quadratic random effects fit worse by Akaike and Bayesian information criteria. Empirical Bayes estimates of 24-month UPDRS scores assuming linear trajectories were all within range of the instrument (range 7.4 to 97.4) even with 85% of the sample being censored prior to the final 24-month observation (median 12 months). Conditional residuals were normally distributed and homoscedastic. Similar assessments and influence statistics will be evaluated in judging the adequacy of the proposed primary analysis in this trial.

10.5.2 Alternative Analyses

If analysis of the mITT sample does not indicate benefit of randomization to oral inosine, it is still possible that (a) inosine supplementation is beneficial if the mITT estimates are affected by non-compliance or that (b) serum urate elevation itself is protective if achieved. As a key secondary analyses, we will analyze the AT sample, censoring follow-up after discontinuation of study drug. This will provide an estimate of the effectiveness of inosine among those tolerant of supplementation. We will also analyze the AT sample with substitution of mean increase in serum urate from baseline to week 12 in place of randomized treatment assignment to estimate method-effectiveness of serum urate elevation.

We will further evaluate robustness of estimates from our primary analysis in a number of other secondary analyses, including: (a) alternative models for the mean temporal profile, (b) alternative models for handling observations made after loss to follow-up or initiation of dopaminergic therapy, and (c) alternative models for handling covariance among repeated observations. To relax the assumption of linear change over time, a model with visit and visit x treatment terms in place of the time and time x treatment terms will be fit to permit an unstructured temporal profile. Rather than censoring observations after initiation of dopaminergic therapy, we will consider several models that include all MDS-UPDRS

observations. If participants randomized to inosine initiate dopaminergic therapy at a slower rate than those on placebo, then lower MDS-UPDRS scores at 24 months irrespective of dopaminergic therapy would suggest that the benefit of inosine supplementation carries over after the need for therapy. Alternatively, we will include either a simple time-dependent offset for measurements made in the ON condition or a time-dependent offset equal to the levodopa equivalent dose prescribed at that time. To avoid bias from potentially informative loss to follow-up, we will consider a pattern-mixture model with the fixed effects stratified by length of follow-up. The Combined Assessment of Function and Survival (CAFS) model[211] described in Section 10.5.5 is constructed similarly by comparing pairs of participants over equal lengths of follow-up. To relax the assumption of linear random slopes, a model with a full 12 x 12 unstructured covariance matrix among repeated assessments of MDS-UPDRS (78 covariance terms) will be explored.

10.5.3 Subgroup Analyses

The potential for differential benefit from serum urate elevation among subgroups of patients will be tested by including subgroup, subgroup x time, and subgroup x time x treatment interaction terms into the primary random-slopes model. A significant subgroup x time x treatment 3-way interaction in combination with significantly slower progression among members of a subgroup randomized to serum urate elevation vs. members of the same subgroup randomized to placebo will be taken as evidence of differential benefit. The following subgroups will be considered: sex, race (classified as Asian, Black or African American, Caucasian, or other, including multiracial; or as Caucasian vs. non-Caucasian if fewer than 10% of our sample is non-Caucasian), ethnicity (classified as Hispanic or Latino vs. non-Hispanic), use of MAO-B inhibitors at baseline, and age (both categorized as <65 years vs. ≥65 years and continuous).

10.5.4 Disability Warranting Dopaminergic Therapy

Treatment differences in time to disability warranting dopaminergic therapy during period 1 will be tested by a Kaplan-Meier logrank test with a two-sided alpha = 0.05. Subjects lost to follow-up will be censored unless available information suggests that a given subject withdrew consent due to increasing disease progression or a desire to seek more aggressive care. Note that subjects will continue to be followed even if they discontinue study drug. As a sensitivity analysis, subjects lost to follow-up will be classified as initiating dopaminergic therapy at the time of their withdrawal.

10.5.5 Combined Function and Treatment

A rank-based test of a single outcome combining function as measured by change in MDS-UPDRS I-III total scores and time to disability warranting dopaminergic therapy will be constructed paralleling the CAFS score methodology developed for ALS.[211] MDS-UPDRS I-III total scores will substitute for ALSFRS-R total scores and time to disability warranting dopaminergic therapy will substitute for time to mortality. The test consists of calculating a rank-sum score for each individual relative to pair-wise comparisons with all other subjects. Subjects are ranked according to time to disability warranting dopaminergic therapy when that is observed for both members of a pair or when one is censored after the observed event time for the other. Pairs that cannot be ranked by time to disability warranting dopaminergic therapy are ranked by absolute change from baseline in MDS-UPDRS I-III total score at the maximum follow-up time at which both subjects have an observation. Inference is drawn by calculating a U-statistic from the rank-sum scores.

10.5.6 Secondary Efficacy Outcomes

Most secondary efficacy measures will be analyzed using the same shared-baseline, random-slopes model described for analyzing our primary outcome. If analyses of the mITT sample do not indicate significant benefit from serum urate elevation, then the AT sample will be analyzed as well. If important differences

in inference of the primary outcome are drawn from the alternative models described in Sec. 10.5.2, then equivalent models will be explore for our secondary efficacy outcomes.

10.5.7 Safety and Tolerability Outcomes

Overall AE and SAE rates will be compared between treatment groups by negative binomial regression. Time to first SAE will be compared between treatment groups by Kaplan-Meier logrank test. The proportion of subjects experiencing (a) each type of AE, classified by MedDRA preferred term and system organ class, (b) clinically-significant abnormal labs, and (c) clinically significant abnormal vital signs, including orthostatic hypotension defined as positional dizziness or other clinical symptoms of orthostatic hypotension or a drop in systolic and diastolic blood pressures of 20 mm Hg and 10 mg Hg or more, respectively, when moving from a supine to a standing position will be compared between treatment groups by Fisher's exact test.

Assuming no administrative censoring, all subjects will be classifiable as tolerant or intolerant, with subjects lost to follow-up or withdrawing consent classified as intolerant. Tolerability will then be estimated by simple proportions tolerant at 12 weeks and 24 months. Serum urate elevation will be declared tolerable at each time point if its exact one-sided lower 95% confidence bound is greater than 50%. If evaluation of tolerance is censored, then Kaplan-Meier product-limit estimates and their complementary log-log confidence bounds will be used. We will explore the relationship between tolerance and use of dopaminergic therapy using Cox regression with use of dopaminergic therapy as a time-dependent covariate.

10.5.8 Symptomatic Effects

The presence of symptomatic effects will be tested using a change-point model constructed as a partial linear spline over time with knots at 12 weeks and 24 months. Both fixed and subject-specific random terms for intercept, slope from baseline to 12 weeks, slope from 12 weeks to 24 months, and slope from 24 to 27 months will be included. Unstructured covariance will be assumed among the random effects (10 terms). The AT sample will be analyzed. Significantly smaller slopes during wash-in or larger slopes during wash-out among subjects treated with inosine using one-sided testing at alpha = 0.025 will be interpreted as symptomatic effects. If symptomatic effects are not found on that basis, the absence of symptomatic effects will be judged based on a non-inferiority test using a non-inferiority bound of 6.3 / 3 = 2.1 points/month in the direction of a symptomatic effect and using one-sided testing at alpha = 0.05.

10.5.9 Disease Modification

A three-part test of time to disability warranting dopaminergic therapy will be used to evaluate whether serum urate elevation is disease-modifying[247-249]. Inference of disease modification would be supported if (a) a Kaplan-Meier logrank test of time to disability warranting dopaminergic therapy during period 1 favors serum urate elevation, (b) the proportion of subjects randomized to serum urate elevation remains significantly lower than placebo at the end of period 2 by Chi-square test, and (c) the time-dependent hazard ratio for time to disability warranting dopaminergic therapy during period 2 is non-inferior to a rate that would lead to equivalence to placebo by 3 months by Cox regression. Inference from each of the three component tests will be evaluated sequentially. Difference in time to disability warranting dopaminergic therapy will be evaluated first. If significant by logrank test with two-tailed alpha = 0.05, then the proportion not yet requiring dopaminergic therapy at the end of period 2 will be tested second. If significant by Chi-square test with two-tailed alpha = 0.05, then non-inferiority of the time-dependent hazard ratio will be tested by Cox regression based on a one-tailed test at alpha = 0.05.

10.5.10 Smart*4*SURE Substudy

The feasibility of implementing Smart*4*SURE will be measured by the following metrics among others: (a) proportion of eligible participants who consent to Smart*4*SURE, (b) proportion of randomized

substudy participants who complete a baseline Smart*4*SURE assessment, and (c) proportion of randomized substudy participants who complete at least monthly motor and cognitive assessments and quarterly self-report MDS-UPDRS, QoL, and apathy evaluations while on-study in the parent trial.

Correlations between Smart*4*SURE measures and related clinical assessments will be evaluated both cross-sectionally and longitudinally. Cross-sectional associations will be estimated both by correlation of baseline data and in mixed models using all contemporaneous assessments. The mixed model will regress clinical measures on contemporaneous Smart*4*SURE measures and will separately estimate within-person and among-person associations by recoding the Smart*4*SURE measures as person-level trends and deviations from a given participant's trend. Adequacy of the Smart*4*SURE measures as proxies for clinical assessments requires strong correlation at both within-person and among-person levels. Longitudinal associations will be estimated similarly, with direct correlation of the final period 1 assessments and by similar mixed models of change-scores calculated as the simple differences from baseline.

Exploratory evaluation of Smart*4*SURE measures as efficacy outcomes will use the same primary, alternative, and subgroup analyses proposed for the primary outcome in Sections 10.5.1-10.5.3 and the same analysis of symptomatic effects specified in Section 10.5.8.

e10.5.11 Serial DAT Scan Substudy

The primary aim of the serial DAT scan substudy is to determine whether serum urate elevation via oral inosine slows the loss of striatal dopamine transporter (DAT) over two years in early PD as measured by a reduction in DAT radioligand uptake on serial SPECT brain scans. In pursuit of secondary aims, we will determine whether and to what extent change in DAT loss correlates with change in smartphone metrics of PD progression as measured in the 'Smart*4*SURE' substudy, and will test whether genetic determinants of PD progression (e.g.,variants of *SNCA*[268] or *SLC2A9*[223]) or of urate-DAT interaction (e.g., *INPP5K* variants[269]) identify PD subpopulations in whom inosine alters the DAT loss in SURE-PD3.

Quantitative estimates of striatal binding ratios in the left and right hemispheres of the caudate and putamen will be averaged, log-transformed, and modeled in a shared-baseline, repeated measures ANOVA with fixed terms for visit (2 levels), post-baseline x treatment interaction (1 term), gender, gender x visit interaction, MAO-B inhibitor, MAO-B inhibitor x visit interaction, and unstructured covariance between each participant's serial assessments. Back-transformed linear functions of the fixed terms will yield estimates of treatment-group specific percent change over 2 years and will be used to test for treatment-dependent differences in 2-year change in DAT ligand uptake. This model construction increases efficiency by adjusting for baseline, controls for any chance differences in DAT uptake at baseline between treatments, and allows inclusion of all participants in the analysis even if some are lost to follow-up. Both mITT and AT samples will be analyzed, with the mITT results considered primary. Associations between 2-year changes in mean striatal binding ratios and functional measures (e.g., MDS-UPDRS) will be estimated overall from simple correlations and separately by gender and baseline MAO-B inhibitor use. We will test for Simpson's paradox (i.e., incongruence between correlations among- vs. within-groups). Gender-specific and genotype-specific effects will be considered in secondary analyses by including subgroup and subgroup x treatment x post-baseline terms.

11. DATA COLLECTION, SITE MONITORING, AND ADVERSE EXPERIENCE REPORTING

11.1 Records to Be Kept

11.1.1 Study File and Site Documents

The Site Investigator should have the following study documents accessible to the Study Monitor during the study.

- Signed Form FDA 1572
- Curriculum vitae for Site Investigator and staff listed on Form FDA 1572
- The signed IRB/IEC form/letter stating IRB/IEC approval of protocol, consent forms and any advertisement notices, documentation of the IRB/IEC composition, and all IRB correspondence including notification/approval of protocol amendments, notification of serious SAEs to the IRB/IEC, and IRB/IEC notification of study termination
- IRB approved consent form (sample) and any advertisement
- Signed protocol (and amendments, if applicable)
- Signed subject consent forms
- Copies of the completed eCRF worksheets (source), supplemental source notes and subject-completed medication logs (Study Drug Record sheets)
- Authorization log (Delegation Log - Study Staff and Staff Related Duties) with names, signatures, initials and functional role of all persons completing protocol assessments, providing back-up to the Site Investigator and Coordinator, if applicable, as well as staff entering data into the eRT system.
- Copies of laboratory reports/printouts
- Any source data/records not kept with the subject's hospital/medical records
- Study Drug Dispensing/Return Log and Dose Management Log
- Signed and dated receipt of supplies
- Record of all monitoring visits made by DCC personnel
- Copies of essential site correspondence to and from CCC and DCC
- Investigator's Brochure and IND safety letters, as applicable
- Certificate for Human Subject Protection Program for each individual named on the Authorization log who has direct subject contact
- Copy of professional licensure/registration, as applicable, for each individual named on the Delegation Log, who has direct subject contact ensuring licensure is in the state in which the study will be conducted
- A Note to File indicating the assessments that will be considered source documents
- Any other documentation as required by the CCC or PSG (e.g., Conflict-of-Interest/Financial Disclosure)

The Site Investigator must also retain all printouts/reports of tests/procedures, as specified in the protocol, for each subject. This documentation, together with the subject's hospital/medical records, is the subject's SOURCE DATA for the study.

11.1.2 Maintenance and Retention of Records

US FDA regulations (21 CFR 312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs (if applicable), consent forms,

laboratory test results, and medical inventory records, must be retained by the Site Investigator for two years after marketing application approval. If no application is filed, these records must be kept for two years after the investigation is discontinued and the US FDA and the applicable national and local health authorities are notified. The CCC or their representative will notify the Site Investigators of these events. The Site Investigators should retain all study documents and records until they are notified in writing by the CCC or their representative.

The Site Investigator will be instructed to consult with the CCC before disposal of any study records and to notify the CCC of any change in the location, disposition, or custody of the study files.

11.2 Role of Data Management and Study Monitoring

11.2.1 Central Data Management

The Unblinded Biostatistician will be responsible for design of the randomization scheme, creation of analytic databases, and the statistical analysis plan. Data management staff at the DCC will be responsible for all other data collection procedures.

An Internet-accessible Electronic Data Capture (EDC) system for data management will be utilized for this study. This system is protected by 128-bit server certificates and utilizes authenticated, password-protected accounts for each site. The EDC system is designed to ensure timeliness and accuracy of data as well as the prompt reporting of data from the study on an ongoing basis to the study Principal and Co-Investigators. The system is compliant with relevant FDA regulatory requirements per 21 CFR Part 11.

Sites will enter subject information and data into an electronic case report form (eCRF) in the EDC application via computer stations connected remotely through an Internet browser to a central server at the DCC. Authorized study personnel at each site will each be granted access to the electronic data capture tool via provision of a unique password-protected user-ID that will limit access to enter and view data specifically for subjects enrolled at their site.

It is the Site Investigator's responsibility to ensure that entries are proper and complete. During entry of data, error checks will be performed by the EDC that will flag problematic (i.e., missing, out of range, inconsistent) data, allowing for sites to correct the data at that time. Error checks will be implemented in the EDC based upon specifications defined in the data management plan.

Once the data are submitted to the EDC system, they are immediately stored in the central study database located at the DCC and are accessible for review by data management staff. Data review, coding and query processing will be done through interaction with the DCC, site personnel and the Study Monitor. Any changes to the data will be fully captured in an electronic audit trail. The cycle of electronic data entry, review, query identification/resolution, and correction occurs over the course of the study period until all subjects have completed the study. Narrative text of AEs and concomitant medications will be periodically coded using established coding mechanisms.

Upon completion of a subject's visit or the study, sites have the option to print the completed eCRFs depicting the data that were entered. At the conclusion of the study, a PDF (portable document format) file on electronic media depicting eCRFs for each site will be provided by the CCC for record keeping at the site.

Once the Unblinded Biostatistician and the DCC, in conjunction with the Principal Investigator, agree that all queries have been adequately resolved and the database has been deemed "clean", the database will be officially signed off and deemed locked. All permissions to make changes (append, delete, modify or update) the database are removed at this time.

All site personnel will remain blinded as to treatment assignments until the conclusion of the entire study. The treatment assignments are not part of the DCC electronic clinical database. The designated unblinded DCC programmer who oversees the dose adjustment algorithm, the drug packaging staff, and unblinded statistician at the DCC will have access to the treatment assignments, and these individuals will not communicate about treatment-specific results to any other staff involved in the study.

11.2.2 Site Investigator Responsibilities

This study will be conducted under the supervision and direction of the Site Investigator designated by the study Steering Committee as the Site Investigator at the address provided to the CCC.

Clinical supplies will be sent to the address specified to the CCC by the Site Investigator.

The Site Investigator must not conduct the study at any sites other than the one designated by the study Steering Committee.

The protocol, informed consent form, and advertisement notices will be approved by the site's specified institutional IRB.

Each Site Investigator is responsible for providing copies of the protocol and all other information relating to the preclinical and prior clinical experience, which were furnished to him/her, to all study personnel responsible to him/her who participate in this study. The Site Investigator will discuss this information with them to assure that they are adequately informed regarding the study drug and conduct of the study. The Site Investigator must assure that all study staff members are qualified by education, experience and training to perform their specific responsibilities.

11.2.3 Case Report Forms

Sites will enter subject information and data into an electronic case report form (eCRF) in the Electronic Data Capture (EDC) application. The eCRFs are used to record study data and are an integral part of the study and subsequent reports. Therefore the eCRFs must be completed for each subject screened or enrolled according to the subject's source data on a per-visit basis. Authorized study personnel will each be granted access to the electronic data capture tool via provision of a unique password-protected user-ID that will limit access to enter and view data specifically for subjects enrolled at their site. **Data should be entered into the EDC system within 3 business days of a subject's visit.**

Sites will be supplied with a set of source document worksheets that correspond to the electronic case report form (eCRF). The worksheets will serve as source documents and are required to be used to enter data into the eCRFs. Sites will initially enter all data into the subject's medical chart and/or onto source documentation worksheets prior to entering data into the eCRFs via computer stations connected remotely to the central server through an Internet browser.

11.2.4 Electronic Signatures

An electronic signature from the Site Investigator is required on the following eCRFs:

- Signature Form (for each visit)
- Adverse Event Form (at the conclusion of the study)

The data entered from the eCRFs will be securely transmitted to a central database stored on a secure server located at the DCC. Sites must print the completed eCRFs depicting the data that were entered, but have the option to do so upon completion of either a subject's visit or the study.

At the conclusion of the study, the site will be provided with a PDF (portable document format) file on electronic media depicting eCRFs for their site. The PDF file should be printed for each subject participating in the study and filed in the subject's binder.

11.2.5 Primary Source Documents

The Site Investigator must maintain primary source documents supporting significant data for each subject in the subject's medical notes. These documents, which are considered 'source data', should include documentation of:

- Demographic information
- Evidence supporting the diagnosis/condition for which the subject is being studied
- General information supporting the subject's consent to participate in the study
- General history and physical findings
- Hospitalization or Emergency Room records (if applicable)
- Each study visit by date, including any relevant findings/notes by the Site Investigator(s), occurrence (or lack) of adverse events, and changes in medication usage including the date the study drug commenced and completed
- Any additional visits during the study
- Any relevant telephone conversations with the subject regarding the study or possible adverse experiences
- Original, signed informed consent forms for study participation

The Site Investigator must also retain all subject specific printouts/reports of tests/procedures performed as a requirement of the study (e.g., laboratory and ECG reports). Laboratory reports from the central laboratory must be signed and dated by the Site Investigator in a timely fashion following review and filed with the subject's source documents. This documentation, together with the subject's hospital/site medical records, is the subject's 'source data' for the study. During monitoring visits the Study Monitor will need to verify data in the eCRFs against these source data.

11.2.6 CRF Worksheets

Sites will be supplied with a set of worksheets that correspond to the electronic case report form (eCRF) for this study. The worksheets will serve as source documents for study observations and assessments and should be used to enter data into the eCRF. Additional source documentation for information not specifically included on the source document may be recorded on a separate document.

11.2.7 Study Monitoring

All aspects of the study will be monitored by authorized individuals in compliance with GCP and applicable regulations. The Study Site Monitors will review, on a regular basis, the progress of the study with the Site Investigator and other site personnel.

11.2.8 Monitoring Visits

To ensure compliance with GCP and other applicable regulatory requirements, the Study Monitor or representative is responsible for monitoring that sites conduct the study according to the protocol, standard operating procedures, and other written instructions and regulatory guidelines.

Monitoring visits by a Study Monitor will be arranged in advance, at a mutually acceptable time, with site personnel. The site personnel must allow sufficient time for the Study Monitor to review CRFs and relevant source documents and queries. The site Coordinator and/or Site Investigator(s) should be available to answer questions or resolve data clarifications. As part of the supervision of the study progress, the CCC and DCC personnel, or Steering Committee members may, on request, accompany the Study Monitor on visits to study sites.

11.2.9 Closeout Visit

Following the completion of the study, Study Monitor(s) may conduct an on-site closeout visit or a telephone closeout visit to ensure that all data queries have been resolved, any protocol deviations are documented appropriately, all relevant study data has been retrieved, that study drug and clinical supplies have been/will be properly returned and that the Site Investigator has copies of all study-related data/information on file and archive responsibilities have been reviewed.

11.2.10 Study Committees

11.2.10.1 Steering Committee

The Steering Committee (SC) is composed of the Principal and Co-Principal Investigators of the study (serving as SC Chair and Co-Chair), blinded biostatisticians, independent Investigator members of the Parkinson Study Group, investigators with expertise in PD and study-related medical (e.g., renal and cardiac) conditions and priorities (trial recruitment and drug supply), and representatives of the CCC, DCC, NINDS and patient advocacy community. The SC is responsible, along with the Study Principal Investigator, for the design of the study protocol and analysis plan, and oversees the clinical trial from protocol development to study analysis and publication.

11.2.10.2 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) has been appointed and is responsible for periodic review of the data related to adverse events throughout the trial. In addition, its responsibilities are to review of the research protocol and ongoing study activities, including review of adequacy of subject recruitment and retention, data quality and completeness, and fidelity to the study protocol. The DSMB will make recommendations to the NINDS and the study PI concerning trial continuation, modification, or conclusion. A DSMB Charter specifying the composition, responsibilities, frequency, and format of DSMB meetings and the content of DSMB reports has been created and is available to sites.

11.3 Quality Assurance

11.3.1 QA Audits/Site Visits

During the course of the study and after it has been completed it is likely that one or more study site visits will be undertaken by authorized representatives of the DCC.

The purpose of the audit is to determine whether or not the study is being, or has been, conducted and monitored in compliance with the protocol as well as recognized GCP guidelines and regulations. These audits will also increase the likelihood that the study data and all other study documentation can withstand a subsequent regulatory authority inspection.

If such audits occur, they will be arranged for a reasonable and agreed time. Site staff will receive feedback after these visits have taken place. Action items noted in this correspondence should be attended to within 1 week of receipt of the correspondence.

11.3.2 Regulatory Inspections

The study may be inspected by regulatory agencies, such as the Food and Drug Administration (FDA). These inspections may take place at any time during or after the study and are based on the local regulations as well as ICH guidelines.

12. HUMAN SUBJECTS

12.1 Compliance Statement

This study will be conducted in accordance with the Good Clinical Practice (GCP) guidelines promulgated by the International Conference on Harmonization (ICH) and the Food and Drug Administration (FDA), and any applicable national and local regulations including FDA regulations under 21 Code of Federal Regulations (CFR) Parts 11, 50, 54, 56, 312 and 314.

All procedures not described in this protocol will be performed according to the study Operations Manual unless otherwise stated. Laboratory tests/evaluations described in this protocol will be conducted in accordance with quality laboratory standards as described in the central laboratory manual unless otherwise stated.

12.2 Informed Consent

This study will be conducted in accordance with the provisions of 21 CFR Part 50. The CCC must be given an opportunity to review the site-specific consent form prior to site IRB submission and before it is used in the study.

In accordance with relevant regulations, an informed consent agreement explaining the procedures and requirements of the study, together with any potential hazards/risks must be read and/or explained to each subject. Each subject will sign such an informed consent form or give oral consent/proxy. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the signed consent form will be given to the subject, and this fact will be documented in the subject's record. The subject must be assured of the freedom to withdraw from participation in the study at any time.

It is the Site Investigator's responsibility to make sure that the subject understands what she/he is agreeing to and that written informed consent is obtained before the subject is involved in any protocol-defined procedures including screening procedures. It is also the Site Investigator's responsibility to retain the original signed consent form and provide each subject with a copy of the signed consent form.

12.3 Institutional Review Board/Independent Ethics Committee

The CCC will supply all necessary information to the Site Investigator for submission of the protocol and consent form to the IRB/IEC for review and approval. The Site Investigator agrees to provide the IRB/IEC all appropriate material. The trial will not begin until the Site Investigator has obtained appropriate IRB/IEC approval. A copy of the approval letter and approved consent form must be submitted to the CCC.

The Site Investigator will request from the IRB/IEC a composition of the IRB members reviewing the protocol and informed consent. Appropriate reports on the progress of this study by the Site Investigator

will be made to the IRB/IEC and the CCC in accordance with institutional and government regulations. The CCC will notify the site when the IRB/IEC may be notified of study completion. It is the Site Investigator's responsibility to notify the IRB when the study ends. This includes study discontinuation, whether it is permanent or temporary. A copy of the site IRB/IEC's acknowledgement of study completion must be submitted to the CCC.

The Site Investigator will discuss any proposed protocol changes with the CCC and no modifications will be made without prior written approval by CCC, except where clinical judgment requires an immediate change for reasons of subject welfare. The IRB will be informed of any amendments to the protocol or consent form, and approval, where and when appropriate, will be obtained before implementation.

12.4 Protocol Amendments

Changes to the protocol should only be made via an approved protocol amendment. Protocol amendments must be approved by the PI, the study's Steering Committee and each respective site's IRB/IEC prior to implementation, except when necessary to eliminate hazards and/or to protect the safety, rights or welfare of subjects.

12.5 Subject Confidentiality

Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, the NINDS, the OHRP, the Sponsor, or the Sponsor's designee.

The Site Investigator must assure that the privacy of subjects, including their personal identity and personal medical information, will be maintained at all times. U.S. sites have additional privacy obligations to study subjects under the Health Insurance Portability and Accountability Act (HIPAA). Subjects will be identified by code numbers on case report forms and other documents submitted to the DCC and CCC.

After a subject signs an informed consent, it is required that the Site Investigator permit the Study Monitor, independent auditor or regulatory agency personnel to review the signed informed consent(s) and that portion of the subject's medical record that is directly related to the study including electronic medical records. This shall include all study relevant documentation including subject medical history to verify eligibility, laboratory test result reports, admission/discharge summaries for hospital admissions occurring while the subject is in the study, and autopsy reports for deaths occurring during the study (when available).

The subject's Authorization allows the CCC and DCC to receive and review the subjects' protected health information that may be re-disclosed to any authorized representative of the PI, CCC or central laboratory facility for review of subject medical records in the context of the study.

12.6 Study Modification/Discontinuation

The study as represented by this protocol may be modified or discontinued for reasonable cause at any time, with approval of IRB as warranted, by the Sponsor, Steering Committee, NINDS, OHRP, FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the Site Investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The IRB/IEC will also be informed promptly and provided the reason(s) for the termination or suspension by the Sponsor or by the Site Investigator/institution, as specified by the applicable regulatory requirement(s). Through timely notification of Site Investigators,

the Sponsor will encourage prompt notification of research subjects with instructions for study drug discontinuation and activities completion to ensure their health and well-being.

13. PUBLICATION OF RESEARCH FINDINGS

Timely publication of the results of this trial is considered a high priority as part of a non-commercial inosine development program for PD. Publication will be governed by the policies and procedures developed by the Steering Committee and guided by publications policy and conflict-of-interest guidelines of the PSG. Results will also be made public through the NIH clinical trials registration website ClinicalTrials.gov. Any presentation, abstract, or manuscript on the primary results will be made available for review by NINDS prior to their initial submission for publication.

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Protocol Change Summary

Below is a summary of the changes that were incorporated into version 2.0 to improve the protocol, prompted primarily by recommendations made by the National Institutes of Health (NIH)/National Institute of Neurological Disorders and Stroke (NINDS) in their scientific review of our funding application for the trial.

All listed changes are included in the protocol version 2.0 and are dated December 1, 2015.

- [pg 1] Addition of Dr. David Oakes as the senior biostatistician of a senior statistician directing the Data Coordination Center for the trial
- [pgs 11, 58] Addition of an additional 'quality of life' outcome measure (Neuro-QOL)
- [pgs 40, 78] Minor modification of the low urine pH criteria that trigger urine alkalinization in the placebo group — in order ensure better matching of alkalinization rates in the placebo and active study drug groups, and so doing to reduce the risk of unblinding by differential rates of alkalinization between the two treatment arms
- [pgs 44-47] Additional recruitment & retention enhancement initiatives, including IRB-approved health care network database-searching methods.
- [pgs 59-60] Addition of several exploratory outcome measures with brief self-administered patient-reported responses (on REM Behavior Disorder, PD risk factors, and study expectations).
- [pg 60] Addition of an optional biomarker blood collection, in which subjects may provide blood samples (in addition to those drawn for serial urate measurement and other safety labs) for DNA extraction and storage serial frozen plasma storage for future research
- [pgs 86-94] Minor refinements of the statistical analysis plan, such as more detailed descriptions of power analyses and of baseline covariates to be incorporated into the primary analysis.
- Other minor administrative changes and corrections were made that do not affect content.

Attached is the full copy of the revised protocol.

Summary and Justification of SURE-PD3 protocol revisions from version 2.0 to 3.0 (12-06-2016)

#	Proposed Change	Section	Purpose
1	Smart*4*SURE substudy addition	6.2.15, 10.5.10	primarily to validate smartphone metrics as outcome measures for future PD trials; to provide secondary outcome measures.
2	Addition of an exclusion criterion: history of nephrectomy	4.2	additional safety measure to avoid increased risk of a potential kidney stone in a subject with a single kidney, per Medical Safety Monitor recommendation
3	Addition of sections that reintroduce language from the phase 2 protocol specifying procedures related to unplanned changes in study drug doses (e.g., missed doses, dose reductions, dose suspensions and dose rechallenges.	5.1.2.4-5.1.2.7	To clarify these procedures for these occasional study drug adjustment scenarios in phase 3.
4	added that sites should refer to MOP for clarification regarding specifics of disallowed medications	5.3.2.2	To avoid confusion over specific types of medications (e.g., non-prescription antioxidants and vitamins)
5	The time window between screening visit 1 and the baseline visit has been extended to 60 days from 45 days.	6.3 (SoA), 7.1.8	This was extended due to logistical issues related to scheduling screening visits and receiving results within 45 days prior to the baseline visit.
6	Replaced Medwatch Form 3500A as the source document used for sites to report SAEs, with the SURE-PD3 SAE Form.	8.3	Due to the fact the Medwatchform 3500A cannot be typed into and saved, and to streamline reporting processes, we are replacing use of the Medwatch Form 3500A as the source document used for sites to report SAEs, with the SURE-PD3 SAE Form.
7	Time of follow-up for Adverse Events has been updated so that it reflects that site's do not need to follow subjects with active adverse events post the 27 month visit unless it is requested by the Medical Safety Monitor or the Principal Investigator.	8.6	To avoid unnecessary subject and site staff burden when not warranted.
8	Minor revisions.	through-out	To improve clarity.

Summary and Justification of SURE-PD3 protocol revisions from version 3.0 to 4.0 (11/28/17)

#	Proposed Change	Section	Purpose
1	Addition of an optional serial DAT scan sub-study which involves a second DAT scan one to two months following a subject's two-year study visit	6.2.11.2, 7.1.8 – 7.2.12, 7.3.1, 10.5.11	To allow for comparative analysis between pre- and post-study drug DAT scan measurements
2	Clarifying language concerning the DAT scan eligibility read process	6.2.11.1	To clarify the process used by the imaging core in determining eligibility
3	Clarifying that the projected enrollment number of 270 is an approximation (where not already indicated)	Synopsis, 3.1, 4.0	To unify the description of the study enrollment goal
4	Reduction in number of consecutive urine pH values ≤5.5 needed to initiate alkalinization therapy from 4 to 3	8.4.2	To lower the threshold for initiating alkalinization treatment to reduce the risk of uric acid kidney stones in those most at risk
5	Expansion of Smart*4*SURE sub-study enrollment	7.1.8 – 7.2.11	To allow subjects who have passed their baseline visit to enroll in the sub-study
6	Updating Schedule of Activities chart to include above-mentioned changes as well as unplanned visits	7.7	To improve clarity
8	Minor revisions	Throughout	To improve clarity