STUDY PROTOCOL

A futility trial of sirolimus in multiple system atrophy

A single center, randomized, double blind, placebo-controlled futility trial to determine if sirolimus is of sufficient promise to slow the progression of multiple system atrophy

Protocol Number: 17-01392

National Clinical Trial (NCT) Identification Number: NCT03589976

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IND #: 136871

Sponsor of the IND:

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Support from:

National Institute of Neurological Disorders and Stroke (NINDS)

Version Number: v.9 (4-JUN-2020)

Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
1.3. Schedule of activities and 4.1, 5.2.	Patients will undergo PCR nasal swab to screen for COVID-19	Sirolimus treatment can cause immunosuppression and, therefore, potentially increase the risk of severe infection. We will ensure that patients with COVID-19 (+) are not enrolled in the study, regardless of their symptoms or severity.

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP), applicable United States (US) Code of Federal Regulations (CFR), and the NINDS Terms and Conditions of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form, recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	A futility trial of sirolimus in multiple system atrophy (MSA)
Study Description:	Single-center, randomized, placebo-controlled, phase-II, futility clinical trial to determine if oral sirolimus is of sufficient promise to slow disease progression in MSA, prior to embarking on a large-scale and costly phase III study to assess its efficacy. A futility design under the null hypothesis assumes that sirolimus will slow the progression of the disease, whereas the alternative hypothesis assumes no benefit of sirolimus. If the null hypothesis is rejected (i.e., futility of sirolimus to slow progression of MSA), a major phase III study will be discouraged, whereas non-futility will offer strong support for a phase III trial to detect clinical efficacy.
Objectives:	*Primary objective:* To determine if oral sirolimus is of sufficient promise or futile to slow disease progression in MSA. *Secondary objectives:* To assess the potential effects of sirolimus on retinal and brain neuroimaging in MSA *Exploratory objective:* To explore the potential effects of sirolimus on blood biomarkers (exosomal alpha-synuclein particles) in MSA.
Endpoints:	*Primary Endpoint:* Change from baseline to 48 weeks (or last available date) in United Multiple System Atrophy Rating Score (UMSARS) total score compared with described progression rates in historical controls. We will assume that patients receiving sirolimus will have a change of 4 points less than those reported in natural history studies (*see statistical section*). *Secondary Endpoints:* 1. Change from baseline to 48 weeks (or last available date) in UMSARS-2 subscale score. We will assume that patients receiving sirolimus will have a change of 2 points less than those reported in historical controls. 2. Change from baseline to 48 weeks (or last available date) in UMSARS-1 subscale score. We will assume that patients receiving sirolimus will have a change of 2 points less than those reported in historical controls. 3. Magnetic resonance changes using dedicated algorithms to evaluate rate of change in diffusivity of defined areas of brain from baseline to 48 weeks. 4. Changes in retinal nerve fiber layer using optical coherence tomography from baseline to 48 weeks. ***Exploratory outcome*** 1. Change in plasma biomarkers (exosomal alpha-synuclein particle concentration) from baseline to 48 months.

Study Population:	A total of 56 patients with multiple system atrophy will be randomized (42 to active agent and 14 to placebo)
Inclusion criteria:	1. Participants aged 30-80 years old with a diagnosis of MSA. Patients have to fulfill current consensus criteria (1) for probable MSA of the parkinsonian subtype (MSA-P) or cerebellar subtype (MSA-C). 2. Participants who are less than 4 years from the time of documented MSA diagnosis. 3. Participants who are still able to walk with or without assistance. 4. Participants with an anticipated survival of at least 3 years in the opinion of the investigator. 5. Participants who are willing and able to give informed consent. 6. Montreal Cognitive Assessment (MoCA) > 20. 7. Ability to take oral medication and be willing to adhere to the study drug regimen 8. For females of reproductive potential: use of highly effective contraception during study participation and for an additional 8 weeks after the end of study drug administration 9. For males of reproductive potential: use of condoms or other methods to ensure effective contraception with partner 10. Agreement to adhere to Lifestyle Considerations (see section 5.3) throughout study duration
Exclusion criteria:	1. Women of childbearing potential who do not practice an acceptable method of birth control. Acceptable methods of birth control in this study are: surgical sterilization, intrauterine devices, partner's vasectomy, a double-protection method (condom or diaphragm with spermicide), hormonal contraceptive drug (i.e., oral contraceptive, contraceptive patch, long-acting injectable contraceptive) with a required second mode of contraception. 2. Participants with a clinically significant or unstable medical or surgical condition that, in the opinion of the investigator, might preclude safe completion of the study or might affect the results of the study. These include but are not limited to severe, uncontrolled heart failure, recent (<6 months) myocardial infarct, severe, uncontrolled cardiopulmonary disease, severe, uncontrolled hypertension (sustained systolic blood pressure above 200 mmHg in any position), thrombocytopenia (< 50 x 10(9)/L), severe anemia (< 8g/dl), immunocompromised state, liver or kidney disease (creatinine > 1.5 mg/dl or proteinuria > 20 mg/dl), uncontrolled diabetes mellitus (HbA1c >10g%), alcoholism, amyloidosis, uncontrolled hypothyroidism, sympathectomy, unstable peripheral neuropathies, concurrent infections, severe orthopedic problems that compromise mobility and activity of daily living, recent (< 6 month) acute cerebrovascular accidents, neurotoxin or neuroactive drug exposure, parkinsonism due to drugs (including neuroleptics, alpha-methyldopa, reserpine, metoclopramide). 3. Participants with high LDL cholesterol levels (LDL > 190 mg/dL) and/or high triglycerides levels (> 500 mg/dL). Patients receiving

	medications for elevated cholesterol or triglycerides are eligible to participate as long as their LDL cholesterol and/or triglyceride levels are below the abovementioned limits. 4. Participants with positive interferon-gamma release-assay (QUANTIferon®) indicating latent tuberculosis infection. Participants with a positive Quantiferon® may be enrolled after the patient has been evaluated by an infectious diseases specialist that has cleared the patient for this trial. 5. Participants with history of untreated tuberculosis. Participants with a history of treated tuberculosis may be enrolled after an infectious diseases specialist evaluates patient and has cleared the patient for this trial. 6. Participants with a history of active, acute or chronic, or latent hepatitis B or hepatitis C. 7. Participants with a nasal swab PCR positive for COVID-19. If positive, participant may be re-screened after 1 month. If the second nasal swab PCR is negative for COVID-19, participant may be enrolled. 8. Participants with human immunodeficiency virus (HIV) infection, or other congenital or acquired causes of immunosuppression. 9. Participants with active malignant neoplasms or history of malignant neoplasm in the last 5 years. 10. Movement disorders other than MSA; e.g., Parkinson disease, dementia with Lewy bodies, essential tremor, progressive supranuclear palsy, spinocerebellar ataxia, spastic paraparesis, corticobasal degeneration, or vascular, pharmacological or post-encephalitic parkinsonism. 11. Dementia (DSM-V criteria). 12. History of electroconvulsive therapy. 13. History of deep brain stimulation surgery. 14. Patients with contraindication for MRI scanning, including those with MRI-incompatible pacemaker 15. History of organ transplant 16. Participants who have taken any investigational products within 60 days prior to baseline. 17. Treatment with cyclosporine, oral or intravenous corticosteroids, methotrexate, rituximab within 3 months prior to baseline. 18. Treatment with inhibitors of CYP3A4 (which may decrease the metabolism of sirolimus and increase sirolimus levels): nicardipine, verapamil, clotrimazole, fluconazole, itraconazole, clarithromycin, erythromycin, troleandomycin, cisapride, metoclopramide, bromocriptine, cimetidine, danazol, HIV-protease inhibitors (e.g., ritonavir, indinavir); grapefruit. 19. Treatment with inducers of CYP3A4 (which may increase the metabolism of sirolimus and decrease sirolimus levels): carbamazepine, phenobarbital, phenytoin, rifabutin, rifapentine. 20. Inability or unwillingness of subject to give written informed consent.

Phase:	Phase II
Description of Sites:	Single-center clinical trial performed at the New York University Langone Medical Center
Description of Study Intervention:	Oral sirolimus capsules at a dosage of 2 mg (one 2 mg capsule), 4 mg (two 2 mg capsules) or 6 mg (three 2 mg capsules) taken once a day. Dosage will be adjusted based on determination of sirolimus levels in blood performed throughout the trial and on the frequency of adverse events related to the study drug.
Study Duration:	3 years
Participant Duration:	48 weeks plus a safety follow-up phone call 2 weeks after the end of the trial.

1.2 SCHEMA

Month -1 Screening

- Total n=56
- Obtain informed consent
- Screen potential participants by inclusion and exclusion criteria
- Obtain history, document concomitant medications, MSA diagnosis and demographics
- Asessments: EKG, MoCA, Physical examination and UMSARS, vital signs
- Safety assessments: CBC, CMP, CPK, urinalysis and 24-hour urine collection, lipid panel, urine pregnacy test, HIV, hepatitis B and C, QuantiFERON

Day 1 Randomization and baseline visit

- Sirolimus (n=42)
- Placebo (n=14)
- Perform randomization
- EKG, MoCA, Physical examination and UMSARS, vital signs
- Safety assessments: CBC, CMP, CPK, urinalysis and 24-hour urine collection, lipid panel
- Collect blood for exosomes
- Perform brain MRI (+/- 5 days)
- Perform retinal OCT (+/- 5 days)
- Administer initial dose of study intervention
- Dispense study drug

Day 8, Day 15, Day 22 Study drug levels

- Measure sirolimus plasma levels (+3 days) and adjust study drug dose accordingly

Month 1 and Month 2 Safety labs (can be performed locally; adverse event recording performed over the phone)

- CBC, CMP, CPK, urinalysis, and 24-hour urine collection, lipid panel, and plasma sirolimus levels.
- Adverse event recording

Month 3 Follow-up assessments of study endpoints and safety

- Concomitant medications
- EKG, physical exam, vital signs, height and weight and UMSARS
- CBC, CMP, CPK, urinalysis and 24-hour urine collection, lipid panel, and plasma sirolimus levels.
- Adverse event recording
- Retrieve study drug
- Dispense study drug

Month 4 and Month 5 Safety labs (can be performed locally; adverse event recording performed over the phone)

- CBC, CMP, CPK, urinalysis and 24-hour urine collection, lipid panel, and plasma sirolimus levels.
  Adverse event recording

Month 6 Follow-up assessments of study endpoints and safety

- EKG, physical exam, vital signs, height and weight and UMSARS
- Adverse event recording
- CBC, CMP, CPK, urinalysis, and 24-hour urine collection lipid panel, and plasma sirolimus levels.
- Obtain blood for exosomes
- Retrieve study drug
- Dispense study drug

Month 7 and Month 8 Safety labs (can be performed locally; adverse event recording performed over the phone)

- CBC, CMP, CPK, urinalysis and 24-hour urine collection, lipid panel, and plasma sirolimus levels.
  Adverse event recording

Month 9 Follow-up assessment

- EKG, physical exam, vital signs, height and weight and UMSARS
- Adverse event recording
- CBC, CMP, CPK, urinalysis and 24-hour urine collection, lipid panel, and plasma sirolimus levels.
- Retrieve study drug
- Dispense study drug

Month 10 and Month 11 Safety labs (can be performed locally)

- CBC, CMP, CPK, urinalysis and 24-hour urine collection, lipid panel, and plasma sirolimus levels.
- Adverse evente recording

Month 12 End of study visit

- MoCA, Physical examination and UMSARS, vital signs
- Safety assessments: CBC, CMP, CPK, urinalysis and 24-hour urine collection, lipid panel
- Collect blood for exosomes
- Perform brain MRI (+/- 5 days)
- Perform retinal OCT (+/- 5 days)
- Retrieve study drug

Month 12.5 Follow-up phone call

- Adverse event recording

Month 13 Final safety assessment (can be performed locally; adverse event recoring performed over the phone)

- Safety assessments: CBC, CMP, CPK, urinalysis and 24-hour urine collection, lipid panel
- Adverse event recording

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	**Screening (V1)** Day -42 to -1	**Baseline / Random.** Visit 2, Day 1	**Study Visit 3** Day 7 +3 day	**Study Visit 4** Day 14 + 3 day	**Study Visit 5** Day 21 +3 day	**Study Visit 6** Day 28 +3 day	**Study Visit 7** Day 56 +15 day	**Study Visit 8** Day 84 +15 day	**Study Visit 9** Day 112 +15 day	**Study Visit 10** Day 140 +15 day	**Study Visit 11** Day 168 +15 day	**Study Visit 12** Day 196 +15 day	**Study Visit 13** Day 224 +15 day	**Study Visit 14** Day 252 +15 day	**Study Visit 15** Day 280 +15 day	**Study Visit 16** Day 308 +15 day	**End of study V17** Day 336 +15 day	**Phone followup V18** Day 350 +/-5 day	**Safety assess. V19** Day 365 +/-5 day
Visit type	S	S	SL	SL	SL	SL	SL	S	SL	SL	S	SL	SL	S	SL	SL	S	P	SL
Informed consent	X																		
Demographics	X																		
Medical history	X																		
Randomization		X																	
Administer study intervention		X																	
Dispense study drug		X						X			X			X					
Retrieve study drug								X			X			X			X		
Conclusion of study																			X
Conc. Medication review	X	X						X			X			X			X		
Physical exam	X	X						X			X			X			X		
Vital signs, height & weight	X	X						X			X			X			X		
MSA criteria	X																X		
MoCA	X	X[e]															X		
UMSARS	X	X[e]						X			X			X			X		
Sirolimus levels in blood [d]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Complete blood count	X	X[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Comprehensive metabolic [a]	X	X[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
CPK	X	X[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Lipid panel	X	X[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Urinalysis	X	X[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
24-hour urine collection	X	X[C]				X	X	X	X	X	X	X	X	X	X	X	X		X
Pregnancy test [b]	X																		
HIV testing, hepatitis B and C testing, QuantiFERON®	X																		
Nasal swab PCR COVID-19	X																		
EKG	X	X[e]						X			X			X			X		
Adverse event review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Brain MRI		X															X		
Retinal OCT		X															X		
Blood sample for exosomes		X									X						X		
Complete Case Report Forms	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose,potassium, total protein, AST, ALT, sodium.
B: Serum pregnancy test (women of childbearing potential).
c. 24-hour urine collection at baseline will be performed only if the baseline visit occurs > 30 days from the 24-hour urine assessment performed as part of the screening visit.
d. Sirolimus blood level should be drawn 7 days after previous dose adjustment has taken place.
e. If baseline visit (Visit 2) occurs within 15 days of the screening visit, the selected procedures can be foregone and the results of the screening day will be used.
S: In-site visit at NYU (denoted in grey)
SL: Safety labs visit, this can be done either at NYU or locally. If done locally, a phone call will be performed to inquire about adverse events. Safety labs should be drawn after a light (low fat) breakfast.
P: Phone call or video-conference call

Procedures	Screening (V1) Day -42 to -1	Baseline / Random. Visit 2, Day 1	Study Visit 3 Day 7 +3 day	Study Visit 4 Day 14 + 3 day	Study Visit 5 Day 21 +3 day	Study Visit 6 Day 28 +3 day	Study Visit 7 Day 56 +15 day	Study Visit 8 Day 84 +15 day	Study Visit 9 Day 112 +15 day	Study Visit 10 Day 140 +15 day	Study Visit 11 Day 168 +15 day	Study Visit 12 Day 196 +15 day	Study Visit 13 Day 224 +15 day	Study Visit 14 Day 252 +15 day	Study Visit 15 Day 280 +15 day	Study Visit 16 Day 308 +15 day	End of study V17 Day 336 +15 day	Phone followup V18 Day 350 +/-5 day	Safety assess. V19 Day 365 +/-5 day
CPK: Creatine Phosphokinase																			

2 INTRODUCTION

2.1 STUDY RATIONALE

Multiple system atrophy (MSA) is an adult-onset, fatal neurodegenerative disease characterized by progressive autonomic failure, parkinsonism, and cerebellar and pyramidal features in various combinations (2). Depending on their presenting predominant motor deficits, MSA is sub-classified into parkinsonian (MSA-P) or cerebellar (MSA-C) variant (3). MSA is an orphan disease (i.e., less than 200,000 cases in the U.S.). The estimated mean incidence is 0.6 to 0.7 cases per 100,000 person-years, with a range of 0.1 to 2.4 cases per 100,000 person-years (4). The estimated point prevalence is 3.4 to 4.9 cases per 100,000 inhabitants, increasing to 7.8 per 100,000 among persons older than 40 years of age (5). Cases of the parkinsonian subtype outnumber cases of the cerebellar subtype in most countries by 2:1 to 4:1, although the cerebellar subtype is more frequent in Japan (2). Disease onset is usually in the sixth decade of life, with both sexes equally affected (6). MSA is a relentlessly progressive disease, and the mean survival from the onset of symptoms is ~8 years (3, 6), with few patients with autopsy-proven MSA surviving more than 15 years (7).

MSA is the most fatally progressive of synucleinopathies, disorders characterized by the abnormal accumulation of the protein α-Synuclein (αSyn) in the nervous system (3, 8). In contrast to Parkinson disease (PD), in which αSyn predominantly accumulates in neurons forming Lewy bodies, in MSA it accumulates predominantly in glia forming glial cytoplasmic inclusions (GCI) (9). Immunohistochemical analysis of the inclusions shows that they stain with antibodies to a variety of proteins in addition to α-syn. These include ubiquitin, tubulin, synphilin-1, and DJ-1. Although GCI are the primary neuropathological hallmark of MSA, neuronal cytoplasmic and nuclear inclusions of αSyn have also been reported. Neuronal loss occurs in the striatum, cerebellum, brainstem, and cortex; and is accompanied by astrogliosis, microgliosis, and myelin loss (10, 11). Neurodegeneration in MSA also affects the retinal ganglion cells, a process that can be non-invasively quantified in-vivo with optical coherence tomography (OCT) (12-14).

Currently there are no disease-modifying treatments for MSA. Therefore, there is an urgent unmet medical need for therapies than can halt or slow the progression of this devastating disease. Many lines of evidence highlight the pathogenic importance of αSyn aggregation and spread in MSA (15-18). Although the mechanisms that lead to this aggregation remain to be elucidated, there is evidence to support roles for neuroinflammation (19), oxidative stress (20), and defective lysosome-mediated clearance of protein aggregates. Therefore, potential therapeutic options for MSA are aimed at correcting these mechanisms (21).

2.2 BACKGROUND

Autophagy is the major cellular digestion process that removes damaged macromolecules, organelles and abnormally misfolded proteins, including αSyn (22). Under normal conditions, autophagy is regulated by the protein mammalian target of rapamycin (mTOR): there is a tight, direct coupling of autophagy induction and mTOR inhibition (23). In synucleinopathies such as MSA, the presence of αSyn aggregates is associated with accumulation of autophagosomes and reduction of lysosomes in neurons, findings indicating a defect in phagosome-lysosome-mediated clearance of αSyn aggregates. In patients

with PD and MSA, misfolded αSyn fails to be processed and αSyn clearance is blocked (24, 25). Pharmacological activation of the autophagy function by inhibition of mTOR with rapamycin (a.k.a. sirolimus) produces neuroprotective effects in animal and cellular models of synucleinopathies (26-28). Thus, inhibition of the mTOR pathway with sirolimus has been proposed to be a highly efficient strategy to block αSyn-induced neurodegeneration (28, 29).

Sirolimus is an orally active macrolide compound and a potent immunosuppressant agent and is currently approved by the U.S. Food and Drug Administration in 1999 to prevent organ transplant rejection and, since 2015, for the treatment of lymphangioleiomiomatosis (LAM), a rare progressive lung disease (30). Thus, sirolimus has been widely used for almost 20 years and its adverse events profile is relatively safe and well known.

Sirolimus is also one of the few pharmacological molecules known to effectively induce autophagy (23).

Of interest to this protocol, there is ample evidence that sirolimus is lipophilic and crosses the brain-blood barrier of murine animals and humans (31-35), the levels of sirolimus in CSF and blood correlate in humans (31), and that sirolimus effectively inhibits mTOR activity in CNS cells of humans (31).
In addition to a potentially promising drug, two developments enhance the probability of success of this study. The first is that NYU is the main site of the international NIH-sponsored Natural History Study of Multiple System Atrophy (PI: Dr. Horacio Kaufmann), which is currently being conducted within the NIH-sponsored Rare Disease Autonomic Disorders Clinical Research Consortium (RDCRC; U54 NS065736; PI: Dr. David Robertson). This Natural History Study includes a cohort of ~200 patients with MSA fully characterized neurologically, being followed closely with validated instruments to fully document and define neurologic and autonomic symptoms and deficits. The second development is the use of instruments and objective outcome measures which have been validated in longitudinal studies of MSA patients. These include the Unified Multiple System Atrophy Rating Scale (UMSARS), measurements of putaminal diffusivity by brain magnetic resonance imaging (MRI), and measurement of retinal nerve fiber layer thickness by optical coherence tomography (OCT).

The UMSARS is a scale that comprises 4 parts. UMSARS-1 scores symptoms of neurological and autonomic dysfunction. UMSARS-2 is a motor examination. UMSARS-3 is a cardiovascular autonomic examination; and UMSARS-4, a global disability scale (36). The UMSARS in the only validated investigator-administered rating scale to reliably measure semi-quantitatively symptoms and signs in MSA. The instrument has high inter-rater reliability, and internal consistency, for instance the UMSARS-1 has a Crohnbach's alpha of 0.84 and the UMSARS-2 of 0.90 (36). The instrument is sensitive in measuring disease progression (37). In a study to assess rates of disease progression in MSA and validating UMSARS for sensitivity to change over time, MSA patients were assessed at two time points 12 months apart using the UMSARS 1-4. Fifty patients were assessed twice with an interval of 12.3 months. UMSARS-2 scores progressed by 57.3% ($P<0.0001$) and the UMSARS-1 scores by 35.6% ($P<0.0001$) in relation to the respective baseline scores with no differences between motor subtypes, diagnostic categories and gender (37). More recently, the minimally clinically important difference (MCID) for the UMSARS scores were determined (38): the MCID for the UMSARS-1 and the UMSARS-2 was 1.5 points, whereas it was of 3.5 points for the UMSARS total score (UMSARS-1 + UMSARS-2). Already completed natural history studies of MSA (8, 39) have reported the annual rates of change of UMSARS. This has also been reported in the placebo group of a previous clinical trial with rifampicin for MSA (40). These developments are relevant because appreciation of the MCID as well as the described annual changes in UMSARS are crucial for statistically significant effects and power analysis calculation (see statistical section).

The rate of progression of objective markers in MSA has been reported in longitudinal studies measuring the thickness of the retinal nerve fiber layer using retinal OCT (12-14), as well as in studies measuring changes in diffusion to water molecules in the pons and the putamen with specific sequences of brain MRI (41, 42). These markers have been specifically measured at baseline and after 12 months, are able to detect change, correlate with disease progression, and appear to be good candidates to be used as objective outcome measures in clinical trials of MSA.

2.2.1. Mechanism of action of sirolimus

Sirolimus inhibits T lymphocyte activation and proliferation that occurs in response to antigenic and cytokine (interleukin [IL]-2, IL-4, and IL-15) stimulation by a mechanism that is distinct from that of other immunosuppressants. Sirolimus also inhibits antibody production. In cells, sirolimus binds to the immunophilin, FK binding protein-12 (FKBP-12), to generate an immunosuppressive complex. The sirolimus:FKBP-12 complex binds to and inhibits the activation of the mTOR. This inhibition suppresses cytokine-driven T-cell proliferation, inhibiting the progression from the G_1 to the S phase of the cell cycle. Studies in experimental models show that sirolimus prolongs allograft (kidney, heart, skin, islet, small bowel, pancreatico-duodenal, and bone marrow) survival and reverses acute rejection of heart and kidney allografts in animal models. In some studies, the immunosuppressive effect of sirolimus lasted up to 6 months after discontinuation of therapy. In animal models of autoimmune disease, sirolimus suppresses immune-mediated events associated with systemic lupus erythematosus, collagen-induced arthritis, autoimmune type I diabetes, autoimmune myocarditis, experimental allergic encephalomyelitis, graft-versus-host disease, and autoimmune uveo-retinitis.

2.2.2. mTOR signaling pathway

mTOR is a serine/threonine protein kinase that oversees multiple functions in cells that involve gene transcription, protein synthesis, cytoskeletal organization, cell metabolism, proliferation and survival. In mammalian cells, there are two mTOR complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 controls protein homeostasis and autophagy, whereas mTORC2 regulates cell survival and proliferation. mTORC1 and mTORC2 share three regulatory components in common, including Deptor, mLST8 and Tti1/Tel2. Despite the presence of a catalytic mTOR subunit in both mTORC1 and mTORC2, mTORC1 (involved in autophagy) is more sensitive than mTORC2 (involved in cell survival) to the inhibition by sirolimus (25).

2.2.3. Pre-clinical data on efficacy of sirolimus in synucleinopathy models

Overall, there is accumulating evidence that treatment with sirolimus exerts neuroprotective effects and improves motor function in synucleinopathy mouse models by increasing αSyn autophagy and, possibly, reducing neuroinflammatory processes. Masliah's group (43) delivered a lentivirus expressing beclin-1, an activator of autophagy, into the brain of a synucleinopathy mouse model. This approach reduced αSyn pathology in the limbic system without deleterious effects. Rapamycin (i.e., sirolimus) enhanced the beneficial effects of beclin-1 (43). Malagelada and colleagues (44) found that treatment with rapamycin (i.e., sirolimus) at a dose of 7.5 mg/kg protected neurons from death in MPTP-induced mice models of synucleinopathy, by sparing phosphorylation of the survival kinase Akt. In an MPTP-induced synucleinopathy mouse model, treatment with rapamycin (i.e., sirolimus) at a dosage of 5 mg/kg/day for up to 4 days restored lysosomal levels, and attenuated αSyn accumulation, which was associated with an attenuation of neurodegeneration in the animals (29). Rapamycin (i.e., sirolimus) at a dosage of 5 mg/kg/day for 7 days protected against neuronal loss, and alleviated mitochondrial ultrastructural damage in a 6-hydroxydopamine rat model of synucleinopathy (45). Moreover, antioxidant activity and expression of anti-apoptotic markers was significantly higher in the synucleinopathy rats treated with rapamycin compared to those treated with placebo (45). Wills and colleagues (46) performed a dual

immunohistochemical staining with co-localization of αSyn with mTOR in striatal and midbrain sections of paraquat-induced synucleinopathy mice or control mice. Dual staining of αSyn and mTOR in striatal and midbrain sections showed increased levels of both proteins that were co-localized with one another in the synucleinopathy mice but not in the control mice. Western blot analysis confirmed increased levels of mTOR only in the synucleinopathy mice (46). To expand their results, the same group analyzed postmortem brain samples of patients with synucleinopathies finding similar results: mTOR levels were increased in the striatum of patients compared to age-matched controls (46). Liu and colleagues (47) showed that the number of dopaminergic neurons in the substantia nigra of MPTP-induced mice with synucleinopathy was significantly higher in animals treated with rapamycin compared to saline-treated controls. αSyn aggregates were significantly decreased in rapamycin-treated mice compared to controls (47). Bai and colleagues (48) studied whether a 24-week course of rapamycin at a dose of 2.25 mg/kg/day had any behavioral or motor effect in αSyn transgenic mice. Rapamycin-treated mice showed improved performance on the forepaw stepping adjustment test, accelerating rotarod and pole test (48). Siracusa and colleagues (49) showed that treatment with temsirolimus (an intravenous analog of sirolimus) at a dosage of 5 mg/kg/day for 8 days significantly ameliorated behavioral deficits, increased tyrosine hydroxylase and dopamine transporter expression, and decreased αSyn in the substantia nigra of MPTP-induced synucleinopathy mice. Western blot and immunohistochemistry showed that temsirolimus increased autophagy significantly. Temsirolimus also had anti-inflammatory properties as assessed by significant inhibition of the expression of mitogen-activated protein kinases such as p-JNK, p-p38, and p-ERK. Collectively, these findings suggest that inhibition of mTOR with sirolimus in MSA may be beneficial (26).

mTOR inhibition with sirolimus or analogs also has demonstrated neuroprotective effects in models of other disorders caused by abnormal accumulation of misfolded proteins such as Huntington's disease or Alzheimer's disease (35, 50-53).

Thus, this clinical trial is based on i) the unmet medical need of a disease-modifying treatment for patients with MSA; ii) the plausible biological mechanism of sirolimus increasing phagocytosis and clearance of αSyn; and iii) robust preclinical data from rigorously performed studies on the effect of sirolimus in cellular and murine models of synucleinopathy demonstrating that there is an adequate scientific foundation to justify this futility trial of sirolimus in patients with MSA.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Specific adverse reactions associated with the administration of sirolimus 2 mg once a day occurred at a significantly higher frequency than in the respective control group. These include hypercholesterolemia, hyperlipemia, hypertension, rash, acne, anemia, arthralgia, diarrhea, hypokalemia, and thrombocytopenia. The elevations of triglycerides and cholesterol and decreases in platelets and hemoglobin occurred in a dose related manner in patients receiving sirolimus (see FDA-approved package insert information (54)). Combination of sirolimus with statins and other medications for dyslipidemia has been shown to increase the risk of rhabdomyolysis. To evaluate the risk of rhabdomyolysis, CPK will be checked at each study visit including safety laboratory assessments, and patients will be specifically asked about new-onset myalgia at each study visit.

Risk involving non-contrast brain magnetic resonance imaging (MRI): There are no known risks or adverse effects resulting directly from exposure to non-contrast MRI. However, subjects who have a pacemaker or metal objects in their body such as shrapnel or metal in the eye should not have the scan performed. Every care will be taken to prevent this, including a thorough interview of each patient and review of his/her medical history to ensure that no metallic implants are present in patient's body.

There is a potential risk of burns in the event that patients are inadvertently carrying metallic ferromagnetic objects or particles in or on the patient. These include but are not limited to pulse 15ximeter, EKG leads, or skin tattoos. Every care will be taken to prevent this, including a thorough screening of each patient to ensure that no metallic materials are present in or on the patient's body. Additionally, the power limit of the brain MRI will be adjusted as necessary. The introduction of metallic ferromagnetic objects in the scanner room may result in magnetic displacement of the metallic object causing injuries to the patient. To reduce this risk, patients and every medical personnel near the MRI magnet will remove metal contents from them.

Some people may feel confined and experience anxiety in the MR scanner. In the patient experiences this during the scanning procedure, the scanner will be stopped immediately. Discussion with the PI and the radiology team will follow, and measures to prevent this could be implemented, such as administering a patient a low dose of anxiolytic medication prior to the scanning.

The MR scanner produces tapping sounds during operation, which may reach very loud levels. To minimize any discomfort from this noise, patients will be given disposable earplugs to reduce the noise levels but will still allow voice communication with the scanner operator.

In extremely rare cases, a magnet can lose its magnetism, in which case cooling fluids may be released noisily through escape valves and may collect in gas form in the scan room. The gas is not harmful in itself as long as fresh air is available. In this very remote event, the patient will immediately be brought out of the magnet room.

Risk involving retinal optical coherence tomography (OCT): The retinal OCT is a non-invasive procedure and there are no known risks or side effects from this procedure. Patients may experience some discomfort due to holding their head still for several minutes in the specific chin-piece while the OCT acquires the images. No pupil dilatation with eye drops is required.

Risks involving those of a blood draw. The most common complication of this procedure is a small bruise. Rarely infection can occur. Standing up can result in a drop in blood pressure.

Risk of orthostatic hypotension. Because selected participants with MSA may have symptomatic orthostatic hypotension, orthostatic syncope is a possibility, but we can almost invariably prevent such an event by having the participant sit back down or lie down. This risk should be no greater than that encountered during a standard clinical visit. Every care will be taken to prevent this occurrence.

2.3.2 KNOWN POTENTIAL BENEFITS

The potential benefits of this study are: The drug could potentially slow, halt or reverse the progression of the neurological abnormalities in MSA, although this is unproven. These include difficulties with speech, walking and autonomic function. There is also the possibility that the drug may be ineffective.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Given the rapidly progressive and fatal nature of multiple system atrophy, there is an urgent need for therapies that can halt or slow the progression of the disease. Thus, performing well-designed clinical trials with medications that have the potential of modifying the natural history of multiple system atrophy is a research priority. Because sirolimus is an FDA-approved medication with a well-known side effects profile, the potential benefits for patients outweigh the potential risks of the study drug.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine if oral sirolimus is of sufficient promise or futile to slow disease progression in patients with MSA	***Primary endpoint:*** Change from baseline to 48 weeks in United Multiple System Atrophy Rating Score (UMSARS) total score in patients taking sirolimus compared with described progression rates in historical controls. We will assume that patients receiving sirolimus will have a change of 4 points less than those reported in natural history studies (*see statistical section*). ***Secondary endpoints:*** Change from baseline to 48 weeks in United Multiple System Atrophy Rating Score (UMSARS) subscores (UMSARS-1 and UMSARS-2) in patients taking sirolimus compared with described progression rates in historical controls. We will assume that patients receiving sirolimus will have a change of 2.5 points less than those reported in natural history studies (*see statistical section*).	UMSARS is a patient-reported, semi-quantitative, validated, disease-specific scale representing the diverse signs and symptoms in MSA (36). The UMSARS in the only validated investigator-administered rating scale to reliably measure semi-quantitatively symptoms and signs in MSA. The instrument has high inter-rater reliability, and internal consistency, for instance the UMSARS-1 has a Crohnbach's alpha of 0.84 and the UMSARS-2 of 0.90 (36). The instrument is sensitive in measuring disease progression (37). It has an Activities of Daily Living score (UMSARS-1, 12 questions) that evaluates motor including autonomic activities and the Motor Examination score (UMSARS-2, 14 questions). UMSARS-3 measures supine/standing BP and UMSARS-4 is a disability scale (1-5). Higher scores on the UMSARS scales mean poorer health.
Secondary		
To assess the potential effects of sirolimus on brain neuroimaging and retinal biomarkers in patients with MSA	Brain magnetic resonance changes using dedicated algorithms to evaluate rate of change in diffusivity of defined areas of brain from baseline to 48 weeks in patients receiving sirolimus compared to the progression of putaminal diffusivity reported in longitudinal studies of MSA.	Increased putaminal diffusivity has been consistently reported, even in early disease stages of MSA (42, 55-59). Increase in putaminal diffusivity has been reported to be a sensitive neuroimaging measures of disease progression in patients with MSA and has been used previously as neuroimaging

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
		outcome measure in previous clinical trials of MSA (41, 42).
	Changes in retinal nerve fiber layer (RNFL) using optical coherence tomography from baseline to 48 weeks in patients receiving sirolimus compared to the rate of progression in longitudinal studies of MSA.	The use of optical coherence tomography (OCT) is emerging as a non-invasive fast technique to measure the thickness of the retinal layers. Even though patients with MSA only rarely have visual complaints, recent studies by our group using OCT showed that the retina of patients with MSA have consistent atrophy of the peripapillary retinal nerve fiber layer (RNFL) and to a lesser extent the macular ganglion cell layer (GCL) complex (13). These abnormalities are progressive overtime and have been recently confirmed by pathological examination in the retinas from three patients with MSA (12, 14).
Tertiary/Exploratory		
To explore the effects of sirolimus on blood biomarkers (exosomal alpha-synuclein particles) in patients with MSA	Change from baseline to week-48 in exosomal alpha-synuclein particle concentration in plasma in patients taking sirolimus versus the placebo group	The concentration of exosomal alpha-synuclein particles in plasma has been proposed as a biomarker of diagnosis of synucleinopathies. We hypothesize that sirolimus will increase autophagy and, therefore, the degradation of these exosomal alpha-synuclein particles.

4 STUDY DESIGN

4.1 OVERALL DESIGN

To determine whether to test sirolimus in a large phase 3 trial, we propose to conduct a clinical trial of sirolimus in MSA using a futility design. A futility design under the null hypothesis assumes that sirolimus will slow the progression of the disease, whereas the alternative hypothesis assumes no benefit of sirolimus. If the null hypothesis is rejected (i.e., futility of sirolimus to slow progression of MSA), major phase 3 studies will be discouraged, whereas non-futility will offer strong support for a phase III trial to detect clinical efficacy.

To test this hypothesis we will perform a single-center, randomized, double blind, placebo-controlled, phase-II, futility trial, chosen as an efficient approach to determine if sirolimus would be worthy of future investigation in larger, more definite trials. This trial was designed to compare the mean value for the primary outcome measure, change in total Unified Multiple System Atrophy Rating Scale (UMSARS) score from baseline to week 48, between the sirolimus arm and a pre-specified fixed value, representing a 4-point reducing in the expected mean decline without treatment; this fixed value was obtained using data from previously completed a large natural history study(39) and the placebo arm of a recently completed clinical trial in MSA (40). The 4-point reduction was based on the recently reported minimally clinically important decline (MICD) in the total UMSARS score of 3.5-points (38).

The null hypothesis is that sirolimus will reduce the mean decline in the total UMSARS score over 12 month by, at least 4-points, compared with the expected (fixed) value obtained from the historical controls. If the null hypothesis is rejected, then the drug would be considered futile for further investigation in MSA. If the null hypothesis is not rejected, sirolimus would be considered for a phase 3 clinical trial.

Thus, the primary goal is to evaluate the effect of sirolimus on progression of MSA, including autonomic, cerebellar and parkinsonian features as measured by the UMSARS, a relevant clinical measure of disease progression. Secondary goals are to determine if there is any effect on neuroimaging (brain MRI) and ophthalmological (retinal OCT) biomarkers of the disease. As exploratory aim we will determine if there are any changes in plasma αSyn exosomes, as surrogate markers of autophagy.

This study will include a placebo group in 3:1 proportion. Our reason for including a placebo arm is to facilitate blinding to treatment assignment and also to permit a descriptive assessment of the validity of the assumed change in UMSARS over time in the historical control group; the placebo group *will not* be used for primary outcome purposes with the active treatment arm.

This will be single-center study performed at the NYU Dysautonomia Center, one of the leading centers in the diagnosis and management of MSA. Dr. Horacio Kaufmann, the director of the Center, is the PI of the international Natural History Study of MSA, within the NIH-funded Rare Diseases Autonomic Disorders Consortium. NYU has the infrastructure and personnel in place to undertake this study and is experienced in the conduct of clinical trials. NYU is in a position to rapidly commence the study and conduct the study efficiently. We will review the option of adding additional sites if recruitment in the first 15 months is slow. Our aim is to complete recruitment within 24 months from commencement of the study.

Eligible participants, who signed the approved informed consent form at the screening visit, will initially receive sirolimus 2 mg/day (one 2-mg capsule/day) or matching placebo in a 3:1 fashion. The dose of sirolimus will be adjusted throughout the trial based on sirolimus blood levels and the presence of drug-related adverse events. The maximum dose of sirolimus will be 6 mg/day (three 2-mg capsules/day) or matching placebo. Patients receiving placebo will undergo analog sham level measurements and the number of capsules will be also adjusted to maintain the blinding of the trial. Patients will receive active agent or placebo for 48-weeks.

Participants will be evaluated at study clinic by the investigator at the screening visit and at baseline visit and will return to the clinic at Day 84 (month 3), Day 168 (month 6), Day 252 (month 9), and Day 363 (month 12) (+/- 10 days) of beginning the study treatment.

Detailed study plan

Screening visit (Visit -1, Day -42 to -1): Prior to performing any study activities/evaluations, the potential participant must be thoroughly informed about all aspects of the study, including scheduled study visits and activities, and must sign the informed consent. A signed copy of the approved informed consent will be given to the participant. Screening procedures will include demography, medical history, inclusion and exclusion criteria assessment, MSA diagnosis confirmation, physical examination, vital signs & height/weight, EKG, concomitant medication, adverse events, laboratory and safety testing (serum pregnancy test, if applicable, CBC, CMP, CPK, urinalysis and 24-hour urine collection (to quantify the protein/creatinine ratio), lipid panel, HIV, hepatitis B and C serologies, and QuantiFERON®), and UMSARS scale.

COVID-19 nasal swab PCR will be performed at screening. If negative, enroll patient. If positive, patient should be quarantined for at least 2 weeks and consult with their PCP to determine if any treatment is necessary. Participants can be re-screened after 4 weeks with a second nasal swab PCR for COVID-19. If negative, the patient can be enrolled. If still positive, patient will be definitely excluded and recommended continued follow-up with an infectious disease specialist.

If the subject has laboratory tests and EKG results available performed within 90 days before the screening date, it will be allowable to use these for the screening visit.

Safety labs should be performed at least 3 hours after a light (low fat) breakfast.

There will be a screening period of up to 42 days.

Baseline visit (Visit 1, Day 1): The following tasks/procedures will be performed at the clinic during the baseline visit:

- EKG
- MoCA
- Concomitant medications
- Physical examination
- Vitals signs
- CBC, CMP, CPK (8 ml of blood) urinalysis
- Lipid panel
- Dispensing of study medication

- UMSARS scale
- Blood sample for exosomes (2 ml of blood)
- Retinal optical coherence tomography
- 24-hour urine collection. This test should be performed only if the baseline visit occurs > 30 days from the screening visit.

If the baseline visit is performed less than 14 days from the screening visit the following procedures can be foregone, and the results will be assumed to be similar to those obtained at the screening visit:

- EKG
- MoCA
- CBC, CMP, CPK (8 ml of blood), urinalysis
- Lipid panel
- UMSARS scale

Participants will be dispensed study medication during the baseline visit. In case this is not possible, participants will be mailed the study medication within 30 days of the baseline visit.

The case report forms will note the date when the subject will start the study medication.

Participants will also undergo a non-contrast brain MRI with specific sequences to evaluate the diffusion at the putamen and pons. The brain MRI will be performed at the NYU Center for Biomedical Imaging. There will be a brain MRI period of up to 7 days after the baseline visit (i.e., subjects must be scanned within 7 days after the baseline visit).

Scheduled site visits: assessment of the primary outcome measure and safety labs will be conducted on-site at study visits at Visit 8 (month 3 after initiation of study drug), Visit 11 (month 6 after initiation of study drug), Visit 14 (month 9 after initiation of study drug), and Visit 17 (month 12 after initiation of study drug) (+ 15 days). We will accept a visit window of 15 days after the target date. The following tasks will be performed:

- EKG
- Concomitant medications
- Adverse event recording
- Physical exam
- Vital signs
- UMSARS scale
- CBC, CMP, CPK (8 ml of blood) and urinalysis with 24-hour urine collection
- Lipid panel
- Sirolimus levels in blood (5 ml of blood)
- Retrieve study drug
- Dispense study drug. If dispensation of study drug is not possible during the visit, the study drug will be mailed within 15 days.

Safety Laboratory Visits: Assessment of safety will be conducted on-site or locally at study visits 7 days (with a window of 3 days after the target date) after initiation of study drug (Study Visit 3), and 7 days after the patient has increased dose of study drug (i.e., Study Visit 4, Study Visit 5 and Study Visit 6, with

a window of 3 days after the target date) during the first month of study drug treatment. We will accept a visit window of 3 days after the target date of these visits.

Assessment of safety visits will also occur on-site or locally on Month 2 (Study Visit 7, this visit may be skipped in previous visits were delayed, to ensure that Visit 8 occurs at Month 3), Month 4 (Study Visit 9), Month 5 (Study Visit 10), Month 7 (Study Visit 12), Month 8 (Study Visit 13), Month 10 (Study Visit 15), and Month 11 (Study Visit 16). We will accept a visit window of 15 days before or after the target date of these visits.

The following safety laboratory assessments will be performed:

- CBC, CMP, CPK (8 ml of blood) and urinalysis with 24-hour urine collection
- Lipid panel (2 ml of blood)
- Sirolimus levels in blood (5 ml of blood)
- Adverse event reporting

In case the safety lab assessments are performed locally (e.g., at a local laboratory near the subjects' home), the PI or a licensed research member of the team will provide the patient with a prescription for the required tests. A research nurse, investigator or study manager will perform a phone call to assess adverse events within 7 days of the study visit.

To account for delays during the fist month of treatment, Visit 7 (2-month) may be skipped to ensure that Visit 8 occurs 3 months (+/- 15 days) after the initiation of the study drug.

To maintain the blinding, the results of the sirolimus levels in blood will remain blinded to the investigators and study coordinators and delivered to the independent pharmacist in charge of determining if the sirolimus dosage should be increased or decreased.

End of study visit/early termination: he following tasks/procedures will be performed at the clinic during the termination visit (Study Visit 17):

- EKG
- MoCA
- Concomitant medications
- Physical examination
- Vitals signs
- CBC, CMP, CPK (8 ml of blood) and urinalysis with 24-hour urine collection
- Lipid panel (2 ml of blood)
- Sirolimus levels in blood (5 ml of blood)
- Retrieve study medication
- Recording of AE
- UMSARS scale
- Blood sample for exosomes (2 ml of blood)
- Retinal optical coherence tomography
- Brain MRI

We will accept a visit window of 15 days before or after the target date of this visit.

The reason for discontinuation of therapy will be documented in the source documents and captured on the CRF. In the event that the study drug is discontinued early, an early termination visit should be

completed as close to the last dose date as possible. Participants will be followed for the duration of 12 months.

If a participant is withdrawn because of an adverse event, the appropriate "Withdrawal Section" of the CRFs should be fully completed in addition to the Adverse Event module. The Independent Medical Monitor should be informed of all participants who are withdrawn for this reason. Participants will be followed for 12 months.

Follow-up phone call: Patients will be contacted 14 days (+/- 5 days) after the End of Study visit to follow up on and record any Aes that were ongoing at the end of their previous visit (Study Visit 18). The follow-up visit may be conducted over the phone; however a response from the patient should be obtained. This response may be obtained by a phone call with the patient and appropriately documented in the source notes. In the event that the patient is unreachable by phone, all reasonable efforts should be made and documented before considering the patient lost to follow-up.

Safety assessment: Assessment of safety will be conducted on-site 30 days (+/- 5 days) after the End of Study visit (Study Visit 19). We accept a visit window of 3 days before or after the target date. The following safety laboratory assessments will be performed:

- CBC, CMP, CPK (8 ml of blood) and urinalysis with 24-hour urine collection
- Lipid panel (2 ml of blood)
- Adverse event reporting

Alternatively, these safety lab assessments can be performed locally (e.g., at a local laboratory near the subjects' home). In this case, the PI or a licensed research member of the team will provide the patient with a prescription for the required tests. Also, a research nurse or study manager will perform a phone call for adverse event reporting.

Unscheduled visit: An un-scheduled visit may be performed in-office or locally, should the investigators determine the need for so. The following safety laboratory assessments may be performed:

- CBC, CMP, CPK (8 ml of blood) and urinalysis with 24-hour urine collection
- Lipid panel (2 ml of blood)
- Sirolimus levels in blood (5 ml of blood)
- Adverse event reporting

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

To determine whether to test sirolimus in a large phase 3 trial, we propose to conduct a clinical trial of sirolimus in MSA using a futility design. This approaches minimizes the number of subjects and resources required for the study and provides an indication of whether or not to pursue larger and more definite studies. A futility design (60) is highly appropriate for MSA given: a) the low prevalence of the disease; b) the availability of historical controls from recent large studies (8, 39); and c) the availability of sensitive outcome measures. A futility design under the null hypothesis assumes that sirolimus will slow the progression of the disease, whereas the alternative hypothesis assumes no benefit of sirolimus. If the null hypothesis is rejected (i.e., futility of sirolimus to slow progression of MSA), a major phase III study will be discouraged, whereas non-futility will offer strong support for a phase III trial to detect clinical efficacy.

This study will also include a placebo group in 3:1 proportion. Our reason for including a placebo arm is to facilitate blinding to treatment assignment and also to permit a descriptive assessment of the validity of the assumed change in UMSARS over time in the historical control group; it will not be used as a basis of comparison for the active treatment arm.

This placebo-controlled futility design comparing outcome measures to historical controls has been used in other neurodegenerative disorders (61-63).

The study population (n=42 active agent, n=14 placebo) was selected based on power analysis calculation using the known annual progression of the UMSARS total score from published natural history studies (8, 39) and the progression in the placebo group of previous clinical trials of MSA(40), as well as from the minimally clinically significant difference in UMSARS (38) (see statistical section).

4.3 JUSTIFICATION FOR DOSE

We will use sirolimus dosages of 2 mg, 4 mg or 6 mg, once a day based on therapeutic drug monitoring in order to maintain sirolimus drug levels within a FDA-recommended target-range: 5-20 ng/ml. This daily dosage is within the range that is used clinically, and we expect this dose to be well tolerated. This is well below the currently FDA-approved maximum sirolimus dose administered per day, which is 40 mg (54). The use of sirolimus for 1 year is known to be safe, based on long experience in the prevention of organ transplant rejection, without significant complications (64, 65). Moreover, there is ample evidence that sirolimus at these dosages crosses the blood-brain barrier (31-35).

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

The end of the study occurs when the following circumstance have ALL occurred:

1. Completion of the last visit or procedure shown in the SoA in the trial globally.
2. Completion of data analysis
3. Completion of final study report

5 STUDY POPULATION

The study will include 56 participants, randomized in a 3:1 ratio to receive oral sirolimus or matching placebo. All participants will fulfill the diagnostic criteria for probable MSA outlined by the MSA Consensus Criteria (1). Participants with MSA-P and MSA-C diagnosed less than 4 years ago will be accepted into the study. This is because natural history studies have found that later stage MSA patients have only modest rates of progression while patients with milder symptoms have a more rapid rate of progression over 12 months. We hypothesize that these milder cases also have a greater probability to respond to effective treatment. We aim to study 56 participants 30-80 years of age. Sex will be approximately evenly represented. It is expected that approximately 10% of participants will be from ethnic minorities.

5.1 INCLUSION CRITERIA

1. Participants aged 30-80 years old with a diagnosis of MSA. Patients have to fulfill current consensus criteria (1) for probable MSA of the parkinsonian subtype (MSA-P) or cerebellar subtype (MSA-C).
2. Participants who are less than 4 years from the time of documented MSA diagnosis.
3. Participants who are still able to walk with or without assistance.
4. Participants with an anticipated survival of at least 3 years in the opinion of the investigator.
5. Participants who are willing and able to give informed consent.
6. Montreal Cognitive Asessment (MoCA) > 20.
7. Ability to take oral medication and be willing to adhere to the study drug regimen
8. For females of reproductive potential: agreement to use highly effective contraception method during study participation and for an additional 8 weeks after the end of study drug administration
9. For males of reproductive potential: use of condoms or other methods to ensure effective contraception with partner
10. Agreement to adhere to Lifestyle Considerations (see section 5.3) throughout study duration

5.2 EXCLUSION CRITERIA

1. Women of childbearing potential who do not practice an acceptable method of birth control. Acceptable methods of birth control in this study are: surgical sterilization, intrauterine devices, partner's vasectomy, a double-protection method (condom or diaphragm with spermicide), hormonal contraceptive drug (i.e., oral contraceptive, contraceptive patch, long-acting injectable contraceptive) with a required second mode of contraception.
2. Participants with a clinically significant or unstable medical or surgical condition that, in the opinion of the investigator, might preclude safe completion of the study or might affect the results of the study. These include severe uncontrolled congestive heart failure, recent (<6 months) myocardial infarct, severe uncontrolled cardiopulmonary disease, severe, uncontrolled hypertension (sustained systolic blood pressure above 200 mmHg in any position), thrombocytopenia (< 50 x 10(9)/L), severe anemia (< 8g/dl), immunocompromised state, liver or kidney disease (creatinine > 1.5 mg/dl or proteinuria > 20 mg/dl), uncontrolled diabetes mellitus (HbA1c >10g%), alcoholism, amyloidosis, uncontrolled hypothyroidism, sympathectomy, unstable peripheral neuropathies, concurrent infections, severe orthopedic problems that compromise mobility and activity of daily living, recent (< 6 months) cerebrovascular accidents, neurotoxin or neuroactive drug exposure, parkinsonism due to drugs (including neuroleptics, alpha-methyldopa, reserpine, metoclopramide).
3. Participants with high LDL cholesterol levels (LDL > 190 mg/dL) and/or high triglycerides levels (> 500 mg/dL) under fasting conditions. Patients receiving medications for elevated cholesterol or triglycerides are eligible to participate as long as their LDL cholesterol and/or triglyceride levels are below the abovementioned limits.
4. Participants with positive interferon-gamma release-assay (QUANTIferon®) indicating latent tuberculosis infection. Participants with a positive Quantiferon® may be enrolled after the patient has been evaluated by an infectious diseases specialist that has cleared the patient for this trial.

5. Participants with history of untreated tuberculosis. Participants with a history of treated tuberculosis may be enrolled after an infectious diseases specialist evaluates patient and has cleared the patient for this trial.
6. Participants with a history of active, acute or chronic, or latent hepatitis B or hepatitis C.
7. Participants with human immunodeficiency virus (HIV) infection, or other congenital or acquired causes of immunosuppression.
8. Participants with a nasal swab PCR positive for COVID-19. If positive, participant may be re-screened after 1 month. If the second nasal swab PCR is negative for COVID-19, participant may be enrolled.
9. Participants with active malignant neoplasms or history of malignant neoplasm in the last 5 years.
10. Movement disorders other than MSA; e.g., Parkinson disease, dementia with Lewy bodies, essential tremor, progressive supranuclear palsy, spinocerebellar ataxia, spastic paraparesis, corticobasal degeneration, or vascular, pharmacological or post-encephalitic parkinsonism.
11. Dementia (DSM-V criteria).
12. History of electroconvulsive therapy.
13. History of deep brain stimulation surgery.
14. Patients with contraindication for MRI scanning, including those with MRI-incompatible pacemaker
15. History of organ transplant
16. Participants who have taken any investigational products within 60 days prior to baseline.
17. Treatment with cyclosporine, oral or intravenous corticosteroids, methotrexate, rituximab within 3 months prior to baseline.
18. Treatment with inhibitors of CYP3A4 (which may decrease the metabolism of sirolimus and increase sirolimus levels): nicardipine, verapamil, clotrimazole, fluconazole, itraconazole, clarithromycin, erythromycin, troleandomycin, cisapride, metoclopramide, bromocriptine, cimetidine, danazol, HIV-protease inhibitors (e.g., ritonavir, indinavir); grapefruit.
19. Treatment with inducers of CYP3A4 (which may increase the metabolism of sirolimus and decrease sirolimus levels): carbamazepine, phenobarbital, phenytoin, rifabutin, rifapentine.
20. Inability or unwillingness of subject to give written informed consent.

5.3 LIFESTYLE CONSIDERATIONS

During this study, participants are asked to:

- Refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice from 30 days before the start of study drug until after the final dose.
- Participants who use tobacco products will be instructed that use of nicotine-containing products (including nicotine patches) will not be permitted while they are in the clinical unit.
- Minimize interactions with household contacts that may be immunocompromised.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to

respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

A screen failure log will be maintained in the study binders.

Individuals who do not meet the criteria for participation in this trial (screen failure) because of an unallowable medication may be rescreened after discontinuation of such medication. Individuals who do not meet the criteria for participation in this trial (screen failure) because of an acute infection may be rescreened after resolution of such infection. Rescreened participants should be assigned the same participant number as for the initial screening.

Individuals with a positive Quantiferon® will be recommended to see an infectious diseases specialist. In the event that the infectious diseases specialist considers that the positive Quantiferon® was a false positive, or that there is negligible safety risks for a patient with positive Quantiferon® to be enrolled in the study, the patient will be re-screened.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Patients with MSA will be recruited from those that we are currently following at the NYU Dysautonomia Center. Our Center currently follows approximately 100 patients with MSA. In addition, we are in close contact with MSA patients advocacy groups (e.g. *Multiple System Atrophy Coalition*) and will collaborate closely with them in order to disseminate information about the trial and increase the likelihood of recruitment, by means of e-mail blast or letter. The recruitment e-mail/letter to be sent to patients with MSA is attached as an appendix. Any recruitment e-mail or letter to be used in this trial will be submitted to the IRB for approval prior to use.

We expect to screen an average of 8 patients per month and accruing an average of 3 patients per month. At this predicted rate, we expect to complete enrollment of the subjects (n=56) within 24 months with patients recruited only at the NYU Dysautonomia Center. A special effort will be made to include women and minorities.

A screening and enrollment log to record the consent and screening of all subjects and the outcome of each screening will be maintained. The log will provide a comprehensive list of all subjects who were screened for eligibility as well as consented, including the screened failures, the reasons for ineligibility, and reasons for nonparticipation of eligible subjects. This information will be collected centrally, will be included in the Essential Documents Binder, and will be used to enhance subject recruitment efforts.

The principal investigators or co-investigators will approach patients with MSA during a routine clinic visit to inquire whether they would be interested in being enrolled in this clinical trial. If so, patients will be given the consent form. The subject and family will be given enough time to read the informed consent in a quiet room to maintain privacy. The principal investigators, co-investigator or clinical study manager will explain the design of the study, discuss it with the patient and answer any questions or concerns. If the patient is satisfied by the responses of the investigators, the principal investigator will ask the patient to sign the consent form before entering the study. The original signed form will be kept within the study files, and the patient and family will be given a copy.

Screening procedures will include demography, medical history, inclusion and exclusion criteria

assessment, disease diagnosis confirmation, physical examination, vital signs & height/weight, EKG, concomitant medication, adverse events, laboratory testing (serum pregnancy test, if applicable, CBC, metabolic panel, CPK, urinalysis and 24-hour urine collection, QuantiFERON®, hepatitis B and C and HIV testing), and UMSARS scale. There will be a screening period of up to 42 days.

To encourage retention, participants will be compensated for the time/travel/meals with $75 per visit. This will be done with a check payable to the subject, mailed to the subject's mail address within 30 days after the study visit.

5.5.1 USE OF DATACORE/EPIC INFORMATION FOR RECRUITMENT PURPOSES

This study will utilize EPIC to identify participants.

Any recruitment information sent by email will utilize Send Safe email.

Once potential participants have been identified, the study team will notify the treating physician (TP) that they have patients eligible to participate as follow:

- TP agrees to permit study team to directly contact potential participants on behalf of TP.
- TP has been notified that the study team will contact potential participants directly, phone, email, and/or the MyChart portal.

Once contact is made, approved recruitment language will be used to communicate the reason they are being contacted and participants will be asked if they are interested in participating in this specific study. Should the potential participants agree, the study team will provide the participants with information regarding the next steps for participation.

If a participant requests information regarding opting out of further recruitment for all research, participants will be directed to contact research-contact-optout@nyumc.org or 1-855-777-7858.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Sirolimus (a.k.a. rapamycin, a.k.a. Rapamune) is an immunosuppressive agent. Sirolimus is a macrocyclic lactone produced by *Streptomyces hygroscopicus*. The chemical name of sirolimus (also known as rapamycin) is (3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-hexadecahydro-9,27-dihydroxy-3-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3*H*-pyrido[2,1-*c*][1,4] oxaazacyclohentriacontine-1,5,11,28,29 (4*H*,6*H*,31*H*)-pentone.

Its molecular formula is $C_{51}H_{79}NO_{13}$ and its molecular weight is 914.2. Sirolimus is a white to off-white powder and is insoluble in water, but freely soluble in benzyl alcohol, chloroform, acetone, and acetonitrile.

Sirolimus is available for administration as an oral solution containing 1 mg/mL sirolimus. Sirolimus is also available as a tan, triangular-shaped tablet containing 0.5 mg sirolimus, as a white, triangular-shaped tablet containing 1 mg sirolimus, and as a yellow-to-beige triangular-shaped tablet containing 2 mg sirolimus. The inactive ingredients in sirolimus tablets include sucrose, lactose, polyethylene glycol 8000, calcium sulfate, microcrystalline cellulose, pharmaceutical glaze, talc, titanium dioxide, magnesium stearate, povidone, poloxamer 188, polyethylene glycol 20,000, glyceryl monooleate, carnauba wax, dl-alpha tocopherol, and other ingredients. The 0.5 mg and 2 mg dosage strengths also contain yellow iron (ferric) oxide and brown iron (ferric) oxide.

In this trial, sirolimus capsules (2 mg) and matching placebo will be manufactured in compliance with current GMP standards and guidelines applicable to study drugs. Sirolimus capsules (2 mg) and their matching placebo will be of identical appearance to maintain study blinding.

This trial will use sirolimus (Rapamune®) 2 mg tablets manufactured by *Pfizer* (NDC #: 0008-1032-05). Matching placebo will be compounded by Joseph Navarra, R.Ph., FACA, Town Total Compounding Center.

The matching placebo will be composed of lactose and encapsulated in an orange gelatin capsule.

6.1.2 DOSING AND ADMINISTRATION

During the baseline visit, participants will be allocated to a treatment group, based on a randomization procedure employing a 3:1 assignment ratio, and a scheme using blocks stratified by MSA type (cerebellar or parkinsonian) and sex. The randomization sequence will be performed by the independent medical monitor (IMM) and delivered, in a closed envelope, to Town Total Compounding Pharmacy (415 Crossways Park Drive, Suite B Woodbury NY 11797). The Town Total Compounding Pharmacy pharmacist will use the randomization order to send the proper medication to the study coordinator. The patient will be then instructed to take one capsule (i.e., 2 mg of sirolimus or matching placebo) daily and have sirolimus levels in blood 7 days (with a window of 3 days after the target date) after start of study drug.

Participants will then undergo a dose-selecting procedure based on therapeutic level measurements. The results of these measurements will be revealed only to an independent study pharmacist (independent from the Town Total Compounding pharmacy) who will make dosing recommendations to maintain sirolimus trough levels between 5 and 20 ng per milliliter, as well as corresponding sham dose adjustments in the placebo group.

Until the dose selection period has been completed, subjects will undergo sirolimus measurements in blood frequently during the first month of study drug treatment. Sirolimus levels will be measured again 7 days (with a window of 3 days after the target date) after a subject has been instructed to increase the dose of study drug. Once the initial dose selection has been achieved, subjects will then undergo measurements of sirolimus in blood levels monthly since the start of the study drug (with a window of 15 days after the target date).

The initial dose selection period is as follows: from day 1 to day 7, all subjects will receive sirolimus 2 mg one capsule daily or matching placebo. Seven days later (+ 3 days), subjects will undergo measurement of sirolimus levels. Once the results of the levels are back, the independent pharmacist will assess and determine if an increase in study drug is required.

- If sirolimus levels are < 5 ng/mL, the participant will be instructed to take sirolimus 2 mg two capsules daily (4 mg total dose) or matching placebo; sirolimus levels will be then measured again 7 days after the patient began taking 4 mg/day (+ 3 days). If sirolimus levels are between 5 and 20 ng/ml, the subject will continue with that dose until the next measurement of sirolimus levels on Visit (+ 3 days).
- If sirolimus levels are > 30 ng/ml with one capsule/day, the subject will be excluded from the trial due to the high risk of adverse events.

Subjects who were instructed to increase their dose to 4 mg will undergo another measurement of sirolimus levels 7 days after the previous dose adjustment (+ 3 days):

- If sirolimus levels are < 5 ng/mL, the participant will be instructed to increase sirolimus to 2 mg three capsules daily (6 mg total dose) or matching placebo. Sirolimus levels will be then measured 7 days after the patient began taking 6 mg/day (+ 3 days).
- If sirolimus levels are between 5 and 20 ng/ml, the subject will continue with that dose until the next measurement of sirolimus. If the sirolimus levels are > 20 ng/ml, the subject will be instructed to go back to the 2 mg/day dose and continue with that dose until the next measurement of sirolimus levels.

From Visit 3 to Visit 6, sirolimus level blood draw will take place 7 days (+3 days) after the previous dose adjustment has taken place (i.e., 7 days after the patient started taking the adjusted dose).

From Visit 7 onwards, subjects will be instructed to increase or decrease their sirolimus dose (between a min dose of 2 mg/day to a max dose of 6 mg/day) depending on blood levels (target: 5-20 ng/ml) and the emergence of adverse events.

Expected dose-limiting effects requiring sirolimus dose reduction are:

- Grade 2 stomatitis or mucositis
- Grade 2 lymphedema
- Grade 2 proteinuria
- Grade 2 renal failure
- Grade 3 pneumonitis
- Grade 2 diarrhea

Every attempt will be made to maintain the participant on the full dose. If the participant is unable to maintain the full dose, the minimum acceptable dose is 2 mg once a day. An attempt will be made to increase the dose with a re-challenge at a later date. Capsules should be swallowed whole with a glass of water or may be taken with food to minimize gastrointestinal side effects.

To maintain investigator and subject blinding during the clinical trial, the results of the sirolimus

measurements in blood will be revealed only to an independent study pharmacist who will make dosing recommendations, and a sham therapeutic drug level will be simulated for those patients receiving placebo (66) (see ***Measures to Minimize Bias*** section).

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Sirolimus capsules (2 mg) and matching placebo will be acquired in randomized per patient bottles from the Town Total Compounding Pharmacy and delivered to the study coordinator/ PI for dispensing to subjects

Town TOTAL Compounding Center
415 Crossways Park Dr., Suite B
Woodbury, N.Y. 11797
516-249-7436

The Town Total Compounding Center pharmacy will randomize, store and dispense study drug to our center.

The study coordinator will maintain drug accountability with a drug accountability log.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Sirolimus capsules (2 mg) and matching placebo capsules will be packaged in tight, light-resistant bottles as defined in the US Pharmacopeia. Sufficient bottles for 3 months of study will be packed for each participant, taking into consideration the potential increases in sirolimus dose after measuring sirolimus levels in blood. Each bottle label will bear the site number, investigator's name, participant number and the bottle number which comprises the participant number followed by a sequential number (01, 02, 03, 04, etc.).

The label on the bottles will include a fixed information section (to include the product name, storage conditions, instructions etc.) and variable information section. The variable information section will include 3 parts: 2 detachable parts, which will be attached to the participant's file upon bottle dispensing and 1 part to remain on the bottle. The variable information section will include the following variable data: site number, investigator name, participant number, bottle number, batch code and expiration date (all to be pre-printed on the label) and blank field for visit number to be manually filled in by the Investigator or designee upon provision of bottle to the participant.

Study drug supplies will be kept at the Total Town Compounding pharmacy, in a secure, limited-access, temperature/humidity-controlled area and a temperature/humidity log will be maintained. Only authorized personnel will have access to the study drug. The Total Town Compounding pharmacy personnel will be responsible for correct storage and handling of the study drug. The study drug bottles will be dispensed to the participant at the study site at each study visit starting at baseline visit (except for termination visit). The site number and investigator's name will be pre-printed on the bottle label. Since the participant number is pre-printed on the bottle label, assigning the right bottles to the

participants is straightforward. After assigning a bottle to the participant, the site personnel should fill in the visit number on the label of the dispensed bottle as well as on the detachable parts that would be attached to the participant's file.

At each dispensing visit, the participant will be dispensed with the study drug with the participant's number printed on the label. Any of the bottles designated for a participant can be dispensed in each of the dispensing visits. The bottle with the bottle number "participant # -R" is designated to replace damaged or lost bottles and should be used only for replacement.

In case a replacement bottle is required, the Total Town compounding pharmacist will be notified.

Participants will be instructed to return all used empty bottles and unused study drug at each visit.

The study coordinator is responsible for performing study drug accountability at the site and the accountability of the returned study drug.

6.2.3 PRODUCT STORAGE AND STABILITY

Capsules will be stored at 20□ to 25□C (US Pharmacopeia Controlled Room Temperature) (68□ - 77□F). If the study drug supplies appear to be damaged/missing upon arrival at the NYU Dysautonomia Center, the study manager should be contacted immediately. Each shipment of study drug supplies for the study will contain a shipment form describing the content of shipment. This form will assist in maintaining current and accurate inventory records. When a shipment is received, the NYU Dysautonomia Center will acknowledge receipt of the study drug supply by signing the acknowledgment of receipt section.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

After a participant meets the eligibility criteria, During the baseline visit, participants will be allocated to a treatment group, based on a randomization procedure employing a 3:1 assignment ratio, and a scheme using blocks stratified by MSA type (cerebellar or parkinsonian) and sex. The randomization sequence will be performed by the independent medical monitor (IMM) and delivered, in a closed envelope, to the Total Town compounding pharmacy. The compounding pharmacist will use the randomization order to send the proper medication to the study coordinator. The patient will be then instructed to take one capsule (i.e., 2 mg of sirolimus or matching placebo) daily and have sirolimus levels in blood at specific times throughout the study. Depending on the sirolimus levels, the sirolimus dose may be increased or decreased throughout the duration of the study.

A participant ID will be assigned by the center and kept in a written log. The participant IDs will be used to code the data obtained during the study visits, including brain MRI, OCT, and blood for exosomes. The participant IDs will follow the following format: three letters followed by three numbers (i.e., NYU001). The three letters correspond to center (i.e., New York University).

Drug Kit numbers will be assigned by the pharmacy and provided to the statistician who will then assign to the randomization block scheme. There will be an electronic validation of the participant's eligibility. If they are found to be eligible the system will send the drug kit number based on the known randomization sequence to the coordinator via electronic format. The drug kit number will also be used

by the pharmacist to send the proper medication. The Drug Kit number will not replace the Participant ID.

Study drug will be stored and dispensed by the study PI or research nurses delegated by the PI, located at:

NYULMC
530 First Ave.
Dysautonomia Center, suite 9Q
New York, NY 10016

Blinding
The participant, as well as investigators and any personnel involved in the participant's recruitment, assessment, monitoring, analysis and data management, will be blinded to the participant's treatment assignment.

The only personnel unblinded to the intervention will be the compounding pharmacist, the independent medical monitor (IMM), the independent pharmacist (independent from the compounding pharmacy and any other study members) for the purposes of sirolimus therapeutic levels monitoring. The independent pharmacist will maintain the blinding of the sirolimus levels and dose escalation during the clinical trial by make dosing recommendations for those patients receiving active agent, and by generating a sham therapeutic drug level for those patients receiving placebo. The independent study pharmacist will then make dosing recommendations to maintain sirolimus trough levels between 5 and 20 ng per milliliter in those patients taking active agent, as well as corresponding sham dose adjustments in patients taking placebo, in order to maintain investigator and subject blinding during the clinical trial.

Sirolimus therapeutic levels monitoring
An independent pharmacist will remain unblinded to each patient allocation and to the sirolimus blood level of each patient to allow the adjustment of such levels. The independent pharmacist will generate a simulated/sham value for patients receiving placebo in order to maintain the blinding of the investigators and the patients (66).

Emergency Unblinding
Unblinding of the participant's drug assignment will occur under any of the following circumstances:

1. Pregnancy.
2. Serious adverse event when the study drug assignment is required to make treatment decisions for the individual participant.
3. Death of participant when the death has been considered to be probably or possibly related to the study drug.
4. Death of participant when the relationship to study drug has been undetermined (e.g., after multiple efforts, the investigator team was unable to obtain medical records and/or death certificate).
5. Other circumstances that, under the opinion of the PI or the overseeing agencies, require emergency unblinding.

Unblinding of the participant's drug assignment will be done by the compounding pharmacist, the independent medical monitor, or the independent pharmacist, after the PI request. The IRB should be notified of the event prior to breaking of the code, if possible. If this is not possible, the IRB should be notified immediately afterwards, and the participant's drug assignment should be revealed. The circumstances leading to the breaking of the code should be fully documented, in the investigator's study files and in the participant's source documentation. The independent medical monitor and the compounding pharmacist will maintain the randomization lists and will be available to break the blind in the event of an emergency. A randomization list will be sent to the compounding pharmacist at the beginning of the study. A backup copy of the complete set of randomization assignments will also be given to the IMM and the PIs in a sealed envelope. We do not anticipate that it will ever be necessary that the PI will have to open this envelope during the trial. We will carefully document the reasons for any emergency unblinding should it ever become necessary to do this.

6.4 STUDY INTERVENTION COMPLIANCE

At each study visit, the investigator/site coordinator will assess the participant's compliance with the prescribed regimen for the study medication. This will include checks of protocol compliance and use of study drug in order to assess the reliability of participant-generated data. Participants who fail to comply with the study requirements may be withdrawn from the study, following consultation with the PI.

Compliance with the dosing regimen will be determined by performing study drug accountability of returned capsules and bottles of the study drug used and unused. The number of used, unused and lost capsules will be recorded on the CRF by the study coordinator.

Participants with less than 75% overall compliance during the entire study will be considered non-compliant. These participants will be included in the intention to treat analysis set and complete analysis set as defined in sections 12.3.1 and 12.3.2 respectively. However, these participants will be excluded as in the per-protocol analysis set.

Accountability records must be maintained at the site at all times. Accountability of the returned study drug should be performed and recorded at the site by the study coordinator. The participant number, the date, batch code, bottle number and quantity of study drug returned by the participant will be checked for correctness and recorded on the appropriate accountability forms.

6.5 CONCOMITANT THERAPY

Prohibited medications

- Intravenous immunoglobulin within 3 months prior to baseline visit
- Rituximab within 3 months prior to baseline visit
- Oral or intravenous corticosteroids within 3 months prior to baseline visit (topical or inhaled corticosteroids are allowable)
- Cyclosporine within 3 months prior to baseline visit
- Methotrexate within 3 months prior to baseline visit
- Azathioprine, mycophenolate or any other immunosuppressants or immunomodulatory drugs within 3 months prior to baseline visit
- Any anti-neoplastic or immunomodulatory agent including but not limited to nilotinib,

natalizumab, infliximab and bevacizumab within 3 months prior to baseline visit
- Any other investigational drug within 3 months prior to baseline visit

Allowed medications
- All anti-parkinsonian medications including but not limited to L-dopa, rasagiline, selegiline, amantadine.
- Fludrocortisone, pyridostigmine and vasopressin and analogs.
- Beta-blockers
- ACE inhibitors
- Alpha adrenergic agonists including but not limited to midodrine
- Norepinephrine precursors including but not limited to droxidopa
- Norepinephrine reuptake inhibitors including but not limited to atomoxetine or venlafaxine
- Serotonin reuptake inhibitors and other antidepressants
- Benzodiazepines
- Baclofen
- CoQ10
- Metformin and other anti-diabetic medications
- Topical or inhaled corticosteroids
- Non-steroidal anti-inflammatory drugs
- Topical anesthetic (e.g., lidocaine patch, lidocaine mouthwash)
- Statins
- Bile sequestrants
- Fibrates
- Trimethoprim/sulfamethoxazole
- Clindamycin
- Primaquine

Efforts will be made to maintain medications and dosages stable until the final study visit.

Standard non-pharmacologic measures to treat orthostatic hypotension are encouraged. These include the use of elastic stockings/abdominal binder, elevation of the head of the bed, increased fluid intake, and liberal salt intake. The participant is encouraged to maintain stable conditions of these measures.

Disallowed concomitant medications during the study:
- Treatment with inhibitors of CYP3A4 (which may decrease the metabolism of sirolimus and increase sirolimus levels) within 1 month prior to baseline visit: nicardipine, verapamil, clotrimazole, fluconazole, itraconazole, clarithromycin, erythromycin, troleandomycin, cisapride, metoclopramide, bromocriptine, cimetidine, danazol, HIV-protease inhibitors (e.g., ritonavir, indinavir); grapefruit.
- Treatment with inducers of CYP3A4 (which may increase the metabolism of sirolimus and decrease sirolimus levels) within 1 month prior to baseline visit: carbamazepine, phenobarbital, phenytoin, rifabutin, rifapentine.

6.5.1 RESCUE MEDICINE

Not applicable

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from study drug does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include the following:

- EKG
- Concomitant medications
- Physical examination
- Vitals signs
- CBC, CMP, CPK, urinalysis
- 24-hour urine collection
- Lipid panel
- Sirolimus levels in blood
- Retrieve study medication
- Recording of AE
- UMSARS scale

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which, in the opinion of the investigator, requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to receive study drug for 15 days or more.

The reason for participant discontinuation or withdrawal from the study will be recorded on the electronic Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for 2 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 10 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls, 3 e-mail messages and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Primary efficacy outcome

The Unified Multiple System Atrophy Rating Scale (UMSARS) total score. The primary outcome measure will be the total UMSARS score progression at week 48 compared to baseline. UMSARS is a validated, disease-specific scale representing the diverse signs and symptoms in MSA (36). It can assess rates of progression and is sensitive to change over time (37). It has an Activities of Daily Living score (UMSARS-1, 12 questions) that evaluates motor including autonomic activities and the Motor Examination score (UMSARS-2, 14 questions). UMSARS-3 measures supine/standing BP and UMSARS-4 is a disability scale (1-5). Higher scores on the UMSARS scales mean poorer health. The UMSARS will be determined by the same investigator at each study visit to ensure consistency. This investigator has undergone specific UMSARS training and has extensive experience in performing this scale. The investigator rating the UMSARS will be blinded to the previous UMSARS score of the patient as well as to the treatment group assignment of the subject.

Secondary efficacy outcomes

The Unified Multiple System Atrophy Rating Scale (UMSARS) part 1 and part 2. Secondary outcome measures include the UMSARS-2 score (neurological examination) progression at week 48 compared to baseline; the UMSARS-1 score (activity of daily living) progression at week 48 compared to baseline.

Neuroimaging: We will compare several diffusion parameters of the individual and combined putamen in subjects using brain magnetic resonance imaging (MRI) obtained at baseline and after 48 weeks. Diffusion-based MRI techniques measure how the random movement of water is altered in normal and disease nervous tissue. Diffusion MRI data can be analyzed with a mathematical tensor that provides

model parameters for the mean diffusion of water (the apparent diffusion coefficient, or "ADC"), the degree of diffusion orientation dependence (fractional anisotropy, or "FA") and the dominant orientation of water diffusion in an individual image voxel (first Eigen vector). In most neurodegenerative conditions, the tissue environment sampled by water becomes less organized over time and this is reflected by increasing ADC and decreasing FA. Compared to PD, increased putaminal ADC in MSA-P has been consistently reported, even in early disease stages (42, 55-59). Putaminal ADC changes have been reported to be a highly sensitive MRI marker of disease progression in patients with MSA and has been used previously as neuroimaging outcome measure in previous clinical trials of MSA (41, 42).

MRI will be carried out with a state-of-the-art 1.5-T MRI system and 24-channel head coil (Aera, Siemens Healthcare, Erlangen, Germany) – note, while 3-T has some signal-to-noise advantages, many MSA patients have cardiac pacer wires that make 3-T imaging difficult or impossible to obtain routinely. First a volumetric 3D T1-weighted MR-RAGE (TR/TE/TI 10/2/600 ms) and volumetric 3D SPACE FLAIR MRI sequences will both be obtained with 1-mm isotropic resolution (10 minutes total). Routine 3D susceptibility-weighted imaging will be obtained (5 min). Finally axial high-angular resolution diffusion MRI will be obtained of the whole brain using 3-mm isotropic resolution using echoplanar imaging, parallel imaging and 2-slice acceleration with simultaneous multi-slice acquisitions (TR/TE = 7000/100 ms, b = 0 and 1500 s/mm2, 30 gradient directions). These data will be analyzed with inline scanner diffusion tensor model and parameter maps of ADC, FA and fiber orientation generated. These will be co-registered with the volumetric data and manual regions-of-interest drawn to include the entire left and right putamen to extract mean ADC and FA. A secondary neuroimaging aim in this study will be to determine whole brain, cortical, white matter and individual structure volumes at baseline and after 48 weeks using automated FreeSurfer analysis as described in the literature (67).

Dr. Timothy Shepherd, Assistant Professor of Radiology at the NYU Center for Advanced Imaging Innovation and Research and Director of the NYU Brain Mapping Service (which performs fMRI and diffusion tractography for neurosurgical planning) will supervise creation of the MRI protocol, data acquisition, diffusion analysis and interpretation of the neuroimaging data. Dr. Shepherd is an academic neuroradiologist with MD/Ph.D. training in diffusion MRI and 6 years of experience performing advanced diffusion acquisitions in patients with MSA, Parkinson disease and other neurodegenerative diseases, brain tumors and epilepsy.

Retinal optical coherence tomography: Retinal nerve fiber layer thickness progression at week 48 compared to baseline. The use of optical coherence tomography (OCT) is emerging as a non-invasive fast technique to measure the thickness of the retinal layers. Even though patients with MSA only rarely have visual complaints, recent studies by our group using OCT showed that the retina of patients with MSA have consistent atrophy of the peripapillary retinal nerve fiber layer (RNFL) and to a lesser extent the macular ganglion cell layer (GCL) complex (13). These abnormalities are progressive overtime and have been recently confirmed by pathological examination in the retinas from three patients with MSA (12, 14). In brief, high definition OCT will be performed in a darkened room by the same trained personnel using standardized Food and Drug Administration (FDA)-approved equipment to evaluate the structure of the eye (Cirrus 4000, Carl Zeiss, CA, USA). Images will be acquired in the seated position with the subjects facing the OCT equipment. Subjects were instructed to fixate their gaze at a green target during the scan. Each subject had both eyes scanned at least three times using two standard acquisition protocols: macular cube (512x128 line scans) and optic disc cube (200x200 line scans). In both protocols the scanned area was a 6-mm cube with no signal averaging. The quality of the obtained images was assessed by evaluation of the signal strength (a value from 0-10 in arbitrary units)

automatically provided by the system. Only scans with signal strength above 6 units were included in the analysis. In some patients with MSA, involuntary eye movements will make the test difficult to obtain. In such cases, repeated scans will be performed to obtain at least three scans without eye movement artifacts. The scan with the best resolution will be used for analysis. For OCT quantification, two automatic segmentation algorithms will be used to determine the RNFL and ganglion cell complex (GCC) thicknesses (Cirrus 4000, Carl Zeiss, CA, USA). For the RNFL analysis, an optic disc cube of data (200x200-line scans) centered in the optic nerve head will be acquired. Subsequently, a recognition algorithm detected the inner (vitreoretinal interface) and outer (inner plexiform layer) border of the RNFL, from a 1.73-mm diameter circle will extract from the optic nerve cube and centered in the optic nerve head. The distance between the two lines will be measured as RNFL thickness at specific quadrants around the optic nerve: temporal, superior, nasal, inferior and global. For the GCC analysis the macular cube 512x128 centered in the foveal pit will be used. A different recognition algorithm will be applied to detect the outer border of the RNFL and the inner plexiform layer. The GCC analysis will evaluate the thickness of the ganglion cell plus inner plexiform layers. The average, minimum and sectorial thicknesses of the GCC will be measured in an elliptical annulus (vertical inner and outer radius of 0.5 mm and 2.0 mm respectively; horizontal inner and outer radius of 0.6 and 2.4 mm, respectively) around the fovea. The GCC thickness will be measured in different locations in the macula, around the foveal center: temporal-superior, superior, nasal-superior, nasal inferior, inferior, temporal-inferior, and global. The global RNFL and GCC thickness values, as well as those at each specific quadrant, expressed in micrometers (μm), will be used for analysis, although only the total RNFL will be used as secondary outcome measure.

Exploratory outcomes

Measurement of αSyn in CNS-derived extracellular vesicles (Evs): As exploratory outcome measure we will measure the concentration of αSyn in neuronal and oligodendroglial Evs isolated from patients' serum at baseline, on week 24, and on week 48. Recently, exosomes have been implicated in the dissemination of misfolded proteins in a variety of neurodegenerative disorders, including Parkinson disease.(68) Exosomes are Evs of 30–200 nm diameters that are released from various cells including neurons.(69) Exosomal release and transport of αSyn followed by intracellular uptake and induction of toxicity and cell death has been reported by several groups *in vivo* and *in vitro.*(70, 71) Several groups also have measured exosomal αSyn particles in cerebrospinal fluid (CSF) of patients with synucleinopathies.(72) However, because the lumbar puncture required for obtaining CSF is rather invasive and patients often refuse it, we opt to use a new methodology in which brain-derived Evs will be isolated from serum. The methodology has been introduced originally in 2014 in studies of Alzheimer's disease and frontotemporal dementia(73-75) and in two recent studies was applied also to patients with Parkinson disease.(76, 77) This methodology has been improved by Professor Gal Bitan's team at UCLA, who has been able to distinguish among healthy controls, patients with Parkinson disease, and patients with MSA with high sensitivity and specificity *(see preliminary data section).* Our group has been collaborating with Dr. Bitan and contributed part of the samples included in the preliminary data. Using the same protocol, we will obtain samples from the patients included in the study proposed here and ship them to the Bitan laboratory for analysis. In brief, 10 ml of blood will be extracted from each patient and centrifuged immediately to extract the serum. Because αSyn is one of the major biomarkers in our analysis, and in addition to neurons it exists in high levels in erythrocytes, it is crucial to separate the serum within a 20-minute window from drawing the blood, before hemolysis begins. The serum samples will be flash frozen immediately and stored at -80C. They will then be shipped on dry ice to Dr. Bitan's laboratory at UCLA, where the quantification of exosomal αSyn particles

will be performed by operators blinded to the study intervention. Dr. Bitan will receive only de-identified samples. He will have no way to link the samples to the subject's identities or PHI.

8.2 SAFETY AND OTHER ASSESSMENTS

The primary safety outcome is number of participants (%) who discontinue the study due to AE.

Secondary safety outcomes include active monitoring for severe adverse events (SAE's) throughout the trial. Specific SAE's are anticipated to be related to sirolimus. These selected SAE's include mucositis, anemia, thrombocytopenia, hyperglycemia, hyperlipidemia, proteinuria, renal failure, pneumonitis, and infection. The occurrences of these safety outcomes by treatment arm will be reported in bi-annual safety reports to the IMM. We will also report counts and proportions of mortality for each treatment arm (by rhythm), along with the number of SAEs that are probably or definitely related to mortality.

Adverse events will be recorded from the time a participant has signed the Informed Consent Form and throughout the 12 months of the study, even if they prematurely discontinue the study drug. They should be reviewed and updated at each subsequent visit and during any phone contact with the participant. Participants will be instructed to call a provided number if they experience AE between scheduled visits. An unscheduled visit may be needed so that the participant can be assessed.

Safety laboratory evaluations. Safety laboratory evaluations will be performed at screening, baseline, during each study visit and at termination visit. In addition, laboratory evaluations at months 2, 4, 5, 7, 8, 10 and 11 will be performed either at the NYU Dysautonomia Center or locally if the patient is unable to come to NYU. HIV testing, hepatitis B and C, QuantiFeron and serum pregnancy testing (women) will be performed only at screening. In addition, the following tests will be performed during all monthly safety assessments (Visit 7 and onwards).

- *Complete blood count with differential – approximately 1 tablespoon of blood will be required.*
- *Comprehensive metabolic panel and CPK– approximately 1 tablespoon of blood will be required.*
- *Lipid panel – approximately 1 tablespoon of blood will be required.*
- *Urinalysis – approximately a cup of urine will be required.*
- *24-hour urine collection – approximately 4 cups of urine, collected throughout a day, will be required*

Physical examination including the cardiovascular, dermatological, respiratory and gastrointestinal and neurological organ systems and ***vital signs, height and weight*** *will be performed at each scheduled and unscheduled visits.*

EKG: EKG will be performed locally at Screening and Termination visits. Twelve-lead ECG should be performed following the participant being in a supine position for 5 minutes. The investigator will evaluate the ECG at time of performance (signed and dated) and the printout should be kept in the source documentation file. If the investigator detects potentially clinically significant findings, a cardiologist should be consulted for a definitive interpretation. All communications and diagnoses should be filed in the source documentation file.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

All reported adverse events will be classified using the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute. The intensity or severity of the AE will be graded as follows:

- **Grade 1:** Mild AE
- **Grade 2:** Moderate AE
- **Grade 3:** Severe AE
- **Grade 4:** Life-threatening or disabling AE
- **Grade 5:** Death related to AE

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AE) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.

- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.3.3.3 EXPECTEDNESS

The Pis will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

Common adverse events of sirolimus that have been described in previous clinical trials (> 10% of patients) include:

- Blood and lymphatic disorders (anemia, leucopenia, thrombocytopenia)
- Dermatological disorders (acneiform rash, oral mucositis)
- Dyslipidemia (high cholesterol, high lipids)
- Hyperglycemia/glucose intolerance/new onset diabetes mellitus
- Hypertension
- Lymphocele
- Pneumonitis
- Renal disorders (proteinuria/increased creatinine/increased blood urea)
- Wound complications (healing problems)
- Infections (urinary tract infection, pneumonia)

In addition, patients with MSA have increased morbidity associated to the motor and autonomic dysfunction inherent to the disease, regardless of any study drug or intervention. Expected events that are expected to occur as part of the natural history of MSA are:

- Urinary tract infection
- Dizziness, lightheadedness or feeling about to faint
- Syncope due to orthostatic hypotension
- Shortness of breath upon standing
- Head or neck pain/discomfort upon standing
- Blurry vision or other vision changes upon standing
- Falls
- Aspiration pneumonia

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All Aes including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All Aes

occurring while on study must be documented appropriately regardless of relationship. All Aes will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 NON-SERIOUS ADVERSE EVENT REPORTING

Non-serious expected adverse events: non-serious expected adverse events that are reported to or observed by the investigator or a member of his research will be presented in tabular form and given to the IMM on a bi-annual basis or as requested.

Non-serious Aes that are expected or unexpected but do not increase the risk of the study drug will be reported in annual reports to the IRB.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

The research study team member will immediately report to the PI any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event.

Serious adverse events (e.g., death, hospitalizations) with evidence suggesting a probable or possible causal relationship between the study intervention and the event (e.g., death from anaphylaxis) will be reported by the PI or study coordinator to the IRB and the IIM **within 7 working days** after the PI's initial receipt of the information. If required, emergency unblinding will follow the criteria specified in section 6.3.

Serious adverse events (e.g., death, hospitalizations) with no evidence of a causal relationship between study intervention and event need to be communicated to the IMM **within 14 working days** after the PI's initial receipt of the information.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by any regulatory agency and should be provided as soon as possible.

The PI will be responsible for notifying the IRB, IMM and Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in **no case later than 7 calendar days** after the PI's initial receipt of the information. Emergency unblinding will follow the criteria specified in section 6.3.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (Ups) to the reviewing Institutional Review Board (IRB) and to the principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, Ups will be reported using the following timeline:

- Ups that are serious adverse events (SAEs) will be reported to the IRB and to the IMM within **7 working days** of the investigator becoming aware of the event.
- Any other UP that significantly increases the risk of the study will be reported to the IRB and to the IMM within **7 working days** of the investigator becoming aware of the problem.
- All UP that do not increase the risk of the study will be reported to the IMM in the bi-annual safety report.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Primary Efficacy Endpoint: We hypothesize that treatment with sirolimus will show promise to slow the progression of the disease of patients with MSA. Specifically, our null hypothesis is that patients receiving sirolimus will have a UMSARS total score progression of only 4.6 points at week 48 higher than baseline, compared to a progression of 8.6 points at week 48 from baseline reported in historical controls from natural history studies and placebo-controlled trials of MSA. Our alternate hypothesis is that sirolimus will be futile to slow the progression of the disease in patients with MSA. The statistical definitions are as follows:

H_0: $\theta_E - \theta_p \leq 4$ where $\theta_p = 8.6$ points increase at week 48 from baseline described in historical controls
H_0: $\theta_E \leq 4.6$ points increase at week 48 from baseline
H_1: $\theta_E > 4.6$ points increase at week 48 from baseline

Secondary Efficacy Endpoints:

Brain MRI: We hypothesize that treatment with sirolimus will slow the increase of putaminal diffusivity in patients with MSA. Specifically, our null hypothesis states that patients receiving sirolimus will have a putaminal Trace (D) total score progression of 0.030 x 10^{-3} mm^2/sec or less at week 48 versus baseline, compared to a progression of 0.070 x 10^{-3} mm^2/sec at week 48 from baseline reported in specific longitudinal studies of MSA (41).

H_0: $\theta_E - \theta_p \leq 0.04$ where $\theta_p = 0.07$ (x 10^{-3} mm^2/sec) increase in putaminal diffusivity at week 48 from baseline described in specific studies
H_0: $\theta_E \leq 0.03$ points increase at week 48 from baseline
H_1: $\theta_E > 0.03$ points increase at week 48 from baseline

Optical coherence tomography: We hypothesize that treatment with sirolimus will slow the thinning of the retinal nerve fiber layer (RNFL) in patients with MSA. Specifically, our null hypothesis states that patients receiving sirolimus will have a RNFL thickness thinning of 1.45 µm at week 48 versus baseline, compared to a decrease of 3.7 µm at week 48 from baseline reported in specific longitudinal studies of MSA (12).

H_0: $\theta_E - \theta_p \leq 2.25$ where $\theta_p =$ 3.7 µm decrease in RNFL at week 48 from baseline described in specific studies
H_0: $\theta_E \leq 1.45$ µm decrease at week 48 from baseline
H_1: $\theta_E > 1.45$ µm decrease at week 48 from baseline

Concentration of exosomal αSyn particles: We hypothesize that treatment with sirolimus will reduce the concentration of exosomal αSyn particles in patients with MSA. Specifically, our null hypothesis is that patients receiving sirolimus will have statistically lower concentration of exosomal αSyn particles at week 48 compared to baseline:

H_0: $\theta_E < \theta_p$
H_1: $\theta_E \geq \theta_p$

9.2 SAMPLE SIZE DETERMINATION

This is study is a randomized, double blind placebo-controlled futility trial in which the primary outcome measure value (UMSARS total score) in a cohort of patients with MSA taking active agent will be compared to a known value from historical controls previously published in the literature. The primary outcome measure was used to calculate the sample size.

The primary statistical analysis will involve a comparison of the mean absolute difference in the active sirolimus group with a pre-specified fixed value, constituting a 4-point reduction in the expected mean increase in the UMSARS total score over 48 weeks without treatment. The expected mean increase in total UMSARS outcomes were obtained from the *U.S. Natural History Study of MSA* (n=96) (39), that reported that the total UMSARS score at 12 months increased a mean of 7.5 (SD: 8.4) absolute points from baseline. Also data from a phase III double-blind placebo-controlled 12-month clinical trial with rifampicin in MSA performed in the U.S. (40) (which was found to be ineffective to slow the progression of the disease) showed that in patients taking placebo (n=50) the total UMSARS score at 12 months increased a mean of 10.8 (SD: 10.7) absolute points from baseline. A weighted analysis of these two populations showd he pooled total UMSARS score at 12-months in patients with MSA in large studies performed in the U.S. had increased a mean of 8.6 (SD: 9.2) points from baseline.

Also, recent studies (38) have determined that the minimally clinically important difference (MCID) in the total UMSARS score is 3.5 points after 48-weeks from baseline.

Taking into consideration this MCID, for this trial, we will assume a promising difference in the UMSARS total score of 4 points between those taking active agent and the historical controls. Specifically, our null hypothesis is that patients receiving sirolimus will have a UMSARS total score progression of only 4.6 points at week 48 higher than baseline, compared to a progression of 8.6 points at week 48 from baseline reported in historical controls from natural history studies and placebo-controlled trials of MSA. Our alternate hypothesis is that sirolimus will be futile to slow the progression of the disease in patients with MSA. The statistical definitions are as follows:

H_0: $\theta_E - \theta_p \leq 4$ where $\theta_p = 8.6$ points increase at week 48 from baseline described in historical controls
H_0: $\theta_E \leq 4.6$ points increase at week 48 from baseline
H_1: $\theta_E > 4.6$ points increase at week 48 from baseline

Therefore, assuming an alpha of 0.05, and a power of 0.8 (one sided, as patients with MSA have not been reported to improve spontaneously) to detect a progression of 4.6 points (i.e., 8.6 minus 4) in the UMSARS total score after 48-weeks from baseline we will need to enroll 38 patients in the active agent arm. Anticipating a 10% for dropouts rate, we will need to enroll 42 patients in the active agent arm.

This trial will include a placebo group to facilitate blinding in a 3:1 proportion. However, statistical comparisons for the primary and secondary efficacy outcome measures will be performed between the active agent arm and the historical controls. We will therefore enroll 14 patients in the placebo arm.

9.3 POPULATIONS FOR ANALYSES

Analyses will be performed according to the intention-to-treat principle and will include all randomized subjects who have completed, at least, 3 months of study drug.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

The two treatment groups (sirolimus and placebo) will be compared for baseline characteristics. This analysis will include demographic data and MSA history. The continuous variables will be examined using the two-sample t-test or Wilcoxon rank sum test when appropriate. For testing the assumption of normality prior to applying parametric approaches (i.e., two sample t-test), visual inspection of the normality plots as well as the Kolmogorov Smirnov test of normality will be used. We will use 5% level of significance for the test of normality. If the underlying data were to be non-normal, we will use non-parametric approaches for analyzing the data (i.e., Wilcoxon rank sum test). Categorical variables will be examined for differences between groups using the Chi-Square test or the Fisher's exact test when appropriate.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT

The primary outcome endpoint (UMSARS total score) will be analyzed by comparing the mean decline in total UMSARS score in the active sirolimus group (UMSARS total score at week 48 minus UMSARS total score at baseline) with the pre-specified fixed value of 8.6 (SD: 9.2) using a one-sample t-test. If the difference between the fixed value of 8.6 minus the UMSARS total score in the active sirolimus group is 4 or less, the null hypothesis will be rejected, indicating futility.

Missing data will be addressed by using a regression-based multiple imputation model (78)

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT

In order to maintain the overall type I error at the 0.05 level, an hierarchy will be employed as follows: If the primary endpoint is found to be significant at a significance level of 0.05, then the first key secondary endpoint will be tested; if this endpoint is found to be significant in a significance level of 0.05, then the second key secondary endpoint will be tested and so on. The key secondary endpoints and their order in the hierarchy are:

UMSARS sub-scores: The secondary outcome endpoint (UMSARS-1 and UMSARS-2 scores) will be analyzed by comparing the mean decline in total UMSARS-1 and UMSARS-2 score in the active sirolimus group (UMSARS-1 and UMSARS-2 at week 48 minus UMSARS-1 and UMSARS-2 at baseline) with the pre-specified fixed value of 4.5 (SD: 5.5) in each score using a one-sample t-test.

Brain magnetic resonance imaging: The secondary outcome endpoint (putaminal diffusivity) will be analyzed by comparing the mean increase in Trace(D) score in the active sirolimus group (Trace(D) score at week 48 minus Trace(D) score at baseline) with the pre-specified fixed value of 0.07 (x 10^{-3} mm^2/sec) using a one-sample t-test.

Retinal optical coherence tomography*:* The secondary outcome endpoint (retinal nerve fiber layer

thickness) will be analyzed by comparing the mean decline in RNFL thickness in the active sirolimus group (RNFL at baseline minus RNFL at week 48) with the pre-specified fixed value of 3.7 μm using a one-sample t-test.

Concentration of exosomal αSyn particles: This exploratory outcome endpoint will be analyzed by comparing the mean change in *αSyn exosomal particles* concentration (week 48 minus baseline) in the active sirolimus group with the placebo group.

In all cases missing data will be addressed by using a regression-based multiple imputation model (78)

9.4.4 SAFETY AND TOLERABILITY ANALYSES

Adverse events: The incidence and frequency of adverse events will be presented by System Organ Class and preferred terminology according to NCI and CTCAE reporting standard. Aes will also be presented by System Organ Class, High Level Term and preferred terminology. Data will be tabulated by treatment group, sex, age, maximal severity, maximal outcome, maximal action taken and maximal relationship to the tested drug. Serious adverse events and seriousness criteria will be listed and discussed on a case by case basis.

Laboratory tests*:* The incidence of laboratory tests outside the normal range and the incidence of measurements of potential clinical significance will be presented by treatment group. Shift analysis of these counts from baseline will also be provided. Descriptive statistics as well as their changes from baseline will also be presented by study group.

Vital Signs & Height/Weight*:* Incidence of measurements of potential clinical significance will be presented by study group. Shift analysis from baseline will be provided as well. Descriptive statistics of vital signs & height/weight, as well as, their changes from baseline, will be presented by study group.

EKG*:* The incidence of abnormal measurements will be presented by treatment group. Shift analysis from baseline will be provided as well. Descriptive statistics, as well as, their changes from baseline, will also be presented by study group.

Tolerability assessments: Tolerability analysis will be based on the number (%) of participants who failed to complete the study, the number (%) of participants who failed to complete the study due to adverse events. Time to withdrawal will be presented by Kaplan-Meier curves. Significance testing of time to withdrawal will be done using Cox's proportional hazards model.

9.4.5 PLANNED INTERIM ANALYSES

Interim efficacy analysis will be performed when 24 patients have completed the trial.

If the efficacy pre-specified criteria are fulfilled, the trial will be stopped for overwhelming efficacy. These pre-specified criteria are:

- The average increase in the total UMSARS score is 4 points or less from baseline to week 48 in patients taking active agent – OR –

- The average increase in the total UMSARS score from baseline to week 48 in patients taking active agent is significantly lower than in patients taking placebo with a P<0.001.

If the futility pre-specified criteria are fulfilled the trial will be stopped for futility. These pre-specified criteria are:

- The average increase in the total UMSARS score is at least 7 points or more from baseline to week 48 in patients taking active agent - OR –
- The average increase in the total UMSARS score from baseline to week 48 in patients taking active agent is significantly higher than in patients taking placebo with a P<0.001.

9.4.6 SUB-GROUP ANALYSES

Not applicable

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol:

- Consent form

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document.

All subjects will have the capacity to provide informed consent. The subject's capacity to provide informed consent will be confirmed by the subject's treating physician in the course of receiving clinical care.

The investigator or research coordinator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the

study with their family or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

After consultation with the PI, the IRB and the IMM the entire trial may be stopped if, at least, 40% of enrolled patients develop either frequent (5 or more events of Grade 2 or above) or severe (at least one event of Grade 3 or above) adverse effects.

Additional circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the NIH, IRB and/or Food and Drug Administration (FDA).

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor-investigator.

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

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not

limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored in TrialMaster This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a randomization number. TrialMaster will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NYU Dysautonomia Center.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the NYU Dysautonomia Center. After the study is completed, the de-identified, archived data will be stored at NYU Dysautonomia Center, for use by other researchers including those outside of the study. Permission to store data at the NYU Dysautonomia Center will be included in the informed consent.

All left over de-identified biological samples will be destroyed upon study completion. No lef over samples will be kept for future analyses or investigations.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Co-Principal Investigator	**Co-Principal Investigator**	**Independent Medical Monitor**
Horacio Kaufmann, MD	Jose-Alberto Palma MD PhD	Amir Steinberg MD PhD
New York University School of Medicine	New York University School of Medicine	Mount Sinai School of Medicine
Professor of Neurology	Associate Professor of Neurology	Associate Professor of Hematology and Oncology
530 First Ave Suite 9Q New York, NY 10016	530 First Ave Suite 9Q New York, NY 10016	Tisch Cancer Institute One Levy Place Box 1410 New York, NY 10029

212 263 7225	212 263 7225	212 241 6021
Horacio.Kaufmann@nyumc.org	*JoseAlberto.PalmaCarazo@nyumc.org*	*amir.steinberg@mountsinai.org*

10.1.6 SAFETY OVERSIGHT AND CLINICAL MONITORING

Because this is a single center study enrolling a small number of subjects and using a study drug that has been FDA-approved for over 10 years with a well-known profile of adverse event, an Independent Medical Monitor (IMM) will oversee the safety and data monitoring procedures of this study. The proposed IMM for this study is Amir Steinberg MD PhD, Associate Professor of Hematology & Oncology at the Mount Sinai School of Medicine. Dr. Steinberg has extensive expertise in sirolimus and its immunosuppressive side effects and has overseen several clinical trials in the past. Dr. Steinberg will be independent from study design and conduct and free from any potential conflict of interest.

The PI, IMM and independent pharmacist will have monthly conference calls to assess AE and the overall safety of the study. During the meeting, the PI will offer information on recruitment, screen failures, withdrawals and AE. The PI will be responsible for identifying AE. Aggregate report -detailed by severity, attribution (expected or unexpected), and relationship to the study drug/study procedures- will be available to the IMM for review on a monthly basis. Additional responsibilities of the IMM will include:

- Ensure that the rights and well being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable.
- The conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirements.
- Initial review of the research protocol and ongoing activities including data integrity, protocol adherence and study participant safety issues.
- Monthly review of adverse events and reasons for losses to follow-up.
- Raising any concern to the sponsor and PI and recommending the sponsor or PI continuation, modification or termination of the trial.
- Protect the confidentiality of the trial data and results of monitoring.
- Submit an annual report to the PI and the NYU IRB.

10.1.7 QUALITY ASSURANCE AND QUALITY CONTROL

The NYU Dysautonomia Center will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Study team weekly meetings will be conducted to review subjects status, enrolment, adverse events and other regulatory issues.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to study personnel for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the IMM will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The NYU Dysautonomia Center will provide direct access to all source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.8 DATA HANDLING AND RECORD KEEPING

10.1.8.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into TrialMaster, a 21 CFR Part 11-compliant data capture system provided by the NYU CTSI. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

Data quality will be assessed at the data entry point using intelligent on-line forms using TrialMaster. Data element constraints, whether independent range and/or format limitations or ‘relative’ referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency. In addition to those described above, we propose to build these checks into the initial tables and cross tabulations that should reveal any remaining data quality issues. All study data will comply with all applicable guidelines regarding participant confidentiality and data integrity.

***Registration*:** A system of coded identifiers will be used to protect participant confidentiality and safety. Each participant enrolled will be assigned a local identifier by the enrollment site. Local IDs should follow the following format: two letters followed by three numbers (i.e., JD001). The two letters correspond to the initials of the first name, and the last name of the patient (e.g., John Doe). When the participant is registered to participate in the study, using TrialMaster, the system will assign a participant ID number.

Data Entry: Data collection for this study will be accomplished with paper and online electronic case report forms by using TrialMaster. Using encrypted communication links, on-line forms will be developed that contain the requisite data fields.

Data Quality Control: As much as possible data quality is assessed at the data entry point. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency. In addition to those described above, we propose to build these checks into the initial tables and cross tabulations that should reveal any remaining data quality issues.

- Data Monitoring: The data manager will identify missing or unclear data and generates a data query to the study manager.
- Data Delinquency Tracking: The data manager will monitor data delinquency on an ongoing basis.

Laboratory data flow
On-line forms and/or electronic data exchange mechanisms to enter, update and obtain relevant data will be also available.
Additional Documents and Records

- Participant Screening and Assignment Log – A listing of all participants who signed the informed consent and were screened.
- Drug Accountability Log – This form documents the total amount of study drug dispensed to and returned by each participant throughout the study.
- Adverse event log
- Protocol deviation log

10.1.8.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

Primary source documents
The investigator must maintain primary source documents to support CRF data entries. These documents, which are considered "source data", may include but are not limited to:

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

The investigator must also retain all participant specific printouts/reports of tests/procedures performed as a requirement of the study. During monitoring visits, the monitor will need to verify data in the CRFs against these source data.

10.1.9 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 30 working days of identification of the protocol deviation, or within 40 working days of the scheduled protocol-required activity.

Protocol deviations that compromise patient safety will be reported to the IRB.

A protocol deviation log will be maintained.

10.1.10 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 10 years after the completion of the primary endpoint by contacting Horacio Kaufmann MD.

10.1.11 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NINDS has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ABBREVIATIONS

AE	Adverse Event
αSyn	Alpha-synuclein
CBC	Complete blood count
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Comprehensive metabolic panel
CNS	Central Nervous System
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CSF	Cerebrospinal Fluid
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
eCRF	Electronic Case Report Forms
EKG	Electrocardiogram
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GCI	Glial Cytoplasmic Inclusions
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IND	Investigational New Drug Application
IRB	Institutional Review Board
IMM	Independent Medical Monitor
ISO	International Organization for Standardization
MCID	Minimally Clinically Important Difference
MRI	Magnetic resonance imaging
MOP	Manual of Procedures
MoCA	Montreal Cognitive Assessment
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSA	Multiple System Atrophy
MSDS	Material Safety Data Sheet
mTOR	Mammalian Target of Rapamycin
mTORC1	Mammalian Target of Rapamycin Complex 1
mTORC2	Mammalian Target of Rapamycin Complex 2
NCT	National Clinical Trial
NIH	National Institutes of Health
NINDS	National Institutes of Neurological Disorders and Stroke
OCT	Optical coherence tomography
OHRP	Office for Human Research Protections
PI	Principal Investigator

PD	Parkinson disease
QA	Quality Assurance
QC	Quality Control
RDCRC	Rare Diseases Autonomic Disorders Clinical Research Consortium
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UMSARS	Unified Multiple System Atrophy Rating Scale
UP	Unanticipated Problem
US	United States

10.3 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change
1	1 June 2018	
2	3 September 2018	Minor changes including clarification of sirolimus levels schedule, clarification that sirolimus levels will be measured in whole blood, extension of the screening period to 42 days (instead of 30), and clarification that sirolimus (Rapamune®) marketed by Pfizer will be used (instead of generic sirolimus)
3	28 September 2018	Specification that the Town Total Compounding Pharmacy will perform the randomization and delivery of study drug, with no involvement of the NYU Investigational Pharmacy
4	15 October 18	24-hour urine collection at baseline will be performed only if baseline occurs > 30 days from the 24-h urine assessment of the screening. Sirolimus levels in blood during the first month will take place 7 days after the previous dose adjustment has taken place. Safety labs to the performed after a light (low fat) breakfast Clarification that the IMM is unblinded to the study allocation
5	19 March 19	Elimination of the need to perform autonomic testing to diagnose MSA as inclusion criteria Clarification of exclusion criteria including: -Specification that heart failure and cardiopulmonary disease should be severe and uncontrolled. - Specification that patients with positive Quantiferon® may be enrolled after an infectious diseases specialist has cleared the patient for this trial owing to the low probability of reactivation when patient is immunosuppressed with sirolimus. - Modification of the unhallowed high levels for LDL cholesterol and triglycerides -Clarification that study intervention will be modified based on study drug levels and the frequency of adverse events related to the study drug. Clarification that the active drug and placebo are administered in capsules, not in tablets Clarification of windows for study visits.

		Clarification on when sirolimus levels will be performed and how the dose adjustment will be performed Specification of Unscheduled Visits. Clarification on reporting of serious adverse events, both expected and unexpected. Clarification on when an interim analysis for efficacy will be performed Modification on how the ID of each subject Specification that a protocol deviation log will be created and maintained for this study will be obtained
6	25 Jul 2019	Clarification of target sirolimus levels to 5-20 ng/ml (instead of 10-20 ng/ml) Visit 7 may be skipped to account for delays in previous visits, to ensure that Visit 8 occurs 3-months after initiation of study drug. Interim analyses will ensure that there is evidence for the study to continue - or to declare it futile earlier than expected. Specification that the study will terminate if 30% of patients early withdraw due to disease progression or side effects
7	29 Oct 2019	The study will be using DataCore/EPIC for recruitment.
8	19 Dec 2019	Clarification of the criteria to unblind the allocation to active agent or placebo Specification that the PI and IMM and the independent pharmacist will have monthly conference calls to evaluate the safety of the study.

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APPENDICES

Appendix 1. Unified Multiple System Atrophy Rating Scale

Appendix 2. Montreal Cognitive Assessment