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Project Title: Safety, Cognitive, and Behavioral Outcomes in Patients with Dementia with Lewy Bodies Treated with Nilotinib

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CLINICAL TRIAL PROTOCOL

A randomized, double blind, placebo-controlled study to evaluate the impact of Nilotinib treatment on safety, tolerability, pharmacokinetics and biomarkers in Dementia with Lewy Bodies (DLB)

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
AD	Alzheimer's Disease
ADCS-ADL	Alzheimer's Disease Co-operative Study-Activity of Daily Living
ADAS-cog	Alzheimer's Disease Assessment Scale-cognition
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
Abl	Abelson Tyrosine Kinase
BBB	Blood Brain Barrier
BUN	Blood Urea Nitrogen
CIB	Clinical Investigator's Brochure
CBC	Complete Blood Count
CNS	Central Nervous System
CPK	Creatine Phosphokinase
CRF	Case Report Form
CSF	Cerebrospinal Fluid
C-SSRS	Columbia Suicide Severity Rating Scale
CRU	Clinical Research Unit
CML	Chronic Myeloid Leukemia
CAF	Clinical Assessment of Fluctuation
DSMB	Data Safety Monitoring Board
DDR1	Discoidin Domain Receptor-1
DLB	Dementia with Lewy bodies
DMSO	Dimethyl Sulfoxide
DOPAC	3.4-Dihydroxyphenylacetic acid
DA	Dopaminergic
EDC	Electronic Data Capture
ELISA	Enzyme-Linked Immunosorbent Assay
FTLD	Fronto-Temporal Lobar Dementia
EKG	Electrocardiogram
GUMC	Georgetown University Medical Center
γ-GT	Gamma-Glutamyl Transferase
HVA	Homovanillic Acid
Hb	Hemoglobin
HCG	Human Chorionic Gonadotropin
HDL	High Density Lipoprotein
ICH	International Conference on Harmonization
IND	Investigational New Drug Application
IHC	Immunohistochemistry
IRB	Institutional Review Board
ITT	Intent to treat
I.P.	Intraperitoneal
IAS	Irritability-Apathy Scale
IV	Intravenous
LP	Lumbar Puncture
LDL	Low Density Lipoprotein

LDH	Lactate Dehydrogenase
LB	Lewy Bodies
MoCA	Montreal Cognitive Assessment
MMSE	Mini Mental Status Exam
MDS	Movement Disorders Society
N	Number (typically refers to subjects)
NPI	Neuropsychiatric Inventory
NSE	Neuron Specific Enolase
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PHI	Protected Health Information
PHRC	Partners Human Research Committee
PDGF	Platelet Derived Growth Factor
PET	Positron Emission Tomography
PI	Principal Investigator
PT-INR	Prothrombin Time-International Normalized Ratio
PK	Pharmacokinetics
PDD	Parkinson's Disease with Dementia
PD	Parkinson's disease
PBAs	Problem Behaviors Assessment –short form
SAE	Serious Adverse Event/Serious Adverse Experience
SBP	Systolic Blood Pressure
SI	Site Investigator
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SN	Substantia Nigra
SCOPA-Cog	Scales for Outcomes in Parkinson's Disease-Cognition
Src	Protein Kinase SRC
TDP-43	Trans-Activation DNA Binding Protein-43
TKI	Tyrosine Kinase Inhibition
TNF	Tumor Necrosis Factor
TREM2	Triggering Receptors on Myeloid Cells
TMT-B	Trail Making Test-B
TUG	Timed-Up and Go
VEGF	Vascular Endothelial Growth Factor
UPDRS	Unified Parkinson's Disease Rating Scale
UIS	University Information Service
WBC	White Blood Cells

1- SUMMARY AND RATIONALE

1.1- Study Title

A randomized, double blind, placebo-controlled study to evaluate the impact of Nilotinib (Tasigna®) on safety, tolerability, pharmacokinetics and biomarkers in Dementia with Lewy Bodies (DLB)

1.2- Version Number

Original Protocol

1.3- Study Indication

Dementia with Lewy Bodies (DLB)

1.4- Phase of Development

II

1.5- Background

Dementia with Lewy Bodies (DLB) is an alpha-synucleinopathy (1,2) and the second most common form of dementia in the elderly. DLB shares striking neuropathological and clinical similarities with both Parkinson's disease (PD) (2) and Alzheimer's disease (AD) (3,4). The core clinical features of DLB, include dementia and Parkinsonism in addition to hallucinations, cognitive fluctuations and rapid eye movement (REM) sleep behavior disorders (RBD) (5-7). DLB and PD are characterized by death of dopaminergic (DA) neurons in the nigro-striatal system (8-13) and formation of inclusions known as Lewy bodies (LBs) that primarily contain aggregated alpha-synuclein (14-16). Misfolded alpha-synuclein accumulates intra-neuronally within LBs and SYN (alpha-synuclein) is the highest genetic risk factor for PD and DLB (1,2) followed by the microtubule associated protein tau (MAPT) (17-21). The neuropathology of DLB overlaps with both PD and AD, and include alpha-synuclein accumulation in LBs, hyper-phosphorylated tau (p-tau) and beta-amyloid deposition (4,22-27). At autopsy alpha-synuclein, p-tau and amyloid plaques may co-exist in the brains of individuals with DLB (26,28). Cerebrospinal fluid (CSF) biomarkers, including alpha-synuclein, dopamine metabolite homovanillic acid (HVA) (9,29), total tau and p-tau and amyloid beta peptides (Abeta40/42) maybe commonly shared in AD, PD and DLB (27,30-32). L-Dopa replacement therapies and acetylcholinesterase inhibitors may partially abate motor and cognitive symptoms, respectively in DLB. Selective Serotonin Re-uptake Inhibitors (SSRIs) and antipsychotics manage the behavioral but worsen motor symptoms in DLB.

A major challenge facing DLB is to develop a therapy that can halt neuronal death and alleviate cognitive, motor and behavioral symptoms. Although DLB is the second most common dementia and a debilitating movement disorder, it stands as a unique disease with complicated pathology and symptomatology; however, research of DLB has been largely overshadowed by AD and PD. No therapeutic approach exists to alter the levels of neurotoxic proteins such as alpha-synuclein, p-tau and Abeta40/42 and halt DA and other neuronal death in DLB. There is a major unmet medical need for further research into DLB to identify potential therapies for this disease and provide significant insights into the treatment of other Parkinsonian and memory disorders. One mechanism to degrade neurotoxic proteins is autophagy (33-37), which is a process by which the cell can degrade its own contents. There is evidence that autophagy is impaired in neurodegeneration (38-45), leading to failure of degradation of protein aggregates, including misfolded alpha-synuclein, beta amyloid and tau. Importantly, autophagy is exploited therapeutically in several diseases, including adult chronic myeloid leukemia (CML). Tyrosine kinase inhibitors (TKIs) induce autophagy (37,46,47), leading to destruction of rapidly dividing tumor cells in CML (46) and degradation of neurotoxic proteins, including alpha-synuclein,

beta-amyloid and p-tau in PD and AD models (37,46-49). Nilotinib (Tasigna®, AMN107, Novartis, Switzerland) is approved by the U.S. Food and Drug Administration (FDA) and is well tolerated for CML treatment at oral doses of 600-800mg daily. Nilotinib penetrates the brain and promotes autophagic degradation of neurotoxic proteins, leading to survival of DA and other neurons and improvement of motor and cognitive behavior in animal models of alpha-synucleinopathy and other neurodegenerative diseases (37,46-50). For these studies, Nilotinib (1-10mg/kg daily) was used at significantly less than the clinically approved dose (up to 1200mg daily) in CML (51-53). Additionally, Nilotinib levels peak in the mouse brain 4hrs after intraperitoneal (I.P) injection and wash out by 8hrs (37,47). This is important because the lower dose and short bio-availability (up to 8hrs) will prevent Nilotinib side effects and induce autophagic degradation of neurotoxic proteins in post-mitotic neurons, providing a strategy to prevent forcing neurons into apoptosis.

Based on strong pre-clinical evidence of the effects of Nilotinib on neurodegenerative pathologies, including autophagic clearance of neurotoxic proteins, neurotransmitters (dopamine and glutamate), immunity and behavior (49,50,54-58), we conducted an open label pilot clinical trial in individuals with advanced PD with dementia (PDD) and Dementia with Lewy Bodies (DLB) (59). Participants (N=12) were randomized 1:1 to once daily oral dose of 150mg and 300mg Nilotinib for 6 months. Our data suggest that Nilotinib penetrates the brain and inhibits brain Abelson (Abl) activity in animal models but achieves CSF concentration that would more preferentially targets Discoidin Domain Receptor (DDR-1) in humans , including PD and AD, independent of Abl activity (37,59-64). Several studies show that CSF alpha-synuclein, Abeta42, total tau and p-tau181 are altered in PD and DLB (65-67). Our data show stabilization of total CSF alpha-synuclein but a reduction in oligomeric alpha-synuclein levels between baseline and 6-months treatment with 150mg-300mg Nilotinib (see data). CSF HVA, which is an end by-product of dopamine, was also significantly increased; and CSF total tau and p-tau were significantly reduced (N=5, $P<0.05$) with 300mg Nilotinib between baseline and 6-month treatment. Despite the reduction of L-Dopa replacement therapies in this study, scores using the Unified Parkinson's Disease Rating Scale (UPDRS) I-IV improved with 150mg (3.5 points) and 300mg (11 points) from baseline to 6 months and worsened (13.7 points and 11.4 points) after 3-month withdrawal of 150mg and 300mg, respectively. Other non-motor functions e.g. constipation was resolved in all patients and cognition was also improved (3.5 points) using both the Mini-Mental Status Exam (MMSE) or the Scales for Outcomes in Parkinson's Disease-Cognition (SCOPA-Cog) between baseline and 6 months. MMSE scores returned to baseline after 3 months of Nilotinib withdrawal. It is important to note that participants who received oral daily dose of 150mg Nilotinib in this phase I open label study were all individuals with DLB. We conducted further phase II dose-finding random multiple dose studies that included placebo, 100mg, 200mg, 300mg and 400mg administered orally once 1-4 hours prior to lumbar puncture (LP) in PD patients. We observed that 200mg oral dose of Nilotinib results in a significant increase in CSF HVA and 3.4-didyroxyphenylacetic acid (DOPAC) concurrent with a decrease in CSF oligomeric alpha-synuclein (63). Recently, two randomized, double-blind, placebo-controlled clinical studies showed that Nilotinib can increase dopamine in the brain and reduce alpha-synuclein and Tau in Parkinson's disease (62,63). Nilotinib can lower CSF Tau and amyloid in the CSF and reduce amyloid burden (plaques) via amyloid Positron Emission Tomography (Amyloid PET) scans In Alzheimer's disease (64). These pathologies including dopamine loss, misfolded of alpha-synuclein and tau and increased amyloid burden are common in Dementia with Lewy Bodies. Taken together, data from our two studies indicate that 200mg Nilotinib may be an optimal dose to study in DLB patients. Our data are very compelling to evaluate the effects of 200mg Nilotinib in a phase II, randomized, double-blinded, placebo-controlled trial in patients with DLB.

We hypothesize that 200mg of Nilotinib is safe and tolerable and will alter CSF and plasma biomarkers in DLB patients.

a- Primary outcomes: We will determine safety and tolerability using the occurrence of adverse events (AEs) of interest, including myelosuppression, QTc prolongation, pancreatic and hepatic disorders as per Nilotinib Investigator Brochure (IB).

b- Secondary outcomes: We will determine 1) Nilotinib levels in CSF and plasma and 2) changes of DLB related CSF and plasma biomarkers, including HVA, DOPAC, Abeta40/42, total tau, ptau231/181 and total and oligomeric alpha-synuclein.

We will quantify brain amyloid burden via Florbetaben PET at baseline and 6 months at end of treatment.

We predict that Nilotinib will stabilize CSF total alpha-synuclein and reduce oligomeric alpha-synuclein, Abeta40/42 and p-tau.

c- Clinical outcomes will include assessment of (**A**) cognitive and behavioral function via Montreal Cognitive Assessment (MoCA), the Trail Making Test (TMT)-B, AD Assessment Scale-Cognitive subscale (ADAS-cog), AD Cooperative Study-Activity of Daily Living (ADCS-ADL), Neuropsychiatric Inventory (NPI), and Clinical Assessment of Fluctuation (CAF), Irritability-Apathy Scale (IAS) and Problem Behaviors Assessment short (PBA-s) form. We will also determine the effects of Nilotinib on (**B**) motor function via the Unified Parkinson's Disease Rating Scale (UPDRS)-I-III and timed-up-and-go (TUG).

1.6- Objectives and Endpoints

1- Primary outcomes: we will evaluate the effects of Nilotinib on safety and tolerability

a- Safety will be measured using the occurrence of adverse events (AEs) and serious adverse events (SAEs) deemed to be possibly, probably, or definitely related to the study drug. AEs of interest are defined as QTc prolongation, myelosuppression, hepatotoxicity and pancreatitis as listed in Table 1. These AEs will be tracked over the course of the trial and reviewed by the data and safety monitoring board (DSMB) at scheduled meetings and in real time. SAEs and AEs are known to be related to drug use at 800mg daily in cancer. A small safety trial using lower oral daily doses of 150 mg and 300 mg Nilotinib in 12 PDD (n=6) and DLB (n=6) patients showed one cardiac SAE over a six-month treatment period. Based on preliminary clinical data, investigator's brochure (IB) and scheduled EKGs and lab tests, SAEs and AEs will be evaluated real-time and on case-by-case basis.

b- Tolerability for a given participant will be defined as the ability of participants to remain on treatment. Overall tolerability of the drug will be defined as an acceptable number of up to 25% discontinuations.

Warnings and precautions: Nilotinib (Tasigna) prescribing information includes risks associated with arterial vascular occlusive events, including ischemic heart disease-related cardiac events, peripheral arterial occlusive disease and ischemic cerebrovascular events.

Nilotinib has been shown to prolong cardiac ventricular repolarization as measured by the QT interval on the surface EKG in a concentration-dependent manner. Prolongation of the QT interval can result in a type of ventricular tachycardia called torsade de pointes, which may result in syncope, seizure, and/or sudden death. **The baseline QTc range for inclusion into this study is 350-460 ms. If QTc prolongs ≥ 60 ms from baseline of individual participants AND to a value ≥ 480 ms, Nilotinib must be held and participants will be re-evaluated in one week on a case-by-case basis in consultation with a cardiologist before continuing on Nilotinib. If QTcF prolongs ≥ 500 ms then Nilotinib will be completely stopped and participants will be withdrawn from the study.**

Nilotinib should not be used in patients who have hypokalemia, hypomagnesemia or long QT syndrome. Before initiating Nilotinib electrolyte, calcium and magnesium blood levels should be tested. Hypokalemia or hypomagnesemia must be corrected prior to initiating Nilotinib and these electrolytes should be monitored periodically during drug treatment [See Table 3].

Gastrointestinal (GI) hemorrhage and effusions (including pleural effusion, pericardial effusion, ascites) or pulmonary edema were also reported in a small percentage of patients taken Nilotinib and GI hemorrhage and effusions should be monitored at every visit.

The bioavailability of Nilotinib is increased with food, thus Nilotinib must not be taken with food. No food should be consumed for at least 2 hours before and for at least 1 hour after the dose is taken.

Significant prolongation of the QT interval may occur when Nilotinib is inappropriately taken with food and/or strong CYP3A4 inhibitors and/or medicinal products with a known potential to prolong QT. Therefore, co-administration with food must be avoided and concomitant use with strong CYP3A4 inhibitors and/or medicinal products with a known potential to prolong QT should be avoided *[see exclusion criteria, section 4.3].*

The presence of hypokalemia and hypomagnesemia may further prolong the QT interval.

Table 1 summarizes some key laboratory tests to monitor AEs and SAEs and includes stopping rules and withdrawal for individual patients.

Table 1- Monitoring laboratory tests for drug safety and stopping rules or withdrawal. Grade 3 classification of organ toxicity is according to standardized definitions of AEs published by the National Cancer Institute (NCI) of the National Institutes of Health (NIH), known as the Common Terminology Criteria for AEs (CTCAE, Version 4.0) or common toxicity criteria.

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.

Grade 4 Life-threatening consequences; urgent intervention indicated.

Grade 5 Death related to AE.

Assessments	Toxicity Criteria	Endpoints
Myelosuppression ≥Grade 3	Thrombocytopenia and Neutropenia	Perform complete blood counts (CBC) every month or as needed. Withhold Nilotinib for hematological toxicities if absolute neutrophil count (ANC) <1.0 x 10^9 /L and/or platelet count <50 x 10^9 /L. Monitor blood counts every 7 days and if problem resolves resume treatment within 2 weeks. Patient's blood counts must be monitored every 7 days. If Myelosuppression recurs patients must be withdrawn.
QT Prolongation ≥Grade 3	EKGs with a QTc ≥480 ms and/or an increase of QTc ≥ 60 ms from baseline	Withhold Nilotinib and perform an analysis of serum potassium and magnesium, and if below lower limit of normal, correct with supplements to within normal limits. Concomitant medication usage must be reviewed. Patients with prolonged QTc ≥ 60 ms change from baseline or QTc ≥480 ms should be withdrawn from the study unless there is a causative electrolyte abnormality that has been corrected or CYP inhibitor that may be withdrawn. Resume within 2 weeks if QTcF returns to <450 ms and to within 20 ms of baseline. Repeat EKG 7 days after dose adjustment and if QTcF returns to ≥ 480 ms following withholding, Nilotinib must be discontinued.
Hepatotoxicity ≥Grade 3	Elevated hepatic transaminases	Withhold Nilotinib and monitor hepatic transaminases, including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase every 7 days. If transaminase level returns to baseline, resume treatment and monitor patients every 7 days. If hepatotoxicity recurs patients must be withdrawn from the study.

Bilirubin ≥Grade 3	Elevated Bilirubin	Withhold Nilotinib and monitor bilirubin every 7 days. If bilirubin level returns to baseline, resume treatment and monitor patients every 7 days. If bilirubin elevation recurs patients must be withdrawn from the study.
Pancreatitis ≥Grade 3	Elevated serum lipase and amylase	Withhold Nilotinib and monitor lipase and amylase every 7 days. If lipase and amylase levels return to baseline, resume treatment and monitor patients every 7 days. If pancreatic toxicity recurs patients must be withdrawn from the study.

2-Secondary outcomes:
a) Pharmacokinetics: We will measure the CSF concentration of Nilotinib.
b) Pharmacodynamics: We will determine the effects of Nilotinib on primary biomarkers, including changes of CSF levels of HVA between baseline and 6 months. Based on the data collected from the proof of concept study in PD we predict an increase in CSF HVA and DOPAC levels following Nilotinib treatment.

We will also measure the CSF concentration of surrogate/exploratory biomarkers: including:

1) Abeta40/42, total tau, ptau231/181 and total and oligomeric alpha-synuclein
2) Quantify brain amyloid burden via Florbetaben PET at baseline and 6 months at end of treatment.

- We will use ELISA to measure exploratory CSF and plasma neuro-inflammatory/modulatory markers, including Triggering Receptors on Myeloid Cells (TREM)-2 interleukins (IL)- 1α&β, 2, 3, 4, 5, 6, 7, 8 (CXCL8), 9, 10, 12, 13, 15, 17 α, and chemokines (C-C) including, CXCL10, CCL2, CL7, CCL22, CCL3, CCL4, platelet-derived growth factor (PDGF)-AA, PDGF-AB/BB, CCL5, CX3CL1 (fractalkine), Tumor necrosis growth factor (TNF)-α, transforming growth factor (TGF)-α, and vascular endothelial growth factor (VEGF). We will also measure NSE and S100B as markers of cell death.

3-Clinical outcomes will include assessment of:

a) cognitive and behavioral function via Montreal Cognitive Assessment (MoCA), the Trail Making Test (TMT)-B, AD Assessment Scale-Cognitive subscale (ADAS-cog), AD Cooperative Study-Activity of Daily Living (ADCS-ADL), Neuropsychiatric Inventory (NPI), and Clinical Assessment of Fluctuation (CAF), Irritability-Apathy Scale (IAS) and Problem Behaviors Assessment short (PBA-s) form.
b) We will also determine the effects of Nilotinib on motor function via the Unified Parkinson's Disease Rating Scale (UPDRS)-I-III and timed-up-and-go (TUG).

Based on preclinical and clinical studies (see below), **we hypothesize that Nilotinib will be safe and tolerated in individuals with DLB.** We also predict that Nilotinib will be detected in the CSF. Nilotinib will also increase CSF HVA and DOPAC levels and improve cognitive and motor symptoms in DLB patients. Nilotinib may also stabilize CSF levels of total alpha-synuclein, and reduce oligomeric alpha-synuclein and p-tau levels and reduce CSF amyloid and brain amyloid burden. Nilotinib may affect cell death markers, including NSE and S100B and phosphorylated neurofilaments. We further hypothesize

that we may see evidence of change in surrogate and biomarkers of pathophysiology as outlined in our secondary outcomes that will help us to build a better clinical development program going forward.

1.7- Rationale to advance to Phase II Nilotinib trial in DLB (Study Rationale)

We performed an open label phase I clinical trial using two commercially available doses of Nilotinib (150 and 300mg capsules) in patients with advanced PDD and DLB. These indications have some overlapping pathologies and clinical symptoms and share common plasma and CSF biomarkers, including loss of dopamine and changes in alpha-synuclein, Abeta42/40, total tau and p-tau (181, 231). We obtained preliminary data showing that Nilotinib crosses the blood-brain-barrier (BBB) and is detected in the CSF, suggesting Abl inhibition and downstream target engagement (alpha-synuclein, tau and Abeta42) in the CNS (pharmacodynamics). Nilotinib increased CSF HVA and DOPAC levels as a downstream biomarker of dopamine metabolism. These data provide strong feasibility to test Nilotinib in a larger placebo-controlled, phase II clinical trial to demonstrate safety, tolerability, pharmacokinetics/pharmacodynamics and changes in disease biomarkers and clinical outcomes in patients with DLB. Recently, two randomized, double-blind, placebo-controlled clinical studies showed that Nilotinib can increase dopamine in the brain and reduce alpha-synuclein and Tau in moderately advanced PD patients (62,63). Nilotinib can lower CSF Tau and amyloid in the CSF and reduce amyloid burden (plaques) via amyloid Positron Emission Tomography (Amyloid PET) scans in participnats with dementia due to mild-to-moderate AD (64). Pharmacokinetics studies showed that alteration of these toxic proteins was independent of Abl Inhibition as Nilotinib reached a CSF concentration sufficient to inhibit DDR1 receptors but not Abl. These pathologies including dopamine loss, misfolded of alpha-synuclein and tau and increased amyloid burden are common in Dementia with Lewy Bodies. Taken together, data from our studies indicate that 200mg Nilotinib may be an optimal dose to study in DLB patients. Our data are very compelling to evaluate the effects of 200mg Nilotinib in a phase II, randomized, double-blinded, placebo-controlled trial in patients with DLB.

3) Study Design

We propose to perform a phase II randomized, double-blinded, placebo-controlled study to evaluate the impact of an oral daily dose of 200mg Nilotinib on safety, tolerability, pharmacokinetics, pharmacodynamics and clinical outcomes in patients with DLB.

2.1- Treatment: Sixty (60) participants will be recruited and randomly assigned 1:1 to placebo (group 1) or 200 mg Nilotinib (group 2). Participants will be treated for 6 months and monitored every month (4 weeks) in a total of up to 11 visits that include screening, baseline, 1, 2, 3, 4, 5, 6 months follow up and 7-month washout. Blood and cerebrospinal fluid (CSF) will be collected at baseline and at 6 months to determine Nilotinib effects on CSF biomarkers. Amyloid PET will also be conducted at baseline and end of treatment.

2.2- Drug Administration: Nilotinib will be taken orally once daily (taken without a meal) for 6 calendar months and 1 month wash out. Study partners must provide written informed consent prior to screening. Based on data obtained in our proof of concept phase I study, a phase II study will primarily examine the effects of Nilotinib on safety and tolerability and clinical outcomes.

2.3- Dosing Guidelines

One 200 mg capsule of Tasigna or matching placebo will be administered orally once a day. Key elements will be detailed in the educational brochure including special warnings and pre-cautions:

- Brief background on Nilotinib, its authorized indication and posology
- Information on the cardiac risks associated with the use of Nilotinib and that Nilotinib can cause prolongation of the QT interval and that patients at risk of arrhythmia, especially torsade de pointes, should not take Nilotinib.

The need to avoid co-prescription with any other medicines that might prolong the QT interval

- Caution in prescribing to patients with a history of or risk factors for coronary heart disease
- That Nilotinib may cause fluid retention, cardiac failure and pulmonary edema
- That Nilotinib is metabolized by CYP3A4 and that strong inhibitors or inducers of this enzyme may significantly affect exposure to Nilotinib.
- That inhibitors may increase the potential for adverse drug reactions in particular QT interval prolongation
- To warn participants about over the counter (OTC) medicines in particular St John's Wort
- The need to inform patients about the effects of food on Nilotinib
- Not to eat within two hours before and one hour after taking Nilotinib
- The need to avoid foods such as grapefruit juice which inhibit CYP3A4 enzymes

2.4- Planned Exposure (e.g. Duration of the study administration)

We anticipate this project to be completed in 4 years (48 months); including 3 years (36 months) for pre-screening and enrolment, 36 months for treatment, neurological and safety examinations and 6 months for post treatment evaluation as well as data analysis.

Table 2- Summary of primary, secondary and exploratory objectives with the associated endpoints and evaluation criteria:

Outcomes	Objective	Endpoint
Primary	Safety and tolerability	1- Safety will be measured using the occurrence of AEs) and SAEs deemed to be possibly, probably, or definitely related to the study drug. AEs of interest are defined as QTc prolongation, myelosuppression, hepatotoxicity and pancreatitis as listed in Table 1 and Novartis IB, on which we base our power consideration. These AEs will be tracked over the course of the trial and reviewed by the data and safety monitoring board (DSMB) at scheduled meetings and in real time. SAEs and AEs are known to be related to drug use at 800mg daily in cancer. A small safety trial using lower oral daily doses of 150mg and 300mg Nilotinib in 12 PD patients showed one cardiac SAE over a six-month treatment period. Based on preliminary clinical data, IB and scheduled EKGs and lab tests, SAEs and AEs will be evaluated real-time on case-by-case basis. 2- Tolerability for a given participant will be defined as the ability of participants to remain on treatment. Overall tolerability of the drug will be defined as an acceptable number of up to 25% discontinuations.
Secondary	Pharmacokinetics and biomarkers	1- Nilotinib level in CSF 2- CSF levels of HVA and DOPAC between baseline and 6 months. 3- CSF alpha-synuclein (total and oligomeric), total tau and p-tau, Abeta40/42, NSE, S100B and phosphorylated neurofilaments. 4- Amyloid burden between Baseline and 6-months using Florbetaben PET 5- CSF ELISA and plasma neuro-inflammatory/modulatory markers, including TREM2.
clinical outcomes	Cognition and behavior	MoCA, ADAS-cog, ADCS-ADL, CAF, TMT-B, NPI, IAS, PBAs.
	Motor	MDS-UPDRS I-III and TUG

2.5- Number of Planned Subjects and Treatment Plan

Up to 100 subjects will be screened for the study with the goal of enrolling 60 for treatment, including 30 participants with DLB on placebo (group 1) and 30 participants on 200 mg Nilotinib (group 2). A sub-group of 50 participants will be enrolled in the amyloid PET study.

2.6- Study Population

This study will be conducted in DLB patients with 2.5≥Hoehn & Yahr≤3 and UPDRS I-III ≤50 and 15≥UPDRS III (motor) ≥40 and MoCA≥18. Eligible participants must not be on MAO-B inhibitors

(Rasagiline or Selegiline) for 4 weeks and must not be on ≥800mg Levodopa daily. Participants must be stable on acetylcholinesterase inhibitors and other medications for at least 6 weeks.

4) VISIT SCHEDULE AND ASSESSMENTS

Screening. This visit (Table 3) will determine study eligibility. Potential participants and their study partners must review and sign an informed consent form (ICF) prior to any study-related procedures. Information regarding demographics, concurrent medications, and medical history will be gathered from the participant and study partner at the screening visit. Prior to any study-related activities, subjects and their legally authorized representatives (LAR) will be thoroughly informed on all aspects of the study and will be requested to sign an ICF. Prior to obtaining written informed consent, information will be given at a complexity level that is understandable by the subject in both oral and written form by staff. Participants will also be informed about the drug safety and side effects.

Visits/month (m)	Screening	Baseline	1m	2m	3m	4m	5m	6m	7m (washout)
Consent	X								
Demographics	X								
Vitals, H&P	X	X	X	X	X	X	X	X	X
Neuro Exam	X	X	X	X	X	X	X	X	X
EKG	X	X	X	X	X	X	X	X	X
Blood draws*	X	X	X	X	X	X	X	X	X
CSSR-S	X	X	X	X	X	X	X	X	X
TMT-B	X	X			X			X	X
MDS-UPDRS	X	X			X			X	X
MoCA	X	X			X			X	X
Hoehn & Yahr	X	X			X			X	X
CAF		X	X	X	X	X	X	X	X
PBAs		X			X			X	X
IAS		X			X			X	X
ADAS-cog		X			X			X	X
ADCS-ADL		X			X			X	X
NPI		X			X			X	X
TUG		X			X			X	X
LP		X						X	
PET		X						X	

Table 3- A detailed schedule of 9 visits (±2 visits) and the assessments done at each visit. History and Physical (H&P), Electrocardiograms (EKG), Columbia Suicide Severity Rating Scale (CSSRS), Montreal Cognitive Assessment (MoCA), Trail Making Test (TMT)-B, AD Assessment Scale-Cognitive subscale (ADAS-cog), AD Cooperative Study-Activity of Daily Living (ADCS-ADL), Neuropsychiatric Inventory (NPI), Irritability-Apathy Scale (IAS), Problems Behavior Assessment Short (PBAs) form and Clinical Assessment of Fluctuation (CAF). Movement Disorders Society-Unified Parkinson's Disease Rating Score (MDS-UPDRS)-I-III, timed-up-and-go (TUG) and Lumbar Puncture (LP).

***Blood draw** will be performed for standard blood chemistry, including Cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), albumin, total protein, alkaline phosphatase, total bilirubin, creatinine, calcium, chloride, sodium, potassium, magnesium, inorganic phosphorus, bicarbonate, creatine phosphokinase (CPK), gamma-glutamyl transferase (γ-GT), lactate dehydrogenase (LDH), lipase, α-amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), thyroid stimulating hormone (TSH), vitamin B12, and glucose. A standard hematology panel including, complete blood count (CBC) with differential counts will be performed. Hemoglobin (Hb), hematocrit, red blood cell count, platelet count, and white blood cell (WBC) with differential count will be measured. In addition, prothrombin time-international normalized ratio (PT-INR) will be measured for assessment of coagulation.

Additional **blood draws** will be performed 30 minutes before LPs at baseline and 6 months. LPs are mandatory at baseline line and end of study visit. LP must be performed within 2 hours from the last Levodopa dose or in ON-STATE.

Study drug will be dispensed at the end of baseline visit, as well as every visit.

Visits should be scheduled on a monthly basis or every 30 days (± 3 days).

Women of child bearing potential must be willing to take a pregnancy test at every visit.

Unscheduled or miscellaneous visits are allowed if necessary.

Amyloid PET imaging can be done as a standalone visit between Screening and Baseline visits or during Baseline upon confirmation of diagnosis and eligibility. PET should be performed before dispensation of study drug at Baseline and within 1 week from last dose at end of study (± 7 days).

Demographics, vital signs, history and physical (H&P) and neurological exams will be performed at the screening visit. The MoCA and TMT-B, Hoehn and Yahr and MDS-UPDRS I-III will be performed at screening to confirm diagnosis of DLB and determine eligibility criteria. Electrocardiograms (EKGs) will be performed with standard blood chemistry, including cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), albumin, total protein, alkaline phosphatase, total bilirubin, creatinine, calcium, chloride, sodium, potassium, magnesium, inorganic phosphorus, bicarbonate, creatine phosphokinase (CPK), gamma-glutamyl transferase (γ-GT), lactate dehydrogenase (LDH), lipase, α-amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), thyroid stimulating hormone (TSH), vitamin B12, and glucose. A standard hematology panel including, complete blood count (CBC) with differential counts will be performed. Hemoglobin (Hb), hematocrit, red blood cell count, platelet count, and white blood cell (WBC) with differential count will be measured. In addition, prothrombin time-international normalized ratio (PT-INR) will be measured for assessment of coagulation. Should treatment with any HMG-CoA (or 3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitor (a lipid lowering agent) be needed to treat lipid elevations, evaluate the potential for a drug-drug interaction before initiating therapy as certain HMG-CoA reductase inhibitors are metabolized by the CYP3A4 pathway. If test results warrant therapy, local standards of practice and treatment guidelines will be followed.

The Columbia Suicide Severity Rating Scale (C-SSRS) will be performed at the screening visit.

Baseline visit will be scheduled 2-4 weeks after screening and results from all screening procedures will be reviewed and all inclusion/exclusion criteria must be met prior to baseline assessments. The Columbia Suicide Severity Rating Scale (C-SSRS) will be performed at baseline visit and at every visit as indicated in Table 3. Vital signs, history and physical (H&P), EKG, neurological exams and blood draws will be performed at baseline and very other visit as indicated in Table 3.

Cognitive assessments will be performed via MoCA (global cognition), and TMT-B (executive function). The MoCA and TMT-B will be done at both screening and baseline to ensure subjects can perform these tests and to minimize practice effects on baseline score. ADCS-ADL will measure activities of daily living and ADAS-cog will measure global cognitive change. Cognitive fluctuations will be measured via CAF. **Motor assessments** will be performed via UPDRS I-III and Timed-Up and Go (TUG). **Psychiatric assessments** will be performed using NPI, PBAs and IAS.
A mandatory blood draw and LP will be performed at baseline. LP must be performed within 2 hours from the last Levodopa dose. CSF collection via LP and blood draw will be performed for biomarker pharmacokinetics, pharmacodynamics and biomarkers measurement (CSF alpha-synuclein (total and oligomeric), CSF total tau/p-tau18, HVA and DOPAC and inflammatory/neurotrophic markers). Blood will also be collected for monitoring laboratory tests as indicated above. A mandatory Amyloid PET must also be performed upon confirmation of diagnosis and eligibility at or before Baseline.
The baseline procedures may be completed over several visits. After recruitment, participants will be randomized into 2 groups (1:1) receiving a single daily dose of 200 mg Nilotinib or matching placebo (group 2).
Drugs will be dispensed at the end of the baseline visit.

At 1, 2, 4 and 5 months visits, vital signs, history and physical (H&P), EKG, neurological exams and blood draws and CSSR-S and CAF will be performed at very other visit as indicated in Table 3.

At 3 and 6 months follow up and 7 months wash out visits, vital signs, history and physical (H&P), EKG, neurological exams and blood draws and CSSR-S will be performed. **Cognitive assessments** will be performed via MoCA, ADCS-ADL, and ADAS-cog. Cognitive fluctuations will be measured via CAF. **Motor assessments** will be performed via UPDRS I-III and Timed-Up and Go (TUG). **Psychiatric assessments** will be performed using NPI, PBAs and IAS. A mandatory blood draw and LP will be performed at 6-month end of treatment visit. LP must be performed within 2 hours from the last Levodopa dose and within 1-4 hours since last Nilotinib dose, because we detected Nilotinib in human CSF up to 4 hours after oral administration (59).

Medication Compliance Check. Site staff will remind subjects to bring unused study drug with them **at each visit** as well as the completed drug compliance diary. Site staff will count the number of capsules remaining at each in-person clinic visit for which the subject brings the unused drug with him/her. This information will be recorded in the subject specific drug accountability form. The total number of returned medication will be reviewed against the drug compliancy diary. If drug compliance is determined to be problematic site staff will review with the subject the importance of using the study drug as per protocol. Any dose adjustments will be documented on the drug exposure form (indicating start and stop date). Additionally, site staff will check the subject's overall compliance with the study requirements. This will also include checks of protocol compliance, and concomitant medication use, in addition to the proper use of study drug in order to assess the reliability of subject generated data.

Drug Related Risks and Potential Side Effects

Nilotinib has a number of side effects as stated in the IB, including gastrointestinal complications, vomiting and nausea, and in some rare cases dizziness (51). Abl has an essential role in mammalian heart growth and development and adult complications with Abl inhibition, include edema, nausea/vomiting, muscle cramps, neutropenia, thrombocytopenia, fever, liver toxicity, arthralgia, and exanthema/rash (68). Side effects are identified for Abl in homozygous Abl mutant mice, which display dramatically enlarged hearts due to abnormally increased cardiomyocyte proliferation during later stages of embryogenesis (69). Disruption of *Abl* in mice results in neonatal lethality accompanied by pleiotropic developmental defects with variable penetrance, including ranting, splenic and thymus atrophy, B cell lymphopenia, dysfunctional osteoblasts, and foreshortened crania (70-72). However, clinical use of Nilotinib (600-800mg daily) is fairly tolerated in CML patients, but no clinical and longitudinal data exist about the long term side effects of this drug since its approval in 2007. Therefore, clinical use of Abl inhibition may have dose-limiting toxicity but we will use much lower doses in the current studies (see study design). Importantly, Nilotinib is washed out of the brain within several hours (37,47), so lower dose and prolonged period of administration may decrease misfolded protein levels, leading to slowing of motor, non-motor and cognitive decline. Furthermore, to evaluate adverse drug effects, all patients will be monitored every month for specific warnings, including prolongation of QT interval. Prior to administration and periodically, patients will be monitored for hypokalemia or hypomagnesaemia. EKGs will also be obtained to monitor the QTc at baseline and periodically thereafter as detailed in Table 3. Nilotinib will not be administered to patients with hypokalemia, hypomagnesaemia, or long QT syndrome. Concomitant drugs known to prolong the QT interval and strong CYP3A4 inhibitors will also be avoided.

Nilotinib should be taken on an empty stomach 2hrs after eating to ensure appropriate absorption and no food should be consumed one hour after Nilotinib. The following SAEs can occur with Nilotinib:

1- Myelosuppression
2- QT Prolongation
1- Sudden Deaths
2- Cardiac and Arterial Vascular Occlusive Events
3- Pancreatitis and Elevated Serum Lipase
4- Hepatotoxicity
5- Electrolyte Abnormalities
6- Hemorrhage
7- Fluid Retention

4- PATIENT POPULATION

4.1- Number of Patients & Centers

This is a single center study at Georgetown University Medical Center (GUMC) Translational Neurotherapeutics Program (TNP) with total duration of 4 years and open enrollment for 3 years. We currently have no competing trials for DLB and we anticipate efficient recruitment to this study. We will recruit DLB patients from our Movement Disorders and Memory Disorders clinics at MedStar Georgetown University Hospital (MGUH). We have a registry of over 400 potential participants diagnosed with mild to moderate DLB and their study partners. Study procedures will be conducted at GUMC Clinical Research Unit (CRU) of Georgetown-Howard Universities Center for Clinical and Translational Science (GHUCCTS). GHUCCTS encompasses MedStar Health Research Institute (10 hospitals), including Georgetown University Hospital (GUH), Howard University, the Washington DC VA Medical Center (with its hospital and five clinics) and the Oak Ridge National Laboratory. CRU GHUCCTS is NIH-funded and combines the five institutions into one research powerhouse, creating one of the largest clinical trials networks in the country

A total of 60 participants with DLB will be enrolled. We anticipate screening 100 individuals to enroll a total of 60 participants over 4 years. There are no competing clinical trials for DLB in the greater Washington-Baltimore area. We will advertise to community support groups and websites (Clinicaltrials.gov). Only patients that meet all inclusion/exclusion criteria and are willing to make all visits will be included in the study. Our program has had significant success with recruitment and retention in clinical trials because of the active clinical involvement at GUH and in the satellite centers, including McLean Medical Center, Montgomery Hospital and Washington Hospital Center. Most patients will be local to study center, but any patient who qualifies and can commit to all study visits will be allowed enrollment into the study regardless of residence. Overall we do not anticipate any issues in recruiting or maintaining 60 patients in this clinical trial for 6-month treatment. We will also advertise in our newsletters and websites.

4.2- Inclusion Criteria

1- Written informed consent
2- Capable of providing informed consent and complying with study procedures. Subjects who are unable to provide consent may use a Legally Authorized Representative (LAR).
3- Clinical diagnosis of DLB according to McKeith et al (32) with both dementia MoCA≥18 and Parkinsonian defined as bradykinesia in combination with rest tremor, rigidity or both UPDRS I-III is less than 50 and/or UPDRS-III between 15 -40 on-state. Dementia and Parkinsonism must be present with at least one other symptom such as fluctuation, visual hallucinations or REM sleep behavioral disorder (RBD)
4- 2.5 ≥Hoehn and Yahr stage ≤3

5- : MDS-UPDRS-III 15-40 on-state (or up to 70 on the off-state)
6- Abnormal DaTScan
7- Stable concomitant medical and/or psychiatric illnesses in the judgement of the PI
8- Patients between the age of 25-90 years, medically stable
9- Must NOT be stable on mono-amine oxidase (MAO)-B inhibitors (Selegeline or rasagiline) for at least 4 weeks before enrollment and during Nilotinib treatment.
10- Must be medically stable on ≤800mg Levodopa daily for at least 4 weeks
11- QTc interval 350-460 ms, inclusive
12- Participants must be willing to undergo LP at baseline and 6 months after treatment
13- 13- Participants must be willing to do an LP and PET scan.

4.3- Exclusion Criteria

1. Patients with hypokalemia, hypomagnesaemia, or long QT syndrome- QTc≥461 ms
2. Concomitant drugs known to prolong the QTc interval and history of any cardiovascular disease, including myocardial infraction or cardiac failure, angina, arrhythmia
3. History or presence of cardiac conditions including:
    a. Cardiovascular or cerebrovascular event (e.g. myocardial infarction, unstable angina, or stroke)
    b. Congestive heart failure
    c. First, second- or third-degree atrioventricular block, sick sinus syndrome, or other serious cardiac rhythm disturbances
    d. Any history of Torsade de Pointes
4. Treatment with any of the following drugs at the time of screening or the preceding 30 days, and/or planned use over the course of the trial:
    a. Treatment with Class IA or III antiarrhythmic drugs (e.g. quinidine)
    b. Treatment with QT prolonging drugs (*www.crediblemeds.org)-* excluding Selective Serotonin Reuptake Inhibitors (SSRIs) (e.g. Citalopram, Paxil, Zoloft, Cymbalta, Sertraline, etc...)
    c. Strong CYP3A4 inhibitors (including grapefruit juice). The concomitant use of strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, voriconazole) must be avoided. Grapefruit products may also increase serum concentrations of Nilotinib. Should treatment with any of these agents be required, therapy with Nilotinib should be interrupted.
    d. Anticoagulants, including Coumadin (warfarin), heparin, enoxaparin, daltiparin, xarelto, etc.
    e. St. John's Wort and the concomitant use of strong other CYP3A4 inducers (e.g., dexamethasone, phenytoin, carbamazepine, rifampin, rifabutin, rifapentine, phenobarbital) must be avoided since these agents may reduce the concentration of Nilotinib.
5. Abnormal liver function defined as AST and/or ALT > 100% the upper limit of the normal
6. Renal insufficiency as defined by a serum creatinine > 1.5 times the upper limit of normal
7. History of HIV, clinically significant chronic hepatitis, or other active infection
8. Females must not be lactating, pregnant or with possible pregnancy
9. Medical history of liver or pancreatic disease
10. Clinical signs indicating syndromes other than DLB, including, PD, PD with Dementia (PDD), corticobasal degeneration, supranuclear gaze palsy, multiple system atrophy,

chronic traumatic encephalopathy, signs of frontal dementia, history of stroke, head injury or encephalitis, cerebellar signs, early severe autonomic involvement, Babinski sign
11. Current evidence or history in past two years of epilepsy, focal brain lesion, head injury with loss of consciousness or DSM-IV criteria for any major psychiatric disorder including psychosis, major depression, bipolar disorder, alcohol or substance abuse
12. Evidence of any significant clinical disorder or laboratory finding that renders the participant unsuitable for receiving an investigational drug including clinically significant or unstable hematologic, hepatic, cardiovascular, pulmonary, gastrointestinal, endocrine, metabolic, renal or other systemic disease or laboratory abnormality
13. Active neoplastic disease, history of cancer five years prior to screening, including breast cancer (history of skin melanoma or stable prostate cancer are not exclusionary)
14. Contraindications to LP: prior lumbosacral spine surgery, severe degenerative joint disease or deformity of the spine, platelets < 100,000, use of Coumadin/warfarin, or history of a bleeding disorder
15. Must not be on any immunosuppressant medications or IVIG
16. Must not be enrolled as an active participant in another clinical study

4.4- Randomization and Registration will be performed by an internet based randomization module. Randomization of the subjects to the 2 treatment groups will be performed in a stratified manner. The chance for randomization to the groups is 1:1 for placebo: 200 mg Nilotinib.

4.5- Blinding. The investigators will be blinded to the dosage. Medications for any patient will be labeled by the CRU with a package medical identification number (Med. Id). A patient specific patient identification number (Pat. Id.) will be assigned to each patient. The investigator will have to note the Pat.Id on the designated medication package number after randomization. Drug dispensation will take place once a month during the monthly visit.

4.6- Un-blinding may occur for emergency purposes in case an AE or SAE makes it necessary for the treating physician to unblind the study treatment– if possible prior contact will be made with the clinical trials co-coordinator (CTC) or project manager. If this is not feasible, the CTC will be contacted within 24 hours after un-blinding. The CTC should not be made aware of what the treatment assignment was. If un-blinding occurs the subject is automatically withdrawn and the procedure for withdrawal will be followed.

4.7- Withdrawal of subjects. In accordance with the Declaration of Helsinki, each subject is free to withdraw from the study at any time without giving reasons for her/his decision. The investigator may also withdraw the subject at any time in the interest of the subject's safety, including severe study-related toxicity as detailed in Table 1 or in the case of un-blinding as described above. The primary reason for withdrawal (e.g. subject wish, safety, withdrawal of consent, etc.) must be recorded in the subject's medical record and on the withdrawal form in the electronic Case Report Form (eCRF). Should a subject decide to withdraw after administration of study drug, or should the investigator decide to withdraw the subject, all efforts will be made to complete and report the observations up to the time of withdrawal as thoroughly as possible.
A study subject will be discontinued from participation in the study if:

- Any clinical AE, laboratory abnormality, concurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.

- The participant meets any exclusion criteria (either newly developed or not previously recognized).

Subjects are free to withdraw from participation in the study at any time upon request.

4.8- Handling of Withdrawals
A subject may choose to discontinue participation in the study at any time. Subjects who permanently discontinue study drug should complete early study drug termination procedures per protocol. The subject should then return any unused study drug and will be asked to return to the study site for a final safety visit.

4.9- Termination of Study
This study may be prematurely terminated if, in the opinion of the principal investigator(s) (PIs), there is sufficient reasonable cause.

Circumstances that may warrant termination include, but are not limited to:
- Determination of unexpected, significant, or unacceptable risk to subjects.
- Enrollment is unsatisfactory.
- Insufficient adherence to protocol requirements.
- Data are not sufficiently complete and/or evaluable.

4.10- Protocol Adherence
The Principal Investigators (PIs) agree to adhere to the protocol detailed in this document and agrees that any changes to the protocol must be approved by the site Institutional Review Board (IRB). The PIs will be responsible for enrolling only those study subjects who have met protocol eligibility criteria.

5- SAFETY MONITORING AND REPORTING

The AE definitions and reporting procedures provided in this protocol comply with all applicable regulations and ICH guidelines. The PI(s) will carefully monitor each subject throughout the study for possible AEs. All AEs will be documented on CRFs designed specifically for this purpose. It is also important to report all AEs, especially those that result in permanent discontinuation of the investigational product being studied, whether serious or non-serious.

5.1- All Non-serious Adverse Events

An AE is any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with a study, use of a drug product or device whether or not considered related to the drug product or device.

Adverse drug reactions (ADR) are all noxious and unintended responses to a medicinal product related to any dose. The phrase “responses to a medicinal product” means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out. Therefore, a subset of AEs can be classified as suspected ADRs, if there is a causal relationship to the medicinal product.

Examples of AEs include: new conditions, worsening of pre-existing conditions, clinically significant abnormal physical examination signs (i.e. skin rash, peripheral edema, etc.), or clinically significant abnormal test results (i.e. lab values or vital signs), with the exception of outcome measure results, which are not being recorded as AEs in this trial (they are being collected, but analyzed separately). Stable chronic conditions (i.e., diabetes, arthritis) that are present prior to the start of the study and do not worsen during the trial are NOT considered AEs. Chronic conditions that occur more frequently (for intermittent conditions) or with greater severity, would be considered as worsened and therefore would be recorded as AEs.

AEs are generally detected in two ways:

Clinical → symptoms reported by the subject or signs detected on examination.

Ancillary Tests → abnormalities of vital signs, laboratory tests, and other diagnostic procedures (other than the outcome measures, the results of which are not being captured as AEs).

If discernible at the time of completing the AE log, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the PI and recorded on the AE log. However, if an observed or reported sign, symptom, or clinically significant laboratory anomaly is not considered by the Site Investigator to be a component of a specific disease or syndrome, then it should be recorded as a separate AE on the AE log. Clinically significant laboratory abnormalities, such as those that require intervention, are those that are identified as such by the PI.

Subjects will be monitored for AEs from the time they sign consent until completion of their participation in the study (defined as death, consent withdrawal, loss to follow up, and early study termination for other reasons or following completion of the entire study).

5.2- All Serious Adverse Events

An SAE is defined as an adverse event that meets any of the following criteria:

1. Results in death.

2. Is life threatening: that is, poses an immediate risk of death as the event occurred.
    a. This serious criterion applies if the study subject, in the view of the PI or Sponsor, is at immediate risk of death from the AE as it occurs. It does not apply if an AE hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization.
    a. Hospitalization for an elective procedure (including elective PEG tube/g-tube/feeding tube placement) or a routinely scheduled treatment is not an SAE by this criterion because an elective or scheduled "procedure" or a "treatment" is not an untoward medical occurrence.
4. Results in persistent or significant disability or incapacity.
    a. This serious criterion applies if the "disability" caused by the reported AE results in a substantial disruption of the subject's ability to carry out normal life functions.
5. Results in congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female).
6. Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure.
7. Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An inpatient hospital admission in the absence of a precipitating, treatment-emergent, clinical AE may meet criteria for "seriousness" but is not an adverse experience, and will therefore, not be considered an SAE. An example of this would include a social admission (subject admitted for other reasons than medical, e.g., lives far from the hospital, has no place to sleep).

The PI is responsible for classifying AEs as serious or non-serious.

5.3- All Reports of Drug Exposure during Pregnancy.
Females, who are lactating/breast feeding, pregnant or with possible pregnancy must not participate in this study. Nilotinib and other drugs and procedures used in this study may be unsafe for a fetus, an infant, sperm and eggs. Women of child bearing potential must agree to avoid pregnancy during participation in this study and for 3 months after the completion of this study. A man must agree not to conceive a child during participation in this study and for 3 months after the completion of this study. If a woman becomes pregnant during the study or if a man fathers a child during the study, they should immediately notify the principal investigator and study coordinator(s) and must be immediately withdrawn from the study.

5.4- All reports of misuse and abuse of Nilotinib and other medication.
Site staff will check the subject's overall compliance with the study requirements. This will also include checks of protocol compliance, Nilotinib and concomitant medication use, in addition to the proper use of study drug in order to assess potential risks of drug abuse or misuse. The PI shall report within 24 hours of discovery any drug misuse experience to the IRB, FDA, Novartis and study monitor.

5.5- Assessment and Recording of Adverse Events
The PIs will carefully monitor each subject throughout the study for possible AEs. All AEs will be documented on CRFs designed specifically for this purpose. All AEs will be collected and reported in

the electronic data capture (EDC) system. The PIs shall promptly review all information relevant to the safety of the investigational product, including all SAEs. Special attention will be paid to those that result in permanent discontinuation of the investigational product being studied, whether serious or non-serious.

5.6- Assessment of Adverse Events

At each visit (including telephone interviews), the subject will be asked if they have had any problems or symptoms since their last visit in order to determine the occurrence of AEs. If the subject reports an AE, the Investigator will probe further to determine:

1. Type of event
2. Date of onset and resolution (duration)
3. Severity (mild, moderate, severe)
4. Seriousness (does the event meet the above definition for an SAE)
5. Causality, relation to investigational product and disease
6. Action taken regarding investigational product
7. Outcome

5.7- Relatedness of Adverse Event to Investigational Product

The relationship of the AE to the investigational product should be specified by the PIs, using the following definitions:

1. Not Related: Concomitant illness, accident or event with no reasonable association with treatment.

2. Unlikely: The reaction has little or no temporal sequence from administration of the investigational product, and/or a more likely alternative etiology exists.

3. Possibly Related: The reaction follows a reasonably temporal sequence from administration of the investigational product and follows a known response pattern to the suspected investigational product; the reaction could have been produced by the investigational product or could have been produced by the subject's clinical state or by other modes of therapy administered to the subject. (Suspected ADR)

4. Probably Related: The reaction follows a reasonably temporal sequence from administration of investigational product; is confirmed by discontinuation of the investigational product or by re-challenge; and cannot be reasonably explained by the known characteristics of the subject's clinical state. (Suspected ADR)

5. Definitely Related: The reaction follows a reasonable temporal sequence from administration of investigational product; that follows a known or expected response pattern to the investigational product; and that is confirmed by improvement on stopping or reducing the dosage of the investigational product, and reappearance of the reaction on repeated exposure. (Suspected ADR)

5.8- Recording of Adverse Events

All clinical AEs are recorded in the AE Log in the subject's study binder. Study staff should fill out the AE Log and enter the AE information into the EDC system within 48 hours of the site learning of a new AE or receiving an update on an existing AE. Entries on GUMC RedCAP database and the AE Log (and into the EDC) will include the following: name and severity of the event, the date of onset, the date of resolution, relationship to investigational product, action taken, and primary outcome of event. The PI shall report within 24 hours of discovery any serious adverse experience whether or not considered related to the study to the IRB, FDA and study monitor who will contact the DSMB. Serious adverse experiences should also be reported immediately by telephone and subsequently in writing within 48 hours of the occurrence to the following:

Robert Schiff, Ph.D., RAC, CQA, FRAPS
Medical Monitor
1120 Bloomfield Avenue
West Caldwell, NJ 07006
Tel: 973-227-1830
Fax: 973-227-5330
Cell: 973-568-3361
Email: *Rschiff13@aol.com*

The Institutional Review Board will be notified of such adverse experiences

The minimum necessary information to be provided at the time of the initial report will include the following:

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

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

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up

6- STUDY MONITORING

6.1- Safety Monitoring

The study PIs will review safety data throughout the trial and may stop the trial for safety if they determine that there is a significant difference in the rate of a particular AE that would indicate a risk that is greater than the possible benefit of the study drug.

Unanticipated problems involving risks to subjects or others including adverse events will be reported to the Partners Human Research Committee (PHRC) in accordance with PHRC unanticipated problems including AEs reporting guidelines.

6.2- Data Safety Monitoring Board (DSMB) will be formed at the time of the initiation of the study and will include medical experts on Nilotinib effects on cardiac toxicity (TBD), neurologist (TBD), a clinical pharmacologist (TBD) and a biostatistician (TBD). No investigator involved in the trial will be a member of the DSMB. The DSMB will review the protocol to identify any necessary modifications. If modifications are necessary, revisions will be reviewed by the DSMB prior to its recommendation on initiation of the project. The DSMB, based on its review of the protocol, will identify the data parameters and format of the information to be regularly reported. The DSMB will be informed of the occurrence of any SAEs and immediately notified of fatal or life-threatening events. The DSMB may at any time request additional information from the PIs. The DSMB will be provided with data blinded to treatment status, but they may request un-blinded data if there is a safety concern. Based on the review of safety data, the DSMB will make recommendations regarding the conduct of the study. These may include amending safety monitoring procedures, modifying the protocol or consent, terminating the study or continuing the study as designed. The discussions and decisions of the DSMB will be summarized in written reports and provided to the PIs. The DSMB will meet in person or by conference call every 6 months or as necessary when requested by the PI or the monitor.
The DSMB will also provide boundaries for when to terminate the study or end participation of an individual patient according to the guidelines of DMSB charters adopted by the NIH. The stopping rules should include stopping rules as in Table 1 and other criteria related to:
Pancreatitis, QT prolongation (select a method for QT correction (typically Frederica's method) and include in the protocol), develop individual stopping rules for withdrawal from the study for QT prolongation, and create algorithms for the follow-up of abnormal blood tests that potentially signal the start of a drug induced event (e.g. neutropenia or thrombocytopenia). The DSMB will monitor each patient in real-time and on a case-by-case basis and unblind any case when necessary.

6.3- Study monitor and auditing will ascertain adherence to the study protocol by investigators and ensure proper documentation and investigator blinding of biomarker data processing and deposition. This study will be monitored by a clinical monitor. The monitors will maintain liaison with the investigators by telephone, letter, email and personal visits in order to assure the sponsors that the clinical study is completed according to the protocol requirements and that Good Clinical Practices are being followed according to 21 CFR parts 50, 56, 812, FDA Guidelines and the ICH E6 Guideline. The PI will allocate adequate time for such monitoring activities. The PI will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (device testing location), and has adequate space to conduct the monitoring visit.

Auditing inspections will serve to verify strict adherence to the protocol and the accuracy of the data management, in accordance with the federal regulations. The PI will permit study-related monitoring and audits by the IRB, the sponsor, and government regulatory bodies, of all study related documents

(e.g. source documents, regulatory documents, data collection instruments, study data etc) at regular intervals throughout the study. The PI will ensure the capability for inspections of applicable study-related facilities (device testing location). Participation as a PI in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.
The investigator should be aware that representatives of the FDA might also inspect the study site and subject records. If contacted for an audit of this study by any regulatory agency, the investigator should notify the sponsor immediately.
Study monitor will ascertain adherence to the study protocol by investigators and ensure proper documentation and investigator blinding of biomarker data processing and deposition.

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7) Institutional Review Board (IRB)

This study will be conducted in compliance with current Good Clinical Practices (GCP) and Title 21Part 56 of the United States of America Code of Federal Regulations (CFR) relating to IRBs.

7.1- Ethical Conduct of Study

The study will be conducted in accordance with GCP defined by the International Conference on Harmonization (ICH) and the ethical principles of the Declaration of Helsinki.

7.2- Subject Information and Consent

This study will be conducted in compliance with Title 21 Part 50 of the United States of America Code of Federal Regulations (CFR), Federal Regulations and ICH Guidance Documents pertaining to informed consent. At the first visit, prior to initiation of any study-related procedures, subjects will be informed about the nature and purpose of the study, participation/termination conditions, and risks and benefits. Subjects will be given adequate time to ask questions and become familiar with the study prior to providing consent to participate. Subjects will give their written consent to participate in the study and will be provided with a copy of the fully executed consent form for their records.

8- Statistical Methods and Data Analysis

8.1- Sample size determination

Safety: No formal sample size calculation is conducted. Sample size of 30 patients in each arm is based on feasibility. Safety of the treatment arm will be judged by the absence of events of major concern, including QTc prolongation and myelosuppression. According to Novartis Investigator Brochure, the frequency of AEs of all grades leading to discontinuation in the Nilotinib 300mg treatment was 14.0% and the incidence of SAEs in the Nilotinib 300 mg treatment recorded 9%. With the Nilotinib 200 mg treatment, the treatment arm is expected to have less than 98.9% probability of observing at least one instance of any AE with an expected incidence of at least 14% in the treatment arm when the binomial distribution is assumed. For SAEs, this probability of at least one event will decrease to at most 94.1% with an expected incidence of at least 9% in the treatment arm.

Tolerability: If we define a dose as tolerable when we observe no more than 25% intolerance or the discontinuation ratio is greater than 25%, the probability of early discontinuation of this trial (>25%), i.e., observing 8 or more AEs out of 30 is 5% (1-95.0%) given 30 participants in the Nilotinib 200mg arm. If we assume 9% of probability of SAEs occurrence, the probability of discontinuation due to SAEs of this trial, i.e., observing 8 or more severe AEs out of 30 is 0.4% (1-99.6%).

Efficacy endpoints. Given sample size of 30 per arm, we calculate power to detect the efficacy of 200mg Nilotinib treatment in terms of primary biomarkers and clinical endpoint between baseline and 6 months. We will test the null hypothesis that the endpoint change in the control group over 6 months equals the endpoint change in the treatment group using a one-sided significance level 0.025.
It is assumed that 10% complete losses and additional 20% partial loss of information due to symptomatic treatment leads to ``drop-out'' rate of 30%. Considering 30% drop-out, a total of 42 patients (21 patients in each arm) will be considered. Given observed statistics on changes of efficacy endpoints in the pilot study, we anticipate powers to detect changes in each endpoint as shown in Table 4.

Table 4- Estimated power based on two endpoints

Endpoint	Placebo	Treatment	Difference	SD	Power (N=30)	Power (N=21)
HVA	0%	50%	50%	50	97%	90%
UPDRS I-IV	3	-7	10	12	90%	77%

We will have 90% power to test our null hypotheses for HVA biomarkers based on comparing means using a normal distribution with 21 patients in each arm. For testing clinical outcomes (MDS- UPDRS), a number of 21 or more patients will achieve at least 77% power to detect the change in UPDRS I-IV. Due to normality given a small sample size, a simulation based non-parametric Mann-Whitney-Wilcoxon test (MWW) power is also computed given the mean change rate and SD. For HVA, MWW power is approximately 87% with 21 patients in each arm and MWW power for UPDRS is approximately 73%.
Amyloid PET power analysis: These imaging studies will significantly add to our understanding of the potential change of CNS amyloid via PET versus CSF in DLB. We anticipate to continue to enroll participants until 2023. We estimate that around 50 participants should be enrolled within the currently approved project period for the existing parent award and we therefore propose to add amyloid imaging to data collection. This is a pilot or roof-of-concept study, but based on the data obtained in the AD study with n=12 patients per group (placebo versus nilotinib), we performed power analysis

considering a similar effect on amyloid PET. Therefore, considering the changes of amyloid in the frontal lobe over 6 months as co-primary endpoints, the study overall type I error of 5% is split into 2.5% for evaluating each endpoint. In a two-sided t-test for two-sample mean, at the significance level of 0.025 and 80% power, **25** subjects for 200mg Nilotinib group and **25** subjects for placebo group will have a minimal detectable effect size of 0.9 SD or 0.9 Cohen's D between 200mg nilotinib and placebo. Considering 20% dropout rate, **20** subjects for 200mg Nilotinib group and **20** subjects for placebo group will have a minimal detectable effect size of 1.03 Cohen's D between 200mg nilotinib and placebo. The results of this proof-of-concept study will guide the development of a larger future clinical trial.

8.2- Analyses plan:

Safety: The frequency of AEs classified by MedDRA system organ class and preferred term and clinically significant changes in EKG and laboratory parameters will be summarized as simple proportions with Clopper-pearson exact 95% confidence bounds. The proportion of participants experiencing each type of event will be compared by Fisher's exact test.
Tolerance: The proportion tolerant of Nilotinib 200mg dose will be estimated with exact confidence intervals.

Target Engagement: The measurements of all endpoints at baseline and the change from baseline to 6 months will be summarized in terms of mean, median, range, and standard deviation. To compare efficacy of Nilotinib, 200mg, a shared-baseline repeated-measures ANOVA will be used with estimates of treatment-specific changes over 6 months and differences between treatments obtained from linear contrasts. This model will include fixed effects of visit (baseline and 6 months) and a dose by post-baseline visit interaction and unstructured covariance for the repeated measurements. One additional baseline covariate may be included if a large difference in a potentially prognostic measure results by chance. A significant dose effect will be judged based on one-tailed testing at a p-value of 0.025. Other CSF and plasma exploratory biomarkers and clinical outcomes will be explored by the same model. The relationship between 6-month changes in tau, alpha-synuclein, dopamine metabolism and amyloid or other biomarkers and 6-month changes in clinical outcomes will be summarized as simple correlations with a visual verification that correlations within each dose roughly match correlations across doses, i.e., absence of Simpson's paradox.

9- STUDY FEASIBILITY AND PRELIMINARY DATA

9.1- Preclinical Evidence

We previously demonstrated that Nilotinib penetrates the brain, inhibits Abl activity and promotes autophagic degradation of amyloid proteins in transgenic mice (37,47,48,50,73). These findings were also reproduced by several other laboratories (37,60,61). To determine whether Nilotinib enters the brain, male and female 2 months old wild type (WT) C57BL/6 mice were intraperitoneally (I.P.) injected with an escalating dose of 1, 10, 20, 40 and 80 mg/kg Nilotinib dissolved in 30µL DMSO (n=3 per group) and sacrificed at 2, 4, 6, 8 and 12hr post-injection (n=3 per time point). The concentration of Nilotinib was measured by mass spectrometry using ^{13}C labeled Nilotinib as a standard control in the plasma (Fig. 1A) and total brain homogenates (Fig. 1B). At 10 mg/kg Nilotinib reached the highest brain/blood ratio (5.08%) at 305nM peak (T_{max}) 4hr post-injection; and 1 mg/Kg brain/blood was 3.06% at 205nM (Fig. 1C). Higher doses of Nilotinib (20, 40 and 80mg/kg) injection did not result in higher brain levels, perhaps due to the role of the ATP-binding cassette (ABC) transporters, which facilitate Nilotinib efflux at the BBB (73).

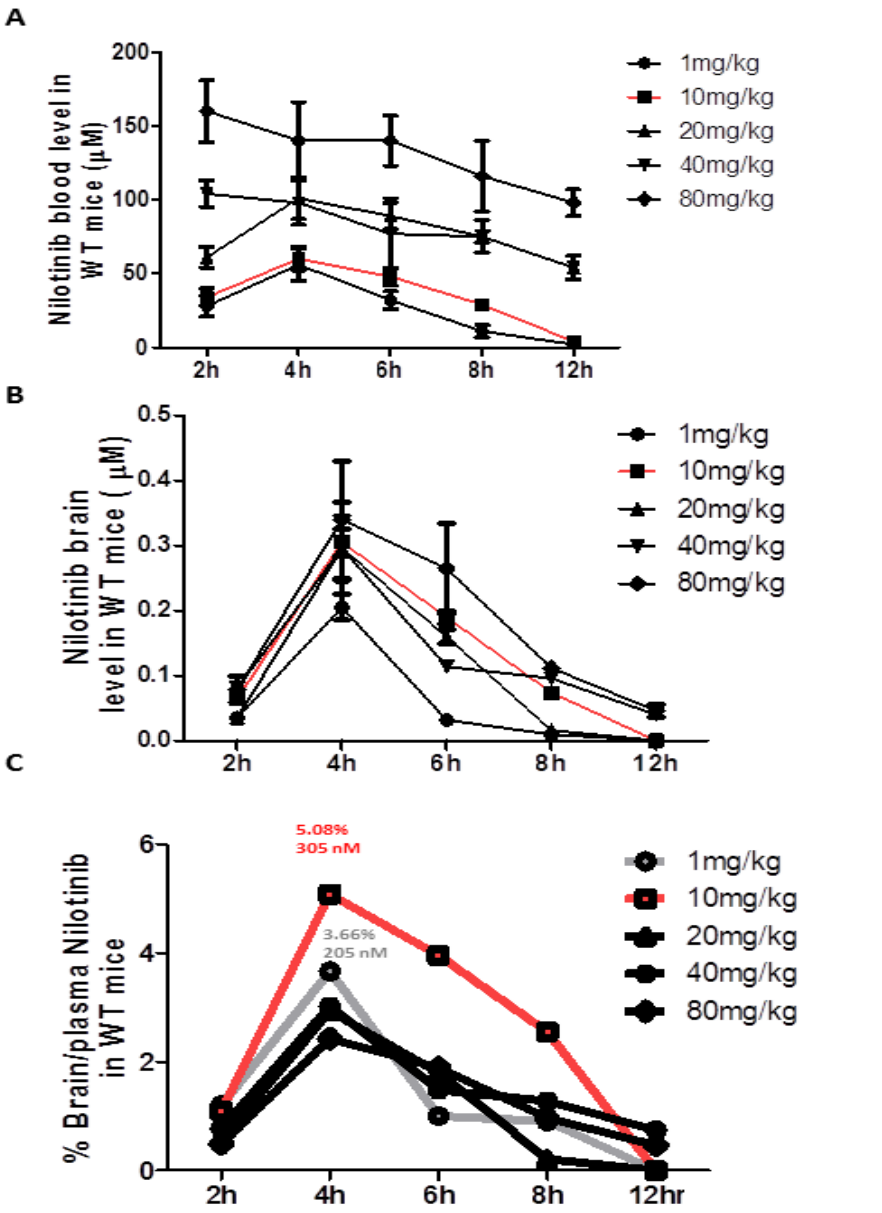


Figure 1. Pharmacokinetics analysis showing Nilotinib concentration in A) plasma, B) brain and C) percentage brain/plasma

Fig. 1- Abl activation is associated with accumulation of alpha-Synuclein. WB on 10% SDS-NuPAGE gel shows **A**) Lentiviral alpha-Synuclein expression (1st blot), total Abl (2nd blot) and tyrosine 412 (T412) phosphorylated Abl (3rd blot) relative to actin (N=9), and **B**) graphs represent densitometry analysis. **C**) Total Abl (1st blot) and tyrosine 412 (T412) phosphorylated Abl (2nd blot), and mouse alpha-Synuclein expression (3rd blot) relative to actin (N=9) in lentiviral Abl and LacZ injected mice, and **D**) graphs represent densitometry analysis. IHC in 20 µm thick brain sections showing **E**) V5, **F**) Abl and **G**) merged V5 and Abl staining in the SN of mice injected with lentiviral Abl. IHC in 20 µm thick brain sections showing **H**) V5, **I**) Abl and **J**) merged V5 and Abl staining in the SN of mice injected with lentiviral LacZ. WB on 4-12% SDS-NuPAGE gel shows **K**) total Abl (1st blot) and T412 Abl (2nd blot) relative to actin in human post-mortem striatal extracts, N= 9 PD and 7 controls, p<0.02, two-tailed t-test, and **L**) Densitometry of human WBs. **M**) Graph represents quantification of Mass Spectroscopy analysis of brain Nilotinib (N=5/time point). **N**). WB on 4-12% SDS-NuPAGE gel shows total Abl (1st blot), T412 Abl (2nd blot) T245 Abl (3rd blot) and phospho-tyrosine (4th blot) relative to parkin in wild type mice injected with DMSO or Nilotinib once daily for 3 weeks and **O**). Graphs represent densitometry analysis. *Significantly different, ANOVA, Neumann Keuls multiple comparison, p<0.05. N= number of animals, and bars are means.

Abl activation is associated with accumulation of alpha-Synuclein. To examine the relationship between Abl and alpha-Synuclein, male C57BL/6 mice were stereotaxically injected with $1x10^4$ multiplicity of infection (m.o.i) lentiviral clones driving the expression of Abl, or alpha-Synuclein (or LacZ) bilaterally into the substantia nigra (SN). Lentiviral injection significantly increased alpha-Synuclein (42%) over LacZ level (Fig. 2A&B, 1st blot, N=9 animals) 6 weeks post-injection. Alpha-Synuclein expression led to an increase in total Abl (110%) relative to actin and tyrosine 412 (T412) phosphorylation (289%) relative to total Abl (Fig. 2A&B, $p<0.05$, N=9) compared to LacZ expressing mice, indicating Abl activation. Conversely, lentiviral expression of Abl in the mouse SN led to an increase (204%) in total Abl relative to actin (Fig. 2D&E, $p<0.05$, N=9) and T412 phosphorylation (231%) relative to total Abl and resulted in increased levels of monomeric (51%) and high molecular weight alpha-Synuclein (relative to actin) 6 weeks post-injection

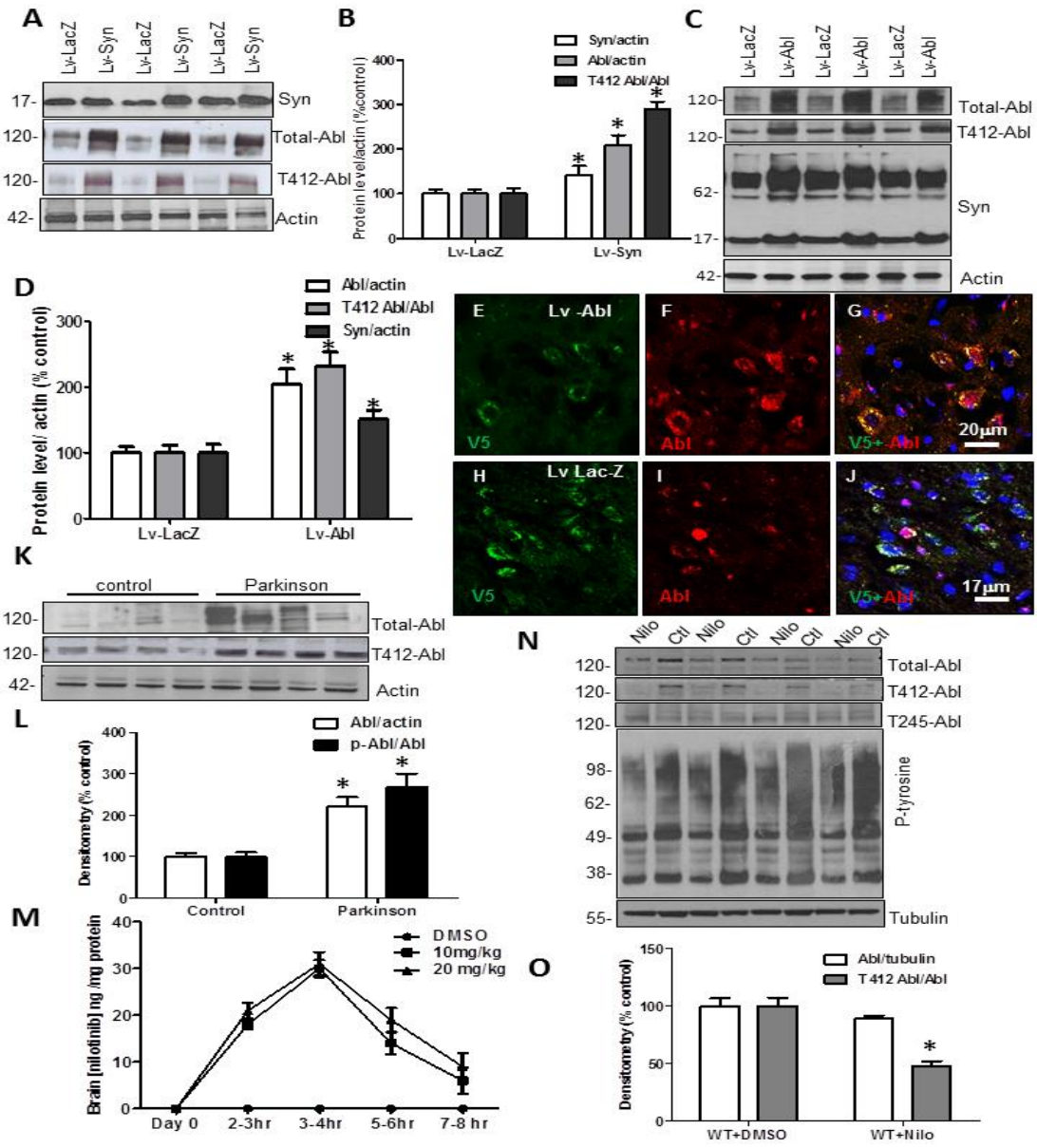


Fig. 2- Abl activation is associated with accumulation of alpha-Synuclein.
Lentiviral alpha-Synuclein expression (1st blot), total Abl (2nd blot) and tyrosine 412 (T412) phosphorylated Abl (3rd blot) relative to actin (N=9), and **B**) graphs represent densitometry analysis. **C**) Total Abl (1st blot) and tyrosine 412 (T412) phosphorylated Abl (2nd blot), and mouse alpha-Synuclein expression (3rd blot) relative to actin (N=9) in lentiviral Abl and LacZ injected mice, and **D**) graphs represent densitometry analysis. IHC in 20 µm thick brain sections showing **E**) V5, **F**) Abl and **G**) merged V5 and Abl staining in the SN of mice injected with lentiviral Abl. IHC in 20 µm thick brain sections showing **H**) V5, **I**) Abl and **J**) merged V5 and Abl staining in the SN of mice injected with lentiviral LacZ. WB on 4-12% SDS-NuPAGE gel shows **K**) total Abl (1st blot) and T412 Abl (2nd blot) relative to actin in human post-mortem striatal extracts, N= 9 PD and 7 controls, $p<0.02$, two-tailed t-test, and **L**) Densitometry of human WBs. **M**) Graph represents quantification of Mass Spectroscopy analysis of brain Nilotinib (N=5/time point). **N**). WB on 4-12% SDS-NuPAGE gel shows total Abl (1st blot), T412 Abl (2nd blot) T245 Abl (3rd blot) and phospho-tyrosine (4th blot) relative to parkin in wild type mice injected with DMSO or Nilotinib once daily for 3 weeks and **O**). Graphs represent densitometry analysis. *Significantly different, ANOVA, Neumann Keuls multiple comparison, $p<0.05$. N= number of animals, and bars are means.

compared to Lac-Z. To verify that both lentiviral LacZ and Abl were expressed, a V5 tag of the lentiviruses were probed with IHC. Staining of 20µm thick SN sections showed V5 (Fig. 2E) and Abl (Fig. 2F) co-localized (Fig. 2G) in lentiviral-Abl injected brains, while staining of Lac-Z injected SN showed V5 (Fig. 2H) and endogenous Abl (Fig. 2I) without co-localization (Fig. 2J), indicating lentiviral expression.

Western Blot (WB) was performed on homogenized frozen striatal (caudate) brain tissues (described in (36)) from 9 sporadic PD patients and 7 age-matched control subjects. Human post-mortem PD striatal extracts showed an increase in total Abl (220%) relative to actin and T412 (267%) relative to total Abl (Fig. 2K&L) compared to control subjects ($p<0.02$, two-tailed t-test), suggesting a relationship between Abl activation and alpha-Synuclein in PD.

Intraperitoneal (I.P.) injection of 10-20 mg/kg Nilotinib into WT mice (N=5 animals/time point), led to detection of up to 30ng Nilotinib (310nM) per mg brain tissue 3-4hr after injection (Fig. 2M) and Nilotinib was still detectable at 3.4ng/mg (35nM) 7-8 hr., indicating that Nilotinib enters the brain and is washed out after several hours. Two months old C57BL6 mice were I.P. injected once a day with 10mg/kg Nilotinib or DMSO (30µL) for 3 consecutive weeks, and total brain extracts were analyzed with WB. Nilotinib led to a slight (11%) decrease in total Abl relative to tubulin but T412 Abl was significantly decreased (52%) relative to total Abl compared to DMSO (Fig. 2N&O, $p<0.05$, N=10 animals). No

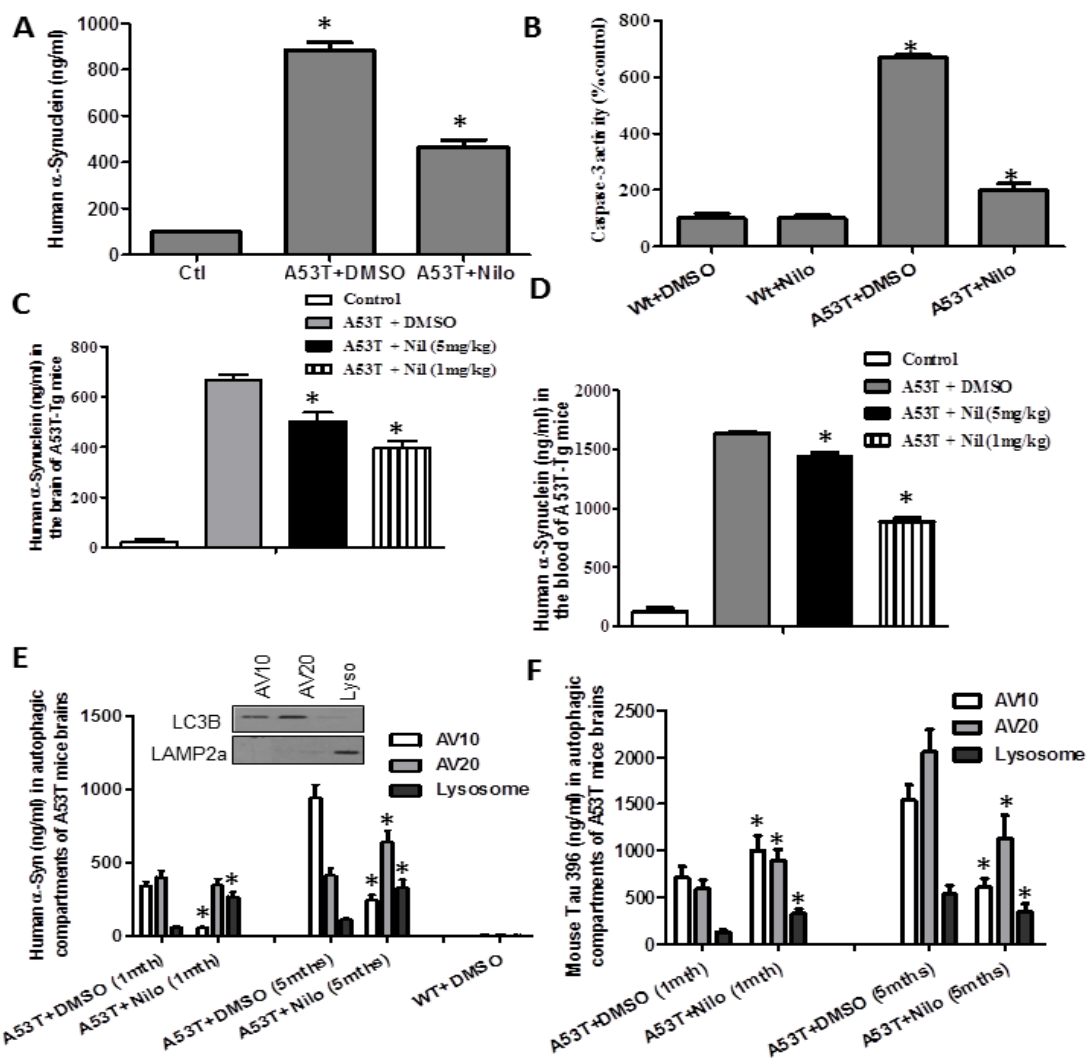


Fig. 3- Abl inhibition via Nilotinib promotes autophagic degradation. ELISA measurement of **A**) human alpha-Synuclein (N=14) and **B**) caspase-3 activity (N=64) in 6-8 months old transgenic A53T mice and wild type age-matched controls injected daily IP with 10mg/kg Nilotinib for 3 weeks. Graphs represent ELISA measurement of **C**) brain levels of human alpha-Synuclein (N=10) and **D**) blood levels of human alpha-Synuclein (N=10) in 5 months old transgenic A53T mice and wild type age-matched controls treated IP with 5mg/kg or 1mg/kg Nilotinib every other day for 6 weeks. Graphs represent ELISA measurement of **E**) human alpha-Synuclein (insert shows WB of AVs) and **F**) p-Tau levels in A53T mice (N=5) treated daily IP with 10mg/kg Nilotinib for 3 weeks. *Significantly different, ANOVA, Neumann Keuls multiple comparison, $p<0.05$. N=number of animals, bars are means.

changes were detected in T245 Abl relative to actin or total Abl (Fig. 2N, 3rd blot). These data were confirmed by other groups (37,60,61).

Nilotinib decreases brain and blood alpha-Synuclein levels. Nilotinib effects on alpha-Synuclein levels were tested in 7-8 months old transgenic alpha-Synuclein mice that harbor the A53T mutation of alpha-Synuclein (74). Total brain lysates showed accumulation (ELISA) of up to 885ng/ml human alpha-Synuclein (Fig. 3A) in 7-8 months mice treated with DMSO compared to wild type control ($p<0.05$, N=10), but daily IP injection of 10mg/kg Nilotinib for 3 weeks significantly decreased alpha-Synuclein to 467ng/ml ($p<0.05$, N=10), indicating that once a day treatment with Nilotinib decreases brain alpha-Synuclein levels. A significant increase in caspase-3 activity (Fig. 3B, 670%, $p<0.05$, N=64), indicating apoptotic death, was observed in A53T mice brains and Nilotinib reversed this increase to 201% compared to wild type age-matched controls with and without Nilotinib (N=64). The

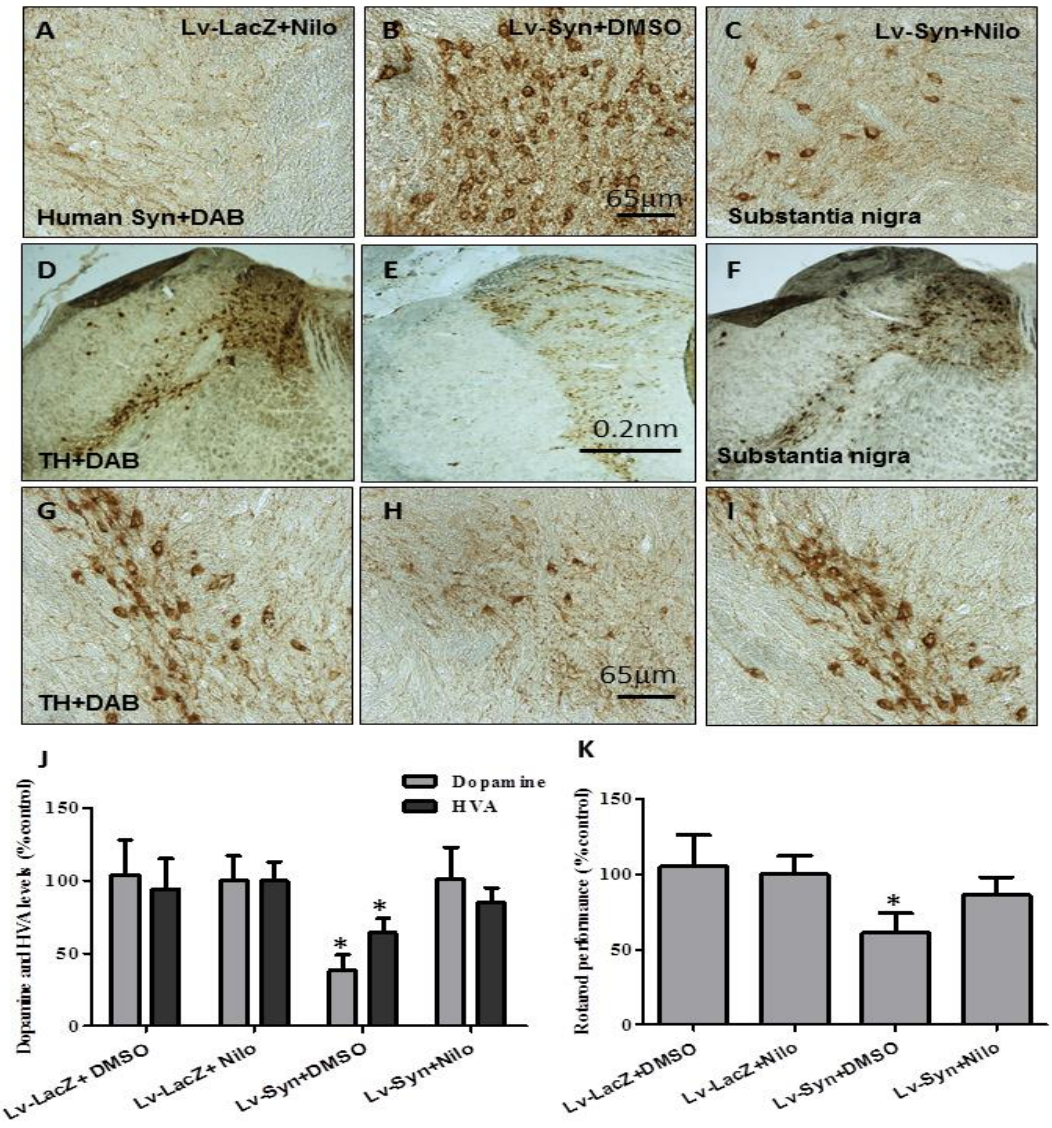


Fig. 4- Nilotinib protects dopamine neurons and improves motor. Staining of 20µM thick brain sections shows human alpha-Synuclein in the SN of **A**) lentiviral LacZ injected mice treated with 10mg/kg Nilotinib, **B**) lentiviral alpha-Synuclein treated with DMSO and **C**). lentiviral alpha-Synuclein treated with 10mg/Kg Nilotinib daily for 3 weeks. Staining of 20 µm thick brain sections show TH in **D**). lentiviral LacZ injected with Nilotinib, and **G**) is higher magnification from a different animal, **E**) lentiviral alpha-Synuclein and treated with DMSO, and **H**) is higher magnification from a different animal. **F**). lentiviral alpha-Synuclein treated with Nilotinib, and **I**) is higher magnification from a different animal. Graphs represent concentrations of **J**). DA and HVA ELISA levels in mesencephalon brain extracts of lentiviral alpha-Synuclein treated with DMSO compared to LacZ mice (N=8) and **K**). shows time spent (%) on rotarod in lentiviral a-Synuclein or LacZ injected mice with and without Nilotinib (N=14). *Significantly different, ANOVA, Neumann Keuls multiple comparison, $p<0.05$. N= number of animals, Bars are means.

effects of lower dose and longer periods of treatment were evaluated in the blood and brain of 5-6 months old A53T mice, which were injected every other day with 1mg/kg or 5mg/kg Nilotinib for 6 weeks (compared to 3 weeks). Whole blood was collected via cardiac puncture and brain tissues were extracted in lysis buffer and analyzed by ELISA. A total of 665ng/ml human alpha-Synuclein was observed in the brain of A53T treated with DMSO compared to C57BL/6 age-matched control (Fig. 3C, $p<0.05$, N=10), but alpha-Synuclein levels decreased to 503ng/ml with 5mg/kg and 344ng/ml with 1mg/kg Nilotinib for 6 weeks. A higher concentration of human alpha-Synuclein (1635ng/ml) was detected in the whole blood of A53T mice treated with DMSO compared to C57BL/6 age-matched control (Fig. 3D, $p<0.05$, N=10), and, again, alpha-Synuclein levels decreased to 1439ng/ml with 5mg/kg and 888ng/ml with 1mg/kg Nilotinib for 6 weeks, suggesting that the decrease in brain levels may also decrease blood alpha-Synuclein. Daily IP injection of 10mg/kg Nilotinib for 3 weeks into 7-8 months A53T mice again decreased monomeric (41%) and high molecular weight human alpha-Synuclein (37) relative to actin compared to A53T mice treated with DMSO.

To ascertain that autophagy is involved in Nilotinib-mediated alpha-Synuclein clearance in A53T mice *in vivo*, autophagic vacuoles (AVs) were isolated via subcellular fractionation using a discontinuous Metrizamide gradient (75) and the levels of alpha-Synuclein and p-Tau were measured via ELISA. Subcellular fractionation was performed in an age-dependent manner to determine whether higher levels of protein accumulation alter autophagic flux through the AV10 or AV20 Metrizamide gradients, which contain LC3B (Fig. 3E, inset), indicating phagophore/autophagosome presence and the lysosomal fraction containing lysosomal associated membrane protein (LAMP)-2a. Human alpha-Synuclein was detected in AV10 (340ng/ml) and AV20 (401ng/ml) in 1 month old A53T brains (Fig. 3E, $p<0.05$, N=5) but 10mg/kg Nilotinib significantly decreased alpha-Synuclein levels (56ng/ml) in AV10 (Fig. 3E, $p<0.05$, N=5). However, Nilotinib significantly increased alpha-Synuclein levels in the lysosomes (268ng/ml) compared to DMSO (59ng/ml). Alpha-Synuclein was even higher in AV10 (940ng/ml) and AV20 (410ng/ml) in 5 months old A53T mice treated with DMSO (Fig. 3G, $p<0.05$, N=5) but alpha-Synuclein levels were decreased by Nilotinib in AV10 (245ng/ml) and increased in AV20 (642ng/ml) compared to DMSO. Nilotinib also significantly increased alpha-Synuclein levels in the lysosomes (333ng/ml) compared to DMSO (109ng/ml) in the same age group. P-Tau was also used as another protein marker that can potentially be degraded by autophagy. P-Tau was detected in AV10 (710ng/ml), AV20 (590ng/ml) and lysosomes (129ng/ml) in 1 month old A53T mouse brain (Fig. 3F, $p<0.05$, N=5), indicating Tau hyper-phosphorylation. However, 10mg/kg Nilotinib increased p-Tau in AV10 (1001ng/ml), AV20 (890ng/ml) and lysosomes (321ng/ml) compared to DMSO within the same age group (Fig. 3F, $p<0.05$, N=5). p-Tau was higher in AV10 (1540ng/ml) and AV20 (2055ng/ml) in 5 months old A53T mice treated with DMSO (Fig. 3F, $p<0.05$, N=5) but Nilotinib decreased p-Tau in AV10 (610ng/ml) and increased it in AV20 (1133ng/ml) compared to DMSO. Nilotinib also decreased p-Tau in the lysosomes (345ng/ml) compared to DMSO (530ng/ml).

These data suggest that Nilotinib inhibition of Abl triggers autophagic protein clearance in vivo.

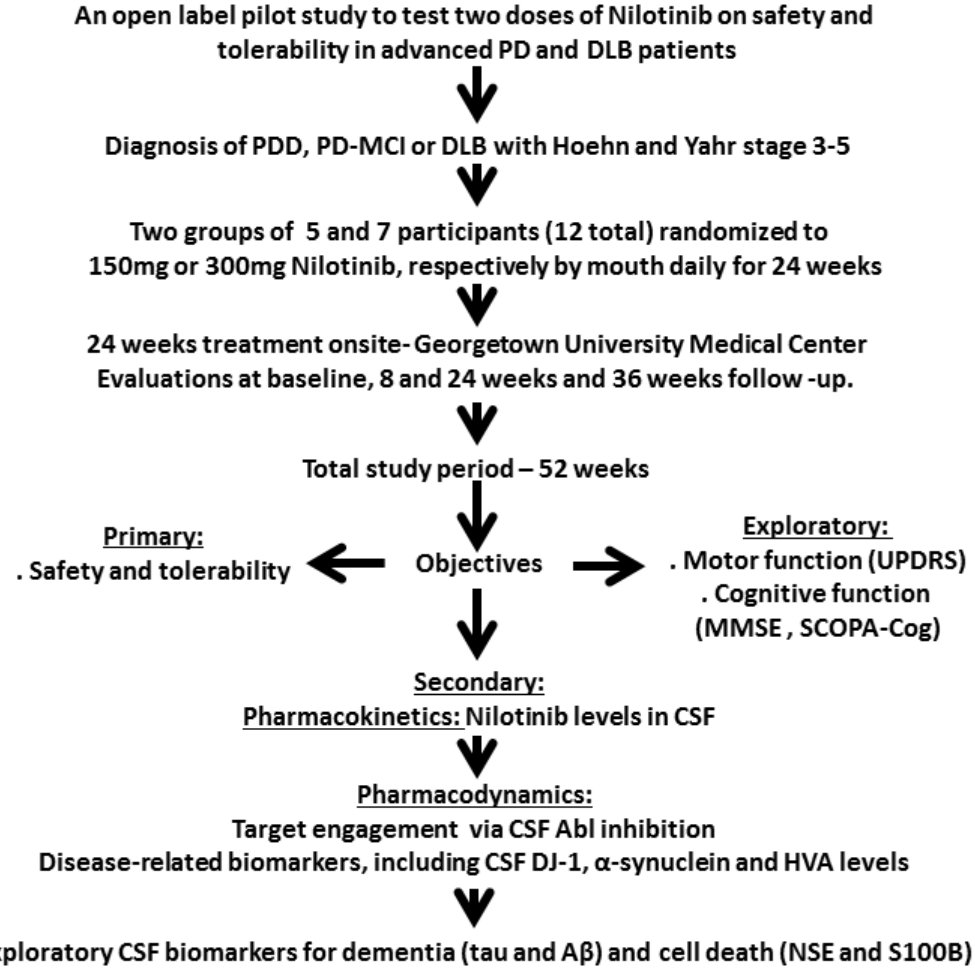
Nilotinib protects SN tyrosine hydroxylase (TH) neurons from alpha-Synuclein toxicity. Staining of 20μm thick brain sections showed human alpha-Synuclein expression in mice injected with lentiviral alpha-Synuclein into the SN and treated with DMSO (Fig. 4B) compared to LacZ treated with Nilotinib (or DMSO data not shown) mice (Fig. 4A, N=12); but Nilotinib led to 84% (by stereology)

decrease of human alpha-Synuclein (Fig. 4C, $p<0.05$, N=12) in SN neurons. A significant decrease in TH^+ neurons (89% by stereology) was observed in lentiviral alpha-Synuclein treated with DMSO (Fig. 4E&H) compared to LacZ treated with Nilotinib (Fig. 4D&G), and Nilotinib treatment of alpha-Synuclein expressing mice reversed TH^+ neuron loss back to 82% (Fig. 4F&I, by stereology) of LacZ level ($p<0.05$, N=12). To evaluate alpha-Synuclein and Nilotinib effects on dopamine (DA) metabolism, DA and its metabolite Homovanilic acid (HVA) in SN brain extracts were measured using ELISA. A significant decrease ($p<0.05$, N=8) in DA (62%) and HVA (36%) were observed in SN extracts of lentiviral alpha-Synuclein treated with DMSO compared to LacZ with and without Nilotinib (Fig. 4J). However, Nilotinib significantly ($P<0.05$, N=8) reversed DA and HVA loss back to control (Fig. 4J, N=8). Alpha-Synuclein expression in SN decreased rotarod performance 39% (Fig. 4K, $p<0.05$, N=14) of LacZ with and without Nilotinib, but Nilotinib reversed motor performance to 86% of LacZ (Fig. 4K, $p<0.05$, N=14).

9.2- Human Studies

To examine the effects of lower doses of Nilotinib on safety and efficacy in PDD and PD with MCI and DLB, twelve late stage (Hoehn and Yahr 3-5) PDD and DLB participants were randomized into 150mg (N=5) or 300mg (N=7) groups and received oral daily doses of Nilotinib for 6 months with 3-month follow up (Chart 3 and Table 6). The primary objective was to determine the safety and tolerability of Nilotinib for 6 months throughout a total of 11 visits. Electrocardiograms (EKG), physical and neurological exams and blood draws for laboratory chemistry were performed every 2 weeks for the first 2 months, and every month thereafter. Participants were on stable medical conditions on L-Dopa treatment and other PD medications for 4 weeks prior to study enrollment as indicated in Table 6. Patients with cardiovascular complications, infarcts, QTc interval >450ms and those who are on drugs that prolong QTc or with potential drug contraindications were excluded as per package insert. Thirty patients were screened and 12 enrolled. The secondary objectives determined whether Nilotinib

Chart 4- Standard CONSORT flow diagram for the trial



crosses the BBB. Nilotinib concentration was measured in plasma and CSF at baseline, 2 months (interim) and 6 months. Pharmacodynamics analysis was performed to determine target engagement via measurement of CSF phosphorylated Abl (activity) in addition to neurodegeneration biomarkers,

including alpha-Synuclein, dopamine metabolite HVA, total Tau and p-Tau181/231, Aβ42 and Aβ40. Cell death markers, including CSF NSE and glial and neuronal marker S100B were measured. Tertiary/exploratory outcomes included measurement of motor and non-motor symptoms using UPDRS I-IV. Montreal Cognitive Assessment (MoCA) in the range of 18-26 was used at screening to determine mild cognitive impairment (MCI), moderate cognitive impairment (17-10) and severe cognitive impairment (<10) in PD and LBD patients. Nilotinib effects on progression of dementia were evaluated

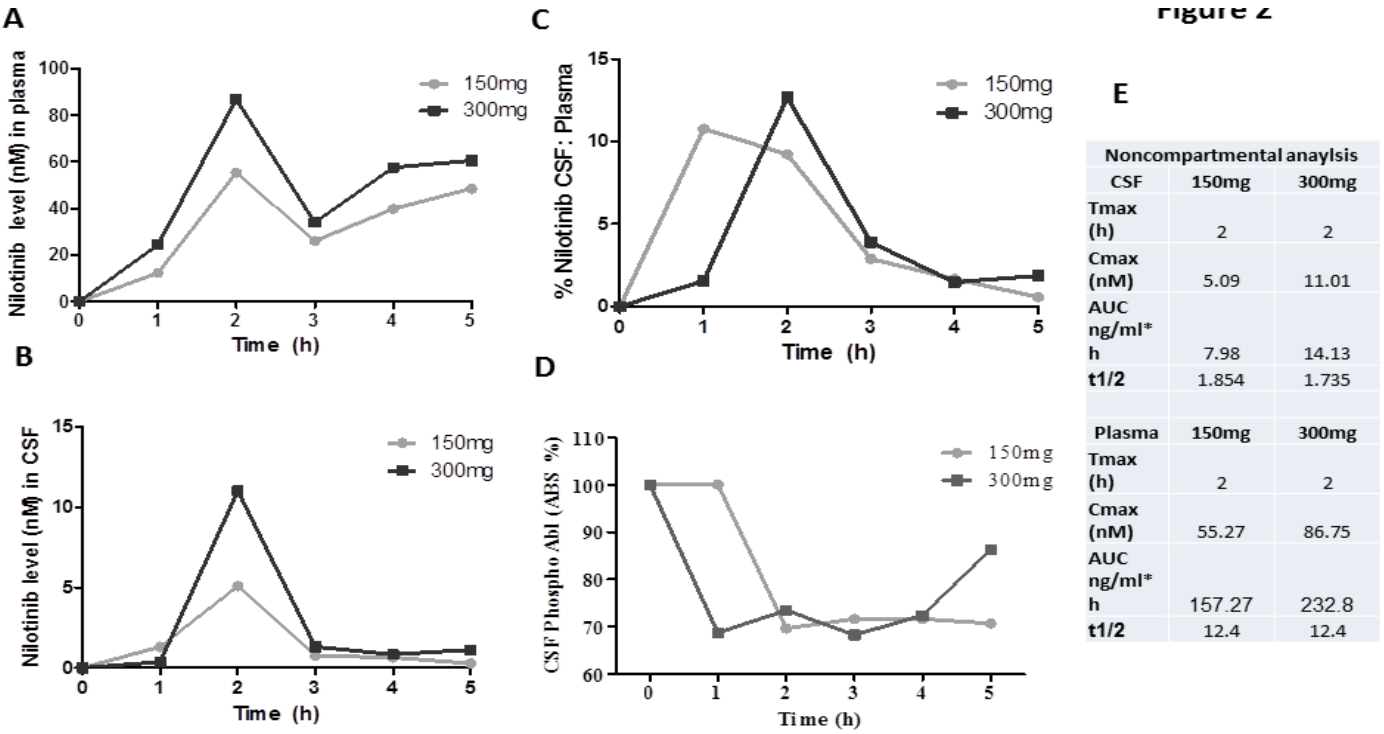
Table 6- Demographics summary of all participants, including 18 "screen fail" and 12 enrollment. PD: Parkinson's disease; DLB: dementia with Lewy body; MCI: mild cognitive impairment; PDD: Parkinson's disease with dementia; MoCA: Montreal Cognitive Assessement.

Total screened	30											
Total enrolled	12											
Total withdrawal due to adverse events	1											
Voluntary discontinuation	1											
Study duration	24 weeks											
Nilotinib study group (mg)	150	150	150	150	150	300	300	300	300	300	300	300
	NIL-01	NIL-05	NIL- 08	NIL-14	NIL-11	NIL-06	NIL-09	NIL-12	NIL-13	NIL-03	NIL-15	NIL-02
Age	69	72	73	74	74	67	81	49	65	74	89	75
Gender	M	M	F	M	M	M	M	M	M	F	F	M
Weight (kgs.)	60.1	71.9	52.3	63.2	76	83.2	78.9	110.2	54.5	69.8	46.7	75.7
Height (cm)	165.8	170.2	142	166.6	175.2	175.1	178.4	170.4	177	168.1	160	178.2
BMI	21.9	24.8	25.9	22.8	24.8	27.1	24.8	38	17.4	24.7	18.2	23.8
Levodopa at baseline	450	500	750	400	1000	500	400	550	500	1150	1160	950
Levodopa at week 24	450	500	650	400	750	500	400	550	500	1025	1160	500
Azilect at baseline	0.5mg	0.5mg	0.5mg	none	1mg	1mg	1mg	1mg	1mg	1mg	1mg	0.5mg
Azilect at week 24	none	0.5mg	none	none	1mg	0.5mg	1mg	none	0.5mg	none	0.5mg	none
Years since diagnosis	10	9	13	3	18	13	20	15	8	11	13	7
Race	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Other	Asian	Caucasian	Caucasian	Caucasian	Caucasian	Other
Diagnosis	DLB	DLB	DLB	DLB	PD-MCI	PDD	PD-MCI	PD, MCI	DLB	PD	PDD	PD-MCI
MoCA	11	Unable	9	None	21	14	19	23	22	28	16	22
Stage	Stage 3	Stage 5	Stage 5	Stage 3	Stage 3	Stage 3	Stage 4	Stage 3	Stage 5	Stage 3	Stage 5	Stage 3

using the MMSE and SCOPA-Cog.

9.2.a- Pharmacokinetic studies

Blood and CSF were collected from twelve patients at baseline prior to treatment (N=12, control) and 2 months into treatment (N=12). Blood and CSF were collected from nine patients at six months (N=9) of Nilotinib treatment (total N=33 samples) and collection time via lumbar puncture (LP) was staggered at 0, 1, 2, 3, 4 or 5hrs after oral administration of Nilotinib. Blood was collected half an hour prior to LP. Two CSF samples were eliminated because they had >25ng/ml hemoglobin.



E

Noncompartmental anaylsis		
CSF	150mg	300mg
Tmax (h)	2	2
Cmax (nM)	5.09	11.01
AUC ng/ml* h	7.98	14.13
t1/2	1.854	1.735
Plasma	150mg	300mg
Tmax (h)	2	2
Cmax (nM)	55.27	86.75
AUC ng/ml* h	157.27	232.8
t1/2	12.4	12.4

Fig. 5- Pharmacokinetic analysis showing A) Nilotinib concentration (T_{max}=2hrs) in human brain. Pharmacological paramaters after treatment with A1) 150 mg and A2) 300 mg Nilotinib. Pharmacodynamics of Nilotinib B) showing direct target engagement via inhibition of CSF Abl.

Pharmacokinetic analysis demonstrates that Nilotinib (Fig. 5A&E) peaks in the plasma 2hrs (T_{max}) after dosing at 55.27nM and 86.75nM (C_{max}) in the 150mg and 300mg groups, respectively. Nilotinib (Fig. 5B&E) also peaks in the CSF 2hrs (T_{max}) after dosing at 5.09nM and 11.1nM (C_{max}) in the 150mg and 300mg groups, respectively. Area under the curve (AUC) is 157.27 and 232.8 ng/ml*h for plasma and 7.98 and 14.13 ng/ml*h for CSF in 150mg and 300mg groups, respectively. Nilotinib was quantified in reference to ^{13}C Nilotinib standard and samples were precipitated and dialyzed to obtain unbound Nilotinib. Nilotinib was not detected in the CSF (t1/2 ~1.8) 3hrs after dosing suggesting short bioavailability of unbound or free Nilotinib compared to plasma t1/2=12.4 (Fig. 5E). The CSF:plasma ratio of Nilotinib (Fig. 5C) is 12% and 5% with 300mg and 150mg Nilotinib, respectively.

9.2.b- Pharmacodynamics. To demonstrate whether Nilotinib inhibits Abl via de-phosphorylation, we measured pan-tyrosine CSF Abl relative to total Abl via ELISA. Phosphorylated Abl was reduced with 150mg and 300mg Nilotinib (Fig. 5B), suggesting that Nilotinib (~12% -22%) decreases CSF Abl (>30%) phosphorylation (inhibition). These data support the pre-clinical results that Nilotinib penetrates the brain. The differences in T_{max} and C_{max} between human and mice can be attributed to several factors, including route of administration (i.e. I.P-mouse versus oral-

human), methods of dissolving the drug (DMSO in mice), integrity of BBB in advanced PDD and DLB, differences in drug metabolism between mice and human, concomitant medication and diet. Additionally, Nilotinib was quantified in whole brain extracts in mice versus human CSF, where drug-protein binding is likely to be different. Collectively these data support further investigation to determine Nilotinib concentration in CSF in larger trials with early PD.

9.3- Safety

9.3.1- Serious Adverse Events (SAEs). Adverse Events (AEs) that required or resulted in hospitalization were classified as SAEs and were listed in Table 7. One patient in the 300 mg Nilotinib group (NIL-9) was withdrawn at week 4 due to moderate cardiac ischemia and myocardial infarct and was double-stented for left bundle block. One participant in the 300mg Nilotinib group was hospitalized with urinary tract infection (UTI) and one participant in the 150mg group was hospitalized with pneumonia and UTI (Table 7).

Table 7- Serious Adverse Events (SAEs) requiring hospitalization in all participants

System Organ Class/Preferred Term	Number of affected participants N=12	Total number of events	Numb1er of affected participants in 150mg group. N=5	Number of affected participants in 300mg group. N=7
Cardiac disorders	1	2	0	1
Moderate cardiac ischemia			0	1
Myocardial Infarct			0	1
Infections and infestations	2	3	1	1
Urinary tract infection			1	1
Pneumonia			1	

9.3.2- Non-serious AEs. Non-serious AEs, which did not require hospitalization were classified per system and/or preferred term and summarized in Table 8 as number of events and number of affected participants in each group. One participant in 150mg and one in 300mg group had slight QTc prolongation>450ms (detailed in Table 9). Participants in the 150mg group reported three UTIs, two cases of pneumonia, one cold virus, one mild back pain, one mild headache, one mild dysgraphia, one mild left foot drag, one mild confusion, one mild hallucination, one mild paranoia, one mild agitation, one moderate anxiety, one mild incontinence, one moderate itching and one skin irritation. Participants in the 300mg group reported one incident of blurry vision, one diarrhea, one nausea, one mild fatigue, and two generalized weakness, three UTIs, one pneumonia, one weight loss, one tooth extraction, one dizziness, two mild hallucination, one mild paranoia, one mild crying episode, one mild urinary urgency, one mild cough and one eczematous lesion.

Table 8- Non-Serious Adverse Events in all participants throughout the study

System Organ Class/Preferred Term	Number of affected participants N=12	Total number of events	Number of affected participants in 150mg group . N=5	Number of affected participants in 300mg group. N=7
Cardiac disorders	2	3	1	1
QTc prolongation			1	2
Eye disorders	1	1	0	1
Mild blurry vision			0	1
Gastrointestinal disorders	2	2	0	2
Diarrhea				1
Nausea				1
General disorders	3	3	0	3
Mild fatigue			0	1
Generalized weakness			0	2
Infections and infestations	8	10	4	4
Urinary tract infection			3	3
Pneumonia			2	1
Cold virus			1	
Metabolism and nutrition disorders	1	1	0	1
Mild weight loss				1
Musculoskeletal and connective tissue disorders	2	2	1	1
Mild back pain			1	
Tooth extraction				1
Nervous system disorders	3	5	2	1
Mild headache			1	
Mild dysphragia			1	
Mild left foot drag			1	
Mild confusion			1	
Dizziness				1
Psychiatric disorders	4	8	3	1
Mild hallucinations			1	2
Mild Paranoia			1	1
Mild crying episodes				1
Mild agitation			1	
Moderate anxiety			1	
Renal and urinary disorders	2	2	1	1
Mild urinary urgency				1
Mild incontinence			1	
Respiratory, thoracic and mediastinal disorders	1	1	0	1
Mild cough				1
Skin and subcutaneous tissue disorders	2	3	1	1
Moderate itching			1	
Moderate skin irritation			1	

9.3.3- QTc prolongation. One participant (NIL-9) in the 300 mg was withdrawn due to moderate cardiac ischemia and myocardial infarct and was double-stented for left bundle block (Table 7&9). NIL-8 had slight QTc prolongation>450ms at week 8, and NIL-15 had two episodes of slight QTc prolongation>450ms at week 2 and week 8 but QTc returned to normal range in subsequent visits. NIL8 withdrew from the study at week 24 due to caregiver burden, but returned for safety follow up visit (week 36) and another patient (NIL-12) did not return at week 36.

Table 9- A detailed list of QTc values throughout the study in the 150mg (top grid) and 300mg (bottom grid) groups. Normal QTc was specified in the study protocol in the range of 350-450ms. EKG: electrocardiogram.

150 mg

EKG QTc (Normal range per study protocol: 350-450 ms)

	Screening	Baseline	Week-2	Week-4	Week-6	Week-8	Week-12	Week-16	Week-20	Week-24	Week-36
NIL-1	421	418	416	421	417	401	428	408	421	419	419
NIL-5	380	411	439	430	445	440	442	435	416	429	429
NIL-8	435	412	419	426	436	456	423	444	441	out	419
NIL-11	399	392	399	411	397	425	417	398	421	410	420
NIL-14	410	424	422	416	424	424	414	424	427	423	426

300 mg

EKG QTc (Normal range per study protocol: 350-450 ms)

	Screening	Baseline	Week-2	Week-4	Week-6	Week-8	Week-12	Week-16	Week-20	Week-24	Week-36
NIL-2	394	409	407	403	387	410	404	414	408	414	414
NIL-3	411	396	405	412	410	409	421	428	407	411	411
NIL-6	410	413	405	434	438	432	444	440	442	432	432
NIL-9	441	430	447	510	out						
NIL-12	431	435	443	440	432	440	444	422	434	424	out
NIL-13	418	415	426	403	409	413	433	412	420	442	388
NIL-15	428	440	456	444	445	463	449	442	446	450	432

9.3.4- Blood chemistry. Laboratory tests (Table 10) were performed throughout eleven visits according to study protocol. Laboratory results of some participants' were outside the normal range at baseline due to comorbidities that were not exclusionary. A total number of three AEs below normal range of ALT level (<100%) were observed in the 150mg group Nilotinib. Two AEs above normal range (>100%), including one ALT and one bilirubin were observed in the 150mg group Nilotinib. Two AEs with an increase of ALT and one AST above normal range (>100%) were observed in the 300mg group Nilotinib.

Table 10- List of results of laboratory tests at baseline and after Nilotinib administration. At baseline, 3 participants had slightly below normal range of ALT, 1 amylase and 1 LDL. A slight increase above the normal range was recorded in WBC in 1 participant, 4 ALT, 1 amylase, 2 cholesterol, 1 HDL, 2 LDL and 3 triglyceride. Low density lipoprotein (LDL): high density lipoprotein (HDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST). white blood cells (WBC).

Normal Range	Lab Test	Baseline (N=12)		Number of events below (<100%) normal range (N-12)		Number of events above (>100%) normal range (N-12)	
		Slightly lower than normal	Slightly higher than normal	Number of events in 150 mg group (N=5)	Number of events in 300 mg group (N=7)	Number of events in 150 mg group (N=5)	Number of events in 300 mg group (N=7)
5-10 k/uL	WBC	0	1	0	0	0	0
12-17 g/dL	Hemoglobin	0	0	0	0	0	0
36-52 %	Hematocrit	0	0	0	0	0	0
145-400 k/uL	Platelet	0	0	0	0	0	0
137-145 mmol/L	Sodium	0	0	0	0	0	0
2.5-4.5 mg/dL	Phosphate	0	0	0	0	0	0
8.4-10.2 mg/dL	Calcium	0	0	0	0	0	0
1.6-2.3 mg/dL	Magnesium	0	0	0	0	0	0
3-34 units/L	AST	0	0	0	0	0	1
15-41 units/L	ALT	3	4	3	0	1	2
0.3-1.9 mg/dL	Total Bilirubin	0	0	0	0	1	0
23-300 units/L	Lipase	0	0	0	0	0	0
30-110 units/L	Amylase	1	1	0	0	0	0
120-200 mg/dL	Total Cholesterol	0	2	0	0	0	0
40-80 mg/dL	HDL	0	1	0	0	0	0
85-125 mg/dL	LDL	1	2	0	0	0	0
50-150 mg/dL	Triglyceride	0	3	0	0	0	0

9.4- Disease related biomarker studies. CSF alpha-Synuclein concentration was significantly reduced at six months compared to baseline in the 150mg group (Table 11, N=4), but alpha-Synuclein did not change in the 300mg group (N=5). CSF HVA also significantly increased in the 300mg group at six-month compared to baseline (Table 11, N=5), but no significant differences were observed in the 150mg group between baseline and six months treatment. CSF total Tau was significantly reduced at six months compared to baseline (Table 11, N=4) in 150mg group and no changes in total Tau were observed in 300mg groups, and these results were confirmed by Invitrogen and Millipore ELISA. CSF pTau181 concentration was increased at six months compared to baseline with 300mg Nilotinib (Table 11, N=5) and the ratio of pTau181:total Tau was increased at six months in 300mg groups (p=0.0225). However, CSF pTau231 was reduced at six months compared to baseline with 150mg (Table, N=4) and 300 mg (Table 11, N=5) Nilotinib and the ratio of pTau23:total Tau did not change.

CSF Aβ40 concentration was reduced at six months compared to baseline with 150mg (Table 11, N=4), but Aβ40 was stable with 300mg Nilotinib. There was no significant change in CSF Aβ42 (Table 11) and the ratio of CSF Aβ42:Aβ40 was not different. CSF concentration of NSE was reduced in 150mg group (Table 11, N=4) and the glial and neuronal cell death marker, S100B was reduced in 300mg group (Table 11 N=5) at six months compared to baseline.

Table 11- Concentration of CSF biomarkers at baseline and after 6-months Nilotinib treatment. Data are summarized as Mean±SD with significance at $p<0.05$. A one tailed paired t-test was used for comparisons between baseline and six months treatment with Nilotinib for both 150mg and 300mg treatment groups (* indicates significantly different between baseline and 6 months). Cerebrospinal fluid (CSF), α-synuclein (α-syn), Homovanillic Acid (HVA), β-Amyloid (Aβ), Neuron Specific Enolase (NSE).

CSF						
Analytes: (pg/ml)	**0 mths: (N=4)**	**6 mths (150mg Nilo): (N=4)**	**p value :**	**0 mths : (N=5)**	**6 mths (300mg Nilo): (N=5)**	**p value:**
α-syn	1831.47±608.08	1633.25±531.72	0.0319*	1263.70±584.80	1464.85±699.64	0.2958
HVA	2.15±2.16	3.62±3.07	0.2414	0.8583±0.23	1.69±0.88	0.0012**
Total tau (Invitrogen)	302.63±156.29	261±135.49	0.0291*	209.7±91.29	203±51.99	0.3744
p-tau (181) (Invitrogen)	22.96±6.34	26±3.12	0.0834	20.87±3.13	25.57±3.30	0.0051**
p-tau (181)/Total tau (Invitrogen)	0.076±0.041	0.996±0.024	0.0671	0.0995±0.034	0.126±0.063	0.0225*
Total tau (millipore)	1.1475±0.63	0.99±0.60	0.0207*	0.764±.33	0.688±0.14	0.234
p-tau (231) (millipore)	2.97±1.74	2.59±1.58	0.0445*	2.30±1.23	2.01±1.00	0.0324*
p-tau (231)/Total tau (millipore)	2.59±2.76	2.61±2.63	0.1873	3.01±3.73	2.992±7.14	0.3068
Aβ42	860.75±562	726.08±525.51	0.0579	811.96±356.38	808.04±429.68	0.4857
Aβ40	2332.6±1228.78	1875.85±1029.95	0.0437*	1817.9±765.70	1951.789±892.16	0.3964
Aβ42/Aβ40	0.369±0.457	0.387±0.510	0.1971	0.447±0.465	0.414±0.482	0.2347
S100B	1531.84±749.65	968.88±202.18	0.1509	1062.80±291.61	912.21±164.07	0.0294*
NSE	96.35±25.48	72.23±36.57	0.0197*	67.20±20.30	74.22±10.82	0.1703

Plasma concentration of alpha-Synuclein was not significantly different at six months compared to baseline in the 150mg and 300mg group (Table 12). Plasma HVA level trended towards an increase after Nilotinib treatment in 300mg and 150mg groups at six months compared to baseline. The plasma level of Aβ40 did not change after Nilotinib treatment in both 150mg and 300mg groups.

We performed further CSF analysis of oligomeric α-synuclein in the same subjects and

Table 12- Concentration of plasma biomarkers at baseline and after 6-months Nilotinib treatment Data are summarized as Mean ± SD with significance at $p<0.05$. A one tailed paired t-test was used for comparisons between baseline and six months treatment with Nilotinib for both 150mg and 300mg treatment groups. Alpha-Synuclein (α-syn), Homovanillic Acid (HVA), β-Amyloid (Aβ).

PLASMA						
Analytes	**0 mths:**	**6 mths (150mg Nilo):**	**p value :**	**0 mths :**	**6 mths (300mg Nilo):**	**p value:**
α-syn	30463.05±18802.60	76103.18±21826.26	0.965	29637.77±16476.64	29886.46±21826.26	0.491
HVA	3.73±1.19	6.89±3.41	0.0758	5.55±4.43	7.70±3.46	0.0812
Aβ40	38.92±18.69	33.91±13.95	0.0998	29.48±16.54	27.91±15.19	0.26

normalized oligomeric to monomeric α-synuclein. The level of oligomeric α-synuclein was increased in the 150mg group (Fig. 6A) but stable in the 300mg group (Fig. 6B), consistent with the changes in monomeric α-synuclein. However the ratio of oligomeric/monomeric α-synuclein was significantly higher in the 150mg group at 6 months compared to baseline (Fig. 6C, $p<0.05$) but did not change in the 300mg group (Fig. 6D). Additionally, the change in CSF oligomeric/monomeric α-synuclein (Fig. 6E) between baseline and 6 months was 40% in the 150mg group but this increase was attenuated (8%) in the 300mg group, suggesting that Nilotinib reduces CSF oligomeric α-synuclein levels. These results are consistent with our preclinical data (37,50,54) and previous evidence that the ratio of oligomeric/monomeric α-synuclein longitudinally increases in the CSF of PD patients (76,77). These data further suggest that Nilotinib reduces α-synuclein levels in PD but a placebo group is needed to better understand the effects of Nilotinib on α-synuclein species.

9.5- Nilotinib ameliorates motor

Table 13- Detailed UPD scores of a participants during ever visit. PD: Parkinson's disease; DLI dementia wi Lewy body; M mild cognitiv impairment PDD: Parkinson's disease with dementia; UPDRS: Unified Parkinson's Disease Rating Score.

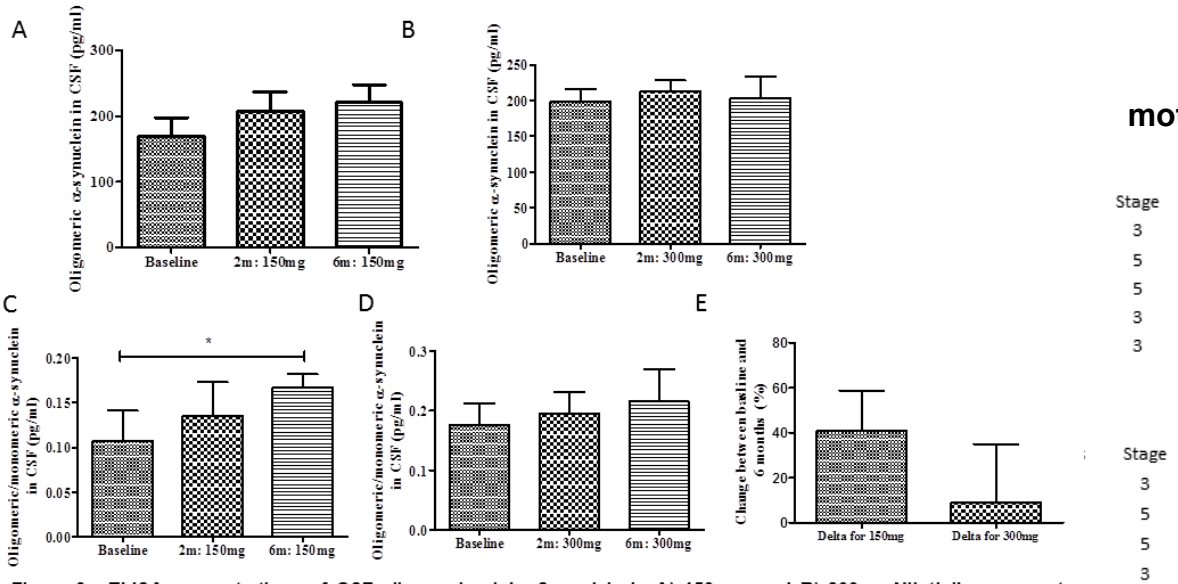


Figure 6- ELISA concentrations of CSF oligomeric alpha-Synuclein in A) 150 mg and B) 300mg Nilotinib groups at baseline, 2 and 6 months treatment. CSF oligomeric alpha-Synuclein normalized to monomeric alpha-Synuclein was significantly increased in C) 150mg group but not the D) 300mg Nilotinib groups between baseline and 6 months treatment. The increase (E) between baseline and 6 months CSF oligomeric alpha-Synuclein is 41% in the 150mg group but this increase was reduced to 8% in the 300mg group. N=5 in 150mg and N=7 in 300mg groups. p<0.05.

	Screening	Week-4	Week-8	Week-12	Week-16	Week-20	Week-24	Week-36	Diagnosis	Stage
NIL- 02	16	11	9	9	13	7	9	10	PD-MCI	3
NIL-03	12	14	11	11	16	16	12	21	PD	3
NIL-06	11	7	11	8	6	7	6	16	PDD	3
NIL-12	16	22	18	18	21	21	21	out	PD-MCI	3
NIL-13	77	67	70	67	67	69	70	70	DLB	5
NIL-15	52	47	49	47	44	41	44	42	PDD	5
AVG	30.6	28	28	26.6	27.8	26.8	27	31.8		

UPDRS-I-IV 300mg

	Screening	Week-4	Week-8	Week-12	Week-16	Week-20	Week-24	Week-36	Diagnosis	Stage
NIL- 02	36	31	28	26	33	23	20	30	PD-MCI	3
NIL-03	33	45	41	41	46	41	28	54	PD	3
NIL-06	28	21	25	19	17	16	15	30	PDD	3
NIL-12	43	49	45	46	49	54	45	out	PD-MCI	3
NIL-13	126	116	118	115	113	106	107	109	DLB	5
NIL-15	109	103	105	97	95	92	95	92	PDD	5
AVG	62.5	60.8	60.3	57.3	58.8	55.3	51.6	63		

Table 14- Summary of statistical analyses of Unified Parkinson's Disease Scores (UPDRS), Mini Mental Status Exam (MMSE) and Scales for Outcomes in Parkinson's Disease-Cognition (SCOPA-Cog) at 6 months compared to baseline in 150mg and 300mg Nilotinib. Data are mean±SD. (*) indicates significantly different to baseline

	150 mg, N=5			300 mg, N=7		
	Baseline	Week-24	p-value	Baseline	Week-24	p-value
UPDRS-III	37.8 ± 10.4	34.4 ± 16.9	0.2087	30.7 ± 27.4	27 ± 25.2	0.07
UPDRS I-IV	72.6 ± 19.1	65.6 ± 29.1	0.1456	62.5 ± 43.2	51.7 ± 39.7	0.0098 **
MMSE	15.4 ± 11	19.3 ± 11.8	0.1332	17 ± 13.1	20.5 ± 10.7	0.013 *
SCOPA-Cog (**/43)	9.4 ± 10.4	11.3 ± 11	0.3188	10.3 ± 10.7	12.3 ± 12.1	0.1082

impairment. Participants' motor symptoms were monitored with UPDRS throughout all visits (Table 13). An average decrease of 3.4 points and 3.6 points in UPDRS-III (motor) was observed at six months compared to baseline with 150mg and 300mg Nilotinib, respectively. An average decrease of 7 points and 11.1 points in UPRDS I-IV was observed at six months in 150mg and 300mg groups, respectively. An average decrease of 7 points and 11.1 points in UPRDS I-IV were observed at 6 months in 150mg and 300mg groups, respectively. In the 300mg Nilotinib group, UPDRSI-IV significantly improved (p=0.0098) at six months compared to baseline (Table 14). The improvement in UPDRS was progressive throughout the six months (24 weeks) trial, and this improvement was reversed in participants (who returned) in the follow up visit off drug at thirty six weeks.

9.6- Nilotinib improves cognitive symptoms. Participants' progression of cognitive decline was monitored with MMSE throughout all visits (Table 15). An average increase of 3.85 (out of 30) and 3.5 points in MMSE was observed at six months compared to baseline with 150mg and 300mg Nilotinib, respectively. In the 300mg group MMSE significantly increased (p=0.013) at six months compared to

Table 15- Detailed MMSE and SCOPA-Cog scores during every visit. PD: Parkinson's disease; DLB: dementia with Lewy body; MCI: mild cognitive impairment; PDD: Parkinson's disease with dementia; MMSE: Mini Mental State Examination. SCOPA-Cog: Scales for Outcomes in Parkinson's Disease-Cognition

MMSE

150 mg

Subjects	Screening	Week-4	Week-8	Week-12	Week-16	Week-20	Week-24	Week-36	Diagnosis	Stage
NIL-01	17	20	22	21	26	26	23	15	DLB	3
NIL-05	0	0	1	1	1	1	2	0	DLB	5
NIL-08	9	11	11	11	8	10	out	4	DLB	5
NIL-11	26	28	29	29	30	30	29	29	PD-MCI	3
NIL-14	25	28	27	28	28	25	23	30	DLB	3
AVG	15.4	17.4	18	18	18.6	18.4	19.25	15.6		

MMSE

300 mg

	Screening	Week-4	Week-8	Week-12	Week-16	Week-20	Week-24	Week-36	Diagnosis	Stage
NIL-02	26	27	28	29	27	26	28	25	PD-MCI	3
NIL-03	30	28	23	26	29	30	30	30	PD	3
NIL-06	22	24	24	25	25	25	25	15	PDD	3
NIL-12	23	28	24	28	14	24	26	out	PD-MCI	3
NIL-13	0	5	2	1	6	2	5	0	DLB	5
NIL-15	1	10	8	6	12	6	9	7	PDD	
AVG	17	20.3	18.1	19.1	18.8	18.8	20.5	15.4		

SCOPA-Cog (**/43)

150 mg

	Screening	Week-8	Week-20	Week-24	Diagnosis	Stage
NIL-01	5	5	3	4	LBD	3
NIL-05	0	0	1	0	LBD	5
NIL-08	1	4	0	out	LBD	5
NIL-11	22	23	26	23	PD-MCI	3
NIL-14	19	18	18	18	LBD	3
AVG	9.4	10	9.6	11.25		

SCOPA-Cog (**/43)

300 mg

	Screening	Week-8	Week-20	Week-24	Diagnosis	Stage
NIL-02	15	22	21	22	PD-MCI	3
NIL-03	28	30	30	31	PD	3
NIL-06	13	11	19	10	PDD	3
NIL-12	6	9	10	10	PD-MCI	3
NIL-13	0	0	0	0	DLB	5
NIL-15	0	1	0	1	PDD	
AVG	10.33	12.17	13.3	12.33		

baseline (Table 14). The increase in MMSE scores was incremental throughout the twenty four-week

treatment, and it declined in the thirty weeks-week follow up visit. Improvement in cognitive symptoms was also observed using Scales for Outcomes in Parkinson's Disease-Cognition (SCOPA-Cog) with an average increase of 1.85 (out of 43) and 2 points at six months compared to baseline with 150mg and 300mg Nilotinib, respectively.

10- TREATMENT ADMINSTERED

10.1- Name of the drug and all active ingredients

One hard white to yellowish powder in red opaque hard gelatin (Tasigna) capsule contains 200 mg Nilotinib (as hydrochloride monohydrate) and 117.08 mg lactose (as monohydrate). The Investigator Brochure (IB) indicating the pharmacological, safety and kinetics of the drug is attached.
In brief, Nilotinib has been clinically prescribed since FDA approval in 2007, and substantial pharmacokinetics data are published (51-53,78). TKIs such as Imatinib (Glevec) are effective in many patients with CML in chronic phase (79,80). Nilotinib (AMN107) is a second generation selective Bcr-Abl inhibitor, which is effective following Imatinib resistance and intolerance (79). Nilotinib was approved by the FDA in 2007 for CML treatment (up to 1200 mg orally daily) (51-53).

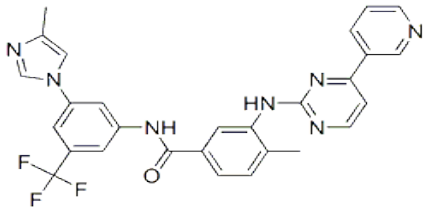
10.2- Pharmacological Class of the Drug

Nilotinib belongs to the pharmacotherapeutic group: Antineoplastic agents, protein kinase inhibitors, ATC code: L01XE08
Nilotinib is a potent inhibitor of the Abl tyrosine kinase activity of the BCR-ABL oncoprotein both in cell lines and in primary Philadelphia-chromosome positive leukemia cells. The substance binds with high affinity to the ATP-binding site in such a manner that it is a potent inhibitor of wild-type BCR-ABL and maintains activity against 32/33 imatinib-resistant mutant forms of BCR-ABL. As a consequence of this biochemical activity, Nilotinib selectively inhibits the proliferation and induces apoptosis in cell lines and in primary Philadelphia-chromosome positive leukemia cells from CML patients. In murine models of CML, as a single agent Nilotinib reduces tumor burden and prolongs survival following oral administration.

Nilotinib has little or no effect against the majority of other protein kinases examined, including Src, except for the platelet derived growth factor (PDGF), Kinase tyrosine kinase (KIT) and Ephrin receptor kinases, which it inhibits at concentrations within the range achieved following oral administration at therapeutic doses recommended for the treatment of CML.

10.3- Structural Formula of the Drug

Nilotinib has the molecular formula $C_{28}H_{22}F_3N_7O$ and a molecular weight of 529.52g/mol. The structural formula of Nilotinib is as follows:



10.4- Formulation of the dosage form to be used

One hard white to yellowish powder in red opaque hard gelatin (Tasigna) capsule contains 200 mg Nilotinib (as hydrochloride monohydrate) and 117.08 mg lactose (as monohydrate). No excipients have any known effects and the full list of excipients in hard capsule includes:
Lactose monohydrate
Crospovidone
Poloxamer 188
Silica, colloidal anhydrous
Magnesium stearate

Hard capsule shell
Gelatin
Titanium dioxide
Red iron oxide
Yellow iron oxide
Printing ink
Shellac
Black iron oxide.

Placebo: Matching placebo that contains all ingredients except Nilotinib will be used. The drug will be procured in 200mg capsules and matching placebo will be manufactured by Sharp Clinical Sevices.

10.5- Summary of Previous Human Experience

Several oncology clinical trials data were reported for Tasigna. Exposure to Tasigna in a total of 717 patients from a randomized Phase III study in patients with newly diagnosed Ph+ CML in chronic phase treated at the recommended dose of 300 mg twice daily (n=279) and from an open-label multicenter Phase II study in patients with imatinib-resistant or intolerant CML in chronic phase (n=321) and accelerated phase (n=137) treated at the recommended dose of 400 mg twice daily. No data were reported on CNS clinical trials. The following safety data were reported:

a- ***In patients with newly diagnosed CML in chronic phase***

The median duration of exposure was 48.0 months (range 0.1-58.7 months). The most frequent (≥10%) non-hematological adverse reactions were rash, pruritus, headache, nausea, fatigue, alopecia and myalgia. Most of these adverse reactions were mild to moderate in severity. Upper abdominal pain, constipation, diarrhea, asthenia, dry skin, muscle spasms, arthralgia, vomiting, abdominal pain and peripheral edema were observed less commonly (<10% and ≥5%) were of mild to moderate severity, manageable and generally did not require dose reduction.

Discontinuation due to adverse drug reactions was observed in 9% of patients.
Treatment-emergent hematological toxicities include myelosuppression: thrombocytopenia (18%), neutropenia (15%) and anemia (7%). Pleural and pericardial effusions, regardless of causality, occurred in 1% and <1% of patients, respectively, receiving Tasigna 300mg twice daily. Gastrointestinal hemorrhage, regardless of causality, was reported in 3% of these patients.

The change from baseline in mean time-averaged QTcF interval at steady state was 6 msec. No patient had an absolute QTcF >500 msec while on the study medicinal product. QTcF increase from baseline exceeding 60 msec was observed in <1% of patients while on the study medicinal product. No sudden deaths or episodes of torsade de pointes (transient or sustained) were observed. No decrease from baseline in mean left ventricular ejection fraction (LVEF) was observed at any time during treatment. No patient had a LVEF of <45% during treatment nor an absolute reduction in LVEF of more than 15%.

b- ***In patients with imatinib-resistant or intolerant CML in chronic phase and accelerated phase***

The data described below reflect exposure to Tasigna in 458 patients in an open-label multicenter Phase II study in patients with imatinib-resistant or intolerant CML in chronic phase (n=321) and accelerated phase (n=137) treated at the recommended dose of 400 mg twice daily. The most frequent (≥10%) non hematological drug-related adverse events were rash, pruritus, nausea, fatigue, headache, vomiting, myalgia, constipation and diarrhea. Most of these adverse events were mild to moderate in severity. Alopecia, muscle spasms, decreased appetite, arthralgia, abdominal pain, bone pain, peripheral edema, asthenia, upper abdominal pain, dry skin, erythema and pain in extremity were observed less commonly (<10% and ≥5%) and have been of mild to moderate severity (Grade 1 or 2).

Discontinuation due to adverse drug reactions was observed in 16% of chronic phase and 10% of accelerated phase patients.
Treatment-emergent hematological toxicities include myelosuppression: thrombocytopenia (31%), neutropenia (17%) and anemia (14%). Pleural and pericardial effusions as well as complications of fluid retention occurred in <1% of patients receiving Tasigna. Cardiac failure was observed in <1% of patients. Gastrointestinal and CNS hemorrhage were reported in 1% and <1% of patients, respectively. QTcF exceeding 500 msec was observed in <1% of patients. No episodes of torsade de pointes (transient or sustained) were observed.

Most frequently reported adverse reactions in Tasigna clinical studies for cancer:

Non-hematological adverse reactions (excluding laboratory abnormalities) are reported in at least 5% of the patients in Tasigna clinical studies. These are ranked under heading of frequency using one decimal precision for percentages and the following convention: very common (≥1/10) or common (≥1/100 to <1/10). Within each frequency grouping, adverse reactions are presented in order of decreasing seriousness.

10.6- Status of Drug in Other Countries

Tasigna is available in the United States, Europe and other countries of the world and requires a prescription. Nilotinib is subject to restricted medical use and the marketing authorization holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal determine compliance.

10.7- Drug Related Risks and Potential Side Effects

Nilotinib has a number of side effects as stated in the IB, including gastrointestinal complications, vomiting and nausea, and in some rare cases dizziness (51). Abl has an essential role in mammalian heart growth and development and adult complications with Abl inhibition, include edema, nausea/vomiting, muscle cramps, neutropenia, thrombocytopenia, fever, liver toxicity, arthralgia, and exanthema/rash (68). Side effects are identified for Abl in homozygous Abl mutant mice, which display dramatically enlarged hearts due to abnormally increased cardiomyocyte proliferation during later stages of embryogenesis (69). Disruption of *Abl* in mice results in neonatal lethality accompanied by pleiotropic developmental defects with variable penetrance, including ranting, splenic and thymus atrophy, B cell lymphopenia, dysfunctional osteoblasts, and foreshortened crania (70-72). However, clinical use of Nilotinib (600-800mg daily) is fairly tolerated in CML patients, but no clinical and longitudinal data exist about the long term side effects of this drug since its approval in 2007. Therefore, clinical use of Abl inhibition may have dose-limiting toxicity but we will use much lower doses in the current studies (see study design). Importantly, Nilotinib is washed out of the brain within several hours (37,47), so lower dose and prolonged period of administration may decrease misfolded protein levels, leading to slowing of motor, non-motor and cognitive decline. Furthermore, to evaluate adverse drug effects, all patients will be regularly (every two week for the first two months) monitored for specific warnings, including prolongation of QT interval. Prior to administration and periodically, patients will be monitored for hypokalemia or hypomagnesaemia. EKGs will also be obtained to monitor the QTc at baseline, 7 days after initiation, and periodically thereafter as detailed in Table 2. Nilotinib will not be administered to patients with hypokalemia, hypomagnesaemia, or long QT syndrome. Concomitant drugs known to prolong the QT interval and strong CYP3A4 inhibitors will also be avoided.

Nilotinib should be taken on an empty stomach. It should be taken one hour before eating, or 2hrs after eating to ensure appropriate absorption.

11-PRIOR AND CONCOMITANT THERAPY

Throughout the study, the subject may be prescribed concomitant medications or treatments deemed necessary to provide adequate supportive care, provided that the medications are licensed in the United States. All concomitant medications and/or treatments received by a subject should be recorded on the appropriate source document and eCRF.
Prohibited and contra-indicated medications in those receiving Nilotinib are listed in the inclusion/exclusion criteria and detailed below.

11.1- Prohibited Medications and Contraindications

Prohibited Medications

Prohibited medications for study subjects are as follows:

- Anti-coagulant medications, including Coumadin, heparin, enoxaparin, fondiparinaux, etc
- CYP3A4 enzyme inhibitors, including grapefruit juice
- St John's Wort

Pregnancy & Nursing Mothers

There are no adequate and well-controlled studies in pregnant women. Subjects or partners of male subjects must not become pregnant during the study or 3 months after stopping study drug. If a female subject becomes pregnant, study treatment must be discontinued immediately. Caution should be exercised; therefore, no subject should nurse their infant while participating in this study.

12-RATIONALE TO USE NILOTINIB TO TREAT NEURODEGENERATIVE DISEASES. Neurodegenerative diseases, including PD, are a group of genetic and sporadic disorders associated with neuronal death and progressive nervous system dysfunction. Cancer is also a collection of related genetic diseases, in which cells begin to divide without stopping and spread into surrounding tissues. Unlike neurodegeneration, in which no regeneration happens when damaged or aging post-mitotic neurons die, damaged cells survive when they should die in cancer, resulting in uncontrolled mitotic cell division to form tumors. Cancerous tumors are malignant as they spread or invade nearby tissues by cellular contiguity or metastasize via blood and/or humoral transport. In neurodegeneration, the spread of disease by contiguity is supported by the hypotheses that toxic or "prion-like" proteins propagate along neuroanatomical pathways (81), leading to progressive spread of disease and cell death. In neurodegeneration, failure of cellular quality control mechanisms leads to inadequate protein degradation via the proteasome or autophagy (82), resulting in intracellular accumulation of neurotoxic proteins. Consequently, these proteins are secreted from a pre-synaptic neuron and can traverse the synaptic cleft and enter a contiguous post-synaptic neuron. Secreted proteins may not penetrate an adjacent cell via the synapse but they may be re-routed into the cell and recycled via the endosomal system to fuse with autophagic vacuoles like the autophagosome or the lysosome (83-85). Microglia, the brain resident immune cells may also phagocytose and destroy toxic proteins (86).

Accumulation of neurotoxic proteins, including alpha-Synuclein (Lewy bodies), beta-amyloid plaques, Tau tangles, Huntington, prions and TDP-43 are major culprits in neurodegeneration. These toxic proteins trigger progressive apoptotic cell death leading to loss of many central nervous system (CNS) functions, including mentation, cognition, language, movement, gastrointestinal motility, sleep and many others. The discoveries of toxic protein propagation from cell to cell (81), leading to progression of neurodegeneration triggered a series of pre-clinical and clinical studies to limit protein propagation via antibodies (active and passive immune therapies) that can capture the protein and destroy it en route to healthy neurons. This approach is fraught with difficulties, including failure to arrest neurocognitive decline and brain edema/inflammation. Manipulation of autophagy is a novel therapeutic approach that focuses on degradation of neurotoxic proteins at the manufacturing site in order to prevent their secretion and propagation. This novel strategy leads to unclogging the cell's disposal machine and degradation of toxic proteins, thus preserving neuronal survival via bulk digestion. Preservation of neuronal survival maintains the level of neurotransmitters that are necessary for cognitive, motor and other CNS functions, leading to alleviation of symptoms as well as arrest of neurodegeneration. As neurons are post-mitotic cells, pulsatile autophagy may promote protein degradation and provide an effective disease-modifying therapy for neurodegenerative diseases.

Autophagy is a double-edged sword in cancer, either preventing accumulation of damaged proteins and organelles to suppress tumors, or promoting cell survival mechanisms that lead to tumor growth and proliferation (87). Leukemia and many other cancer treatments have been revolutionized by manipulation of autophagy, which leads to bulk degradation of unwanted or toxic molecules (88). For example in leukemia, genetic mutations and DNA damage can lead to large numbers of abnormal white blood cells (leukemia cells and leukemic blast cells) to accumulate in the blood and bone marrow, crowding out normal blood cells. Autophagy can lead to the degradation of the products of cancer-causing genes (oncogenes), tumor suppressor genes, damaged DNA and essential components of the cytosol, thereby controlling abnormal mitotic division and limiting tumor growth. Autophagy can also lead to self-cannibalization via promotion of programmed cell death, or apoptosis (89,90). Activation of the tumor suppressor p53 in response to DNA damage leads the cell to arrest proliferation, initiate DNA repair, and promote survival. However, if the DNA damage cannot be resolved by p53, it can trigger

apoptotic death (89,90). Cell division and apoptosis are mediated by signaling mechanisms via the endosomal (early and recycling) system (83). Tyrosine kinases are activated via auto phosphorylation, triggering various signaling mechanisms that mediate cell division and/or apoptosis (91,92). Tyrosine kinase inhibition via de-phosphorylation leads to signaling via the late endosomal-lysosomal pathway, thus increasing autophagic degradation (83-85) and tumor growth.

TKIs have significantly improved the life quality and expectancies in many cancers, including CML (93,94). CML is characterized by the translocation of chromosomes 9 and 22 to form the "Philadelphia" chromosome resulting in the expression of a constitutively active Breakpoint Cluster Region-Abelson (BCR-Abl) tyrosine kinase. This oncogenic protein activates intracellular signaling pathways and induces cell proliferation. Our laboratory investigated TKIs that activate autophagy and are FDA-approved for CML, thus significantly reducing research and development efforts and cost by re-purposing (reviewed in (57)) for new indications. Abl is activated in neurodegeneration (48,56,89,90). A fraction of Nilotinib crosses the blood-brain-barrier (BBB), inhibits Abl and facilitates autophagic amyloid clearance, leading to neuroprotection and improved cognition and motor behavior (49,50,54-58). Mice treated with a much lower dose of these drugs (<25% of the typical CML dose) show significant motor and cognitive improvement and degradation of alpha-Synuclein, beta-amyloid, Tau and TDP-43 without evidence of increased inflammation (49,50,54-58). There was also significant reversal of neurotransmitter alterations, including dopamine and glutamate in several models of neurodegeneration. As a modulator of myeloid cells (95), Nilotinib may also positively regulate neuronal death and produce neuro-restorative effects via increased production of necessary growth factors and proliferation of myeloid-derived glia. Autophagic toxic protein clearance and production of growth factors may restore loss of neurotransmitters, leading to improved motor and cognitive functions. Nilotinib provides a double-edge sword via manipulation of autophagy to inhibit cell division and tumor growth in CML on one hand, and promote toxic protein degradation and neuronal survival in neurodegeneration on the other hand.

13-CLINICAL ASSESSMENTS AND OUTCOME MEASURES

13-1. Clinical Variables

Assessments will be performed at visits as noted above throughout the study for clinical evaluation. In addition to the assessments evaluated below, subjects will provide information on their demographics, past medical history, including PD, as well as concomitant medication usage.

13.2- Vital Signs, Height & Weight

Vital signs, including systolic and diastolic blood pressure, pulse rate (radial artery)/minute, respiratory rate/minute, temperature and weight will be assessed at specified visits. Height will be measured and recorded at the Screening Visit only.

Medical history will be assessed as follows:

- History of PD (inquiring on date of diagnosis and first symptoms)
- PD treatment history
- Smoking history
- Important medical information for inclusion/exclusion
- Significant medical and surgical history (e.g. Allergy/Immunologic, Cardiovascular, Dermatological, ENT, Gastrointestinal, Gynecologic/Urologic, Hepatobiliary, Hermato/Lymphatic, Metabolic/Endocrine, Musculoskeletal, Neurologic, Ophthalmologic, Psychiatric, Pulmonary, renal, and Other)

Questioning of Comorbidities will be done using the Charlston Comorbidity Index and should be done at every visit after Baseline:

Metastatic solid tumor:	no/yes
AIDS:	no/yes
Moderate-to-severe liver disease:	no/yes
Hemiplegia:	no/yes
Moderate-to-severe renal failure:	no/yes
Moderate-Diabetes with endorgan damage:	no/yes
Neoplasia:	no/yes
Leukemia:	no/yes
Lymphoma:	no/yes
Myocardial infarct:	no/yes
Congestive heart failure:	no/yes
Peripheral vascular disease:	no/yes
Cerebrovascular disease:	no/yes
Dementia:	no/yes
Chronic pulmonary disease:	no/yes
Connective tissue disease:	no/yes
Ulcer disease:	no/yes
Mild liver disease:	no/yes
Diabetes:	no/yes

- Concomitant medications will be recorded at every visit after baseline.

13.3- Clinical Laboratory Assessments

The following safety laboratory tests will be performed during the study:

Standard blood chemistry, including Cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), albumin, total protein, alkaline phosphatase, total bilirubin, creatinine, calcium, chloride, sodium, potassium, magnesium, inorganic phosphorus, bicarbonate, creatine phosphokinase (CPK), gamma-glutamyl transferase (γ-GT), lactate dehydrogenase (LDH), lipase, α-amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), thyroid stimulating hormone (TSH), vitamin B12, and glucose. A standard hematology panel including, complete blood count (CBC) with differential counts will be performed. Hemoglobin (Hb), hematocrit, red blood cell count, platelet count, and white blood cell count with differential count will be measured. In addition, prothrombin time-international normalized ratio (PT-INR) will be measured for assessment of coagulation.

- Serum hCG for women of childbearing potential (WOCBP)

Additional testing may be ordered if needed, to further assess an AE, or if there is any suspicion that a subject may be pregnant, throughout the course of the study.

13.4- Physical Examination

A physical examination will be performed and recorded. A full physical examination (including vital signs) will be performed by a trained physician as indicated in the schedule of assessments (Table 2). The Physical examination will include:

- General appearance
- Abdomen
- Cardiovascular systems
- Lungs
- Lymph nodes
- Musculoskeletal systems
- Skin (with special attention to dermatological tolerability)
- Extremities
- Head, ears, eyes, nose, throat, and mouth
- Thyroid gland
- Vital signs:

  Body weight (Kg or pound)
  Height (cm or inch) (at the Screening Visit only)
  Blood pressure systolic and diastolic (mmHg)
  Heart rate (bpm)

13.5- Neurological Examination

A neurological examination will be performed and recorded as detailed in Table 2. Neurological examination will include:

- Cranial nerves
- Motor functions
- Sensation
- Coordination
- Speech

13.6- Adverse Events
AEs will be documented at each study visit, including the Screening Visit once the ICF has been signed by the subject. Information on AEs of study medication and on inter-current events will be determined at each visit by direct questioning of the subjects' review of concomitant medications, and vital sign results. AEs are incidents or complaints that do not require hospitalization, SAEs require hospitalization (see section 13).

13.7- MDS-UPDRS (includes Hoehn & Yahn staging) is used to follow the longitudinal course of PD and it is the most commonly used scale in the clinical study of PD. UPDRS motor section in particular is used to follow the progression of a person's PD. The UPDRS is made up of these sections: Part I: evaluation of mentation, behavior, and mood. Part II: self-evaluation of the activities of daily life (ADLs) including speech, swallowing, handwriting, dressing, hygiene, falling, salivating, turning in bed, walking, and cutting food. Part III: clinician-scored monitored motor evaluation. Part IV: complications of therapy. Part V: *Hoehn and Yahr* staging of severity of Parkinson's disease. Part VI: Schwab and England ADL scale

13.8- TMT-B. The Trail Making Test (TMT) is a *neuropsychological test* of visual attention and task switching (Reitan, 1958). It consists of two parts in which the subject is instructed to connect a set of 25 dots as quickly as possible while still maintaining accuracy. The test can provide information about visual search speed, scanning, speed of processing, mental flexibility, as well as executive functioning. The Trail Making Test B (TMT-B) has been shown to be sensitive to changes in cognitive ability in HD, and has been used in an HD clinical trial (Huntington Study Group Reach2HD Investigators, 2015).

13.9- MOCA is designed as a rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains, including attention and concentration, executive functions, memory, language, visuo-constructional skills, conceptual thinking, calculations and orientation. It has excellent psychometric properties and has become a widely used screening instrument for mild cognitive impairment (Smith, Gildeh & Holmes, 2007). It is administered by a nurse or clinician and takes 10 - 20 minutes.

13.10- C-SSRS
The US FDA recommends the use of a suicidality assessment instrument that maps to the Columbia Classification Algorithm for Suicide Assessment (C-CASA). The C-CASA was developed to assist the FDA in coding suicidality data accumulated during the conduct of clinical trials of antidepressant drugs. One such assessment instrument is the Columbia Suicide Severity Rating Scale (C-SSRS). The C-SSRS involves a series of probing questions to inquire about possible suicidal thinking and behavior. The US FDA also recommends assessment of depression during every visit, so the Geriatric Depression Scale (GDS) will also be performed.
Only investigators who have been fully trained in the administration of the C-SSRS and GDS will assess subject suicidality and depression. As part of training, investigators are prepared to respond to and manage instances in which patients express suicidal ideation or exhibit suicidal behavior.
At the Baseline Visit, the C-SSRS *Baseline* version will be administered. This version is used to assess suicidality over the subject's lifetime and specifically for the previous 6-month time period.
At each visit and the Final Safety visit, as applicable, the *Since Last Visit* version of the C-SSRS will be administered. This version of the scale assesses suicidality since the subject's last visit.
Information obtained from: *http://www.cssrs.columbia.edu/*

13.11- TUG- Timed Up and Go (TUG) is an assessment of mobility, balance, walking ability, and fall risk (Podsiadlo et al, 1991). It uses the time that a person takes to rise from a chair, walk three meters, turn around, walk back to the chair, and sit down. It requires both static and dynamic balance.

13.12- NPI. The behavioral outcome measure for this trial is the NPI. The NPI is a well-validated, reliable, multi-item instrument to assess psychopathology in AD based on interview with the study partner. The NPI evaluates both the frequency and severity of 10 neuropsychiatric disturbances. Frequency assessments range from 1 (occasionally, less than once per week) to 4 (very frequently, once or more per day or continuously) as well as severity (1=mild, 2=moderate, 3=severe). The overall score and the score for each subscale are the product of severity and frequency.

13.13- ADAS-Cog aims to evaluate cognitive impairment in the assessment of AD. Recommended for second stage or more detailed assessments and/or for particular research evaluations rather than for applications in routine care settings. It takes 30-45 min and administered by an interviewer. Requires additional training. ADAS-cog was included in this LBD study to better capture potential changes in ADL and non-ADLs and severity of cognitive impairment.

13.14- ADCS-ADL is an activity of daily living inventory developed by the ADCS to assess functional performance in participants with AD. Using a structured interview format, study partners are queried as to whether participants attempted each item in the inventory during the prior 4 weeks and their level of performance. The ADCS-ADL scale discriminates well between normal participants and those with mild AD and it has good test-retest reliability. The ADCS-ADL includes some items from traditional basic ADL tests (e.g., grooming, dressing, walking, bathing, feeding, toileting) as well as instrumental (complex) activities of daily living (e.g., shopping, preparing meals, using household appliances, keeping appointments, reading).

13.15- CAF consists of seven items of confusional behaviour (falls, fluctuation, drowsiness, attention, disorganised thinking, altered level of consciousness, communication), scores for which are summed to provide a severity score for fluctuating confusion ranging from 0 to 21.

13.16- IAS was developed to measure apathy and irritability in patients with dementia, including AD and HD (Chatterjee 2005). The IAS is a 28-item self-administered questionnaire collecting information about different aspects of irritability and apathy utilizing a 0-3 scale for each item to indicate severity. Both a patient and a study partner version can be administered. The IAS will be completed separately by Subjects and Study Partners (Chatterjee 2005).

13.17- PBA-s is a structured interview in which a trained interviewer rates the frequency and severity of neuropsychiatric symptoms through observation and the reporting of the Subject and Study Partner. Symptoms rated include depressed mood, suicidal ideation, anxiety, irritability, angry or aggressive behavior, apathy, perseverative thinking or behavior, obsessive-compulsive behaviors, delusional or paranoid thinking, hallucinations, and disoriented behavior. Each behavioral problem is rated for both severity and frequency on a 0-4- point scale; severity and frequency ratings are then multiplied to provide an overall score for each symptom.

13.18- CSF and Blood Biomarkers

Subjects will also be asked to provide blood samples for biomarker analysis per Schedule of Activities. Blood samples will be stored in a sample repository at Georgetown University Medical Center (GUMC)

laboratory for Dementia and Parkinsonism, where bio fluids will be indefinitely stored and may be used for further biomarker validation studies. All samples will be labeled with a code. The code will not include any identifiable information. Any analysis performed on these samples is for research purposes only. Unused samples will remain in the biorepository for future PD-related research. There is no scheduled date on which the samples will be destroyed. Samples may be stored for research until they are used, damaged, decayed or otherwise unfit for analysis. Subjects have the option of declining participation in this portion of the study at any time by withdrawing their consent to have their sample used. However, it will not be possible to destroy samples that may have already been used.

14- BIOMARKERS

14-1- Rationale for CSF and plasma biomarkers. The biomarkers proposed include CSF levels of alpha-Synuclein, total Tau and p-Tau$_{181/231}$, which yielded useful results in our open label pilot trial and are identified as potential CSF biomarkers for PD and DLB pathology.
Several studies indicate that reduction of CSF alpha-Synuclein is associated with alpha-Synuclein pathology in the CNS (96-98). CSF alpha-Synuclein, which probably comes from a neuronal source, has a lower level than the highly abundant presence of peripheral alpha-Synuclein (99). However, only a few studies have investigated CSF alpha-Synuclein levels in patients with neuropathologically confirmed alpha-Synucleinopathies (65,100). CSF alpha-Synuclein is reduced in de novo PD patients compared with healthy individuals (101), and it is lower in patients with advanced alpha-Synucleinopathies compared to other neurological disorders (100). Our pilot study showed that alpha-Synuclein is significantly decreased with 150mg Nilotinib between baseline and 6 months, perhaps reflecting the natural progression of the disease. However, 300mg Nilotinib attenuates the loss of CSF alpha-Synuclein between baseline and 6 months treatment. Therefore, we expect Nilotinib to reduce the decrease in CSF alpha-Synuclein level indicating reduced cell death, which will also be compared with direct markers of cell death, including NSE and S100B.

Tau pathology is also frequently found in the CNS of PD patients and have been associated with the development of cognitive impairment and PD dementia (102). Several studies also demonstrate increased levels of CSF total Tau and p-Tau181 levels (65-67) in PD patients, but another study report slightly decreased or normal levels of CSF total Tau and p-Tau (103). The CSF variably as reported in the literature in CSF Tau levels may be due to disease stage and/or differences in diagnostic criteria. Therefore, based on the preliminary data we obtained from our pilot trial showing changes in CSF Tau with Nilotinib treatment, we will measure CSF Tau in this study as another marker of cell death and compare Tau with NSE and S100B at baseline and 6 months in a placebo-controlled study.

The R47H and other variants of triggering receptors on myeloid cells (TREM)-2, which result in loss of TREM2 function, are strong risk factors for PD and DLB (104-106). Activated microglia in the SN proliferate and produce reactive oxygen species and pro-inflammatory cytokines, resulting in progressive degeneration of DA neurons in PD (107,108). TREM2 may regulate microglial response and phagocytosis. TREM2 inhibits inflammatory responses in microglia via suppression of NF-kB pathways and activation of innate immunity (109), while TREM2 loss of function results in reduced microglial phagocytosis (110-112). Therefore, measuring TREM2 levels in the CSF may provide another important pharmacodynamics effect indicating neuro-inflammation and the phagocytic activity of microglia to potentially reduce alpha-synuclein levels after Bosutinib treatment of DLB patients. The biomarkers proposed include CSF levels of total and oligomeric alpha-Synuclein, total tau and p-tau$_{181/231}$, which are identified as potential CSF biomarkers for PD and DLB pathology.

Other candidate biomarkers for PD include catecholamines, such as dopamine and noradrenaline, and their metabolites. Levels of CSF HVA, which is the end byproduct of the neuronal metabolite of dopamine is reduced in PD (113,114), reflecting diminution of stores of central dopamine. We will measure CSF HVA and quantify DaTscan to measure dopamine transporters activity in collaboration with GE Healthcare at baseline, 3 months (interim) and 6-months to correlate imaging and CSF biomarkers of dopamine metabolism. DaTscan is clinically useful in early stages of PD and since we observe an increase in HVA level with Nilotinib treatment, we expect Nilotinib to increase HVA levels, reflecting an increase in central dopamine stores. We also expect Nilotinib to increase dopamine transporters activity, which will be quantified at baseline and compared to 3 and 6 months Nilotinib treatment. We will also measure changes in exploratory biomarkers of pathophysiology, which may show Nilotinib effects on biomarker levels that will help us to build a better clinical development program going forward.

CSF, plasma DLB biomarkers: Blood draw (15ml) and lumbar puncture (LPs) to obtain CSF (~15ml) will be performed on all patients at baseline and 12-months after treatment. Blood will be drawn 0.5hr before LP s outlined above. Plasma will be isolated immediately after blood draw and will be aliquoted and stored at -80^{o}C. CSF will be aliquoted and stored at -80^{o}C. Freeze and thaw cycles will be avoided. To avoid CSF contamination with blood, the first 1 mL of CSF collection will be discarded and all samples will be centrifuged at 1000g for 15 minutes. Samples that contain a detectable level of Hemoglobin will be eliminated from alpha-Synuclein and HVA evaluation.

Plasma and CSF sample preparation for Mass Spec to determine Nilotinib pharmacokinetics

Plasma and CSF samples (20 µl) will be thawed initially on ice at room temperature and transfused to Eppendorf tubes containing 100µl of water. 500µl extraction solvent, Acetonitrile/Methanol (50:50) containing the internal standard (5ng/mL of Nilotinib_13C_2H3) will be added to the sample. The mixture will be vortexed and incubated for 20min on ice to accelerate protein precipitation. After incubation, the samples will be vortexed and centrifuged at 13,000 rpm for 20 min at 4^{O}C. The supernatant is freeze- dried using speed vacuum and reconstituted in 200µL of Methanol: Water (50:50) and processed by Mass Spectrometry.

14.2- Pharmacokinetics. Plasma and CSF will be collected 2hrs (T_{max} Figure 5) after oral administration of Nilotinib to determine the pharmacokinetic parameters of Nilotinib after dosing with 150 mg and 300 mg. Note that no Nilotinib was detected in CSF after 4 hrs. in our prior study (Figure 5 A). Quantitation of Nilotinib will be performed using multiple reactions monitoring mass spectrometry. The samples will be resolved on an Acquity UPLC BEH C18 1.7µm, 2.1 x 50 mm column online with a triple quadrupole mass spectrometer (Xevo-TQ-S, Waters Corporation, USA) operating in the multiple reaction monitoring (MRM) mode (The sample cone voltage and collision energies will optimized for both analytes to obtain maximum ion intensity for parent and daughter ions using “IntelliStart” feature of MassLynx software (Waters Corporation, USA). The instrument parameters will be optimized to gain maximum specificity and sensitivity of ionization for the parent [m/z = 530.27 (Nilotinib), 438.25 and daughter ions [m/z = 289.01 (Nilotinib). Signal intensities from all MRM Q1/Q3 ion pairs for both analytes are ranked to ensure selection of the most intense precursor and fragment ion pair for MRM-based quantitation. The metabolite ratios are calculated by normalizing the peak area of endogenous metabolites within tissue samples normalized to the internal standard Nilotinib_13C_2H3.

14.3- Amyloid PET. Abeta positron emission tomography (PET) imaging Florbetaben F18 (Neuraceq™; Piramal Imaging) is a radioactive tracer to help visualize beta-amyloid plaque deposits in AD. Florbetaben F18 PET amyloid imaging offers a unique opportunity to detect the presence of Abeta in the human body during life. This technology is FDA-approved to detect Abeta neuritic plaque density

in adult patients with cognitive impairment who are being evaluated for AD and/or other causes of cognitive decline. The objective of Abeta image interpretation "is to estimate beta-amyloid neuritic plaque density in brain gray matter. Florbetaben is not intended for use in predicting development of dementia or other neurological condition, its use in the current studies is intended to monitor responses to Nilotinib treatment.

14.4- Cell death and exploratory biomarkers. NSE and S100B (glia and neurons), total Tau and phosphorylated Tau will be measured using Millipore ELISA at baseline and after 12 months Nilotinib treatment. Nilotinib may have a modulatory effect on myeloid cells (95), which can either proliferate and differentiate into peripheral macrophages or become myeloid-derived glia that cross the BBB and produce neurotrophic and/or inflammatory markers. Nilotinib may affect CNS glial progenitor cells. Our preliminary preclinical and clinical data show Nilotinib-induced alterations of peripheral and CNS inflammatory makers. Therefore, we will perform unbiased multiplex ELISA (Millipore) to profile a panel of 44 plasma and CSF markers, including TREM2, interleukins (IL)- 1α&β, 2, 3, 4, 5, 6, 7, 8 (CXCL8), 9, 10, 12, 13, 15, 17 α, and chemokines (C-C) including, CXCL10, CCL2, CL7, CCL22, CCL3, CCL4, platelet-derived growth factor (PDGF)-AA, PDGF-AB/BB, CCL5, CX3CL1 (fractalkine), Tumor necrosis growth factor (TNF)-α, transforming growth factor (TGF)-α, and vascular endothelial growth factor (VEGF), GFAP, neurofilaments, glial fibrillary acidic protein (GFAP) and TDP-43.

14.5- Alpha-Synuclein ELISA. Solid phase alpha-Synuclein sandwich ELISA (Cat#SIG38974, Biolegend) will be performed on CSF and plasma. To avoid repetitive freeze and thaw cycles, immediately after LP and blood draws, 15 mL CSF and 5mL plasma will be aliquoted on ice into 0.5mL tubes and stored at -80^{o}C. Fresh aliquots were used to perform or repeat ELISA. Total alpha- Synuclein rabbit monoclonal antibody (amino acids 118-123) will be coated on the microwells and 200µl CSF or plasma will be added to designated wells. CSF samples will be diluted 1:10 while plasma samples will be diluted 1:50. After overnight sample incubation at 2-8^{o}C, alpha-Synuclein will be captured by the coated antibody. After washing, a biotinylated mouse monoclonal alpha-Synuclein (amino acids 103-107) detection antibody will be added to each well to detect the captured alpha-Synuclein (amino acids 118-123). Samples will be incubated with 50µl of detection antibody for 2hrs at room temperature. After washing, 200µl of streptavidin HRP will be added and incubated for 1hr at room temperature to recognize the bound biotinylated detection antibody. Samples will then washed and incubated with 100µl of chemiluminescent substrates. Plates will be shaken for 10-15 seconds and read immediately by a luminometer. The magnitude of the luminescence is proportional to the quantity of alpha-Synuclein in the samples.

14.6- Homovanillic Acid and DOAPC ELISA. A 100µl CSF or plasma samples will be incubated with 100µl HRP-conjugate reagent and incubated for 1hr at 37^{o}C using solid phase sandwich ELISA (MyBioSource, Cat# MBS064661). All samples at baseline and 6 months will be analyzed side-by-side using same reagents. After washing, 50µl of chromogen solution A and 50µl of chromogen solution B will be added to the solution and incubated for 15min at 37^{o}C. The reaction will be stopped with 50µl stop solution and the optical density will be read at 450nM. The magnitude of the absorbance is proportional to the quantity of CSF and plasma HVA.HVA and DOPAC levels will be confirmed by LC-MS.

14.7- Total Tau and p-Tau181 measurement. Solid phase human total Tau sandwich ELISA (Invitrogen, Cat# KHB0042) and p-Tau181 (Invitrogen, Cat# KH0061) will be performed on CSF samples. All samples at baseline and 6 months will be analyzed side-by-side using same reagents. A monoclonal Tau or p-Tau181 capture antibodies will be coated onto micro-wells. 50µl of CSF will be

added to each well, allowing human Tau or p-Tau181 antigen to bind to the immobilized capture antibody, and incubated for 2hrs at room temperature. After 2hr incubation, samples will be washed and incubated with 100µl of total Tau or p-Tau181 detection antibody and incubated for 1hr at room temperature. After washing, 100µl of HRP labeled anti-rabbit IgG will be added to each well and incubated for 30min at room temperature. Samples will be washed to remove all unbound enzyme and100µl TMB, a HRP substrate, will be added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of CSF total Tau or p-Tau181.

14.8- Human Neurodegenerative Disease Panels. We will use a multiplex Xmap technology that uses magnetic microspheres internally coded with two fluorescent dyes to measure markers of neurodegeneration. All samples at baseline and 6 months will be analyzed side-by-side using same reagents. Through precise combinations of these two dyes, multiple proteins are measured within the sample. Each of these spheres is coated with a specific capture antibody. The capture antibody binds to the detection antibody and a reporter molecule, completing the reaction on the surface of the bead. 25µL of CSF or plasma will be incubated overnight at 4^{o}C with 25µL of a mixed bead solution, containing human total Tau, pTau231 and NSE (Millipore, Cat#: HND1MAG-39K) or S100B, Aβ42, and Aβ40 (CSF Aβ40 is diluted 1:10). After washing, samples will be incubated with 25µL detection antibody solution for 1.5hrs at room temperature (Millipore, CAT#: HND4MAG-36K), 25µL of Streptavidin-Phycoerythrin will be added to each well containing the 25µl of detection antibody solution. Samples were then washed and suspended in 100µl of sheath fluid. Samples will be then run on MAGPIX with Xponent software. The Median Fluorescent Intensity (MFI) data will be analyzed using a 5-parameter logistic or spline curve-fitting method for calculating analyte concentrations in samples.

15- DATA COLLECTION

All data collected as part of this study will be entered into a secure data management site maintained by Georgetown University Information Service (UIS). All data will be stored on a UIS and IRB approved databases, including long-term storage on Amazon Web Service (WAS) and will be deposited on RedCAP for an external and independent data analysis and monitoring. Data will be stored in EDC maintained by GUMC. This platform facilitates:

1. Capture of clinical and research data from neurologic patients for individual projects in a structured and secure system;
2. Aggregating and sharing uniform, de-identified and/or anonymized datasets for secondary analyses.

15.1- Role of Data Management

Data Management (DM) is responsible for the development, execution and supervision of plans, policies, programs, and practices that control, protect, deliver, and enhance the value of data and information assets.

All data will be managed in compliance with applicable Sponsor and regulatory requirements. Site personnel will collect, transcribe, correct, and transmit the data onto source documents, Case Report Forms (CRFs), and/or other forms used to report, track and record clinical research data. DM is responsible for developing, testing, and managing clinical data management activities.

15.2- Data Entry and Checks

The site personnel are instructed to enter information into the EDC. Data capture is the responsibility of the staff at the site under the supervision of the PIs. During the study, the PIs must maintain complete and accurate documentation for the study. The EDC provides password protection. An edit checking and data clarification process will be put in place to ensure accuracy of the data. Logic and range checks as well as more sophisticated rules may be built into the eCRFs to provide immediate error checking of the data entered. The system has the capability to automatically create electronic queries for forms that contain data that are out of range, out of window, missing or not calculated correctly.

15.3- Data Lock Process

The platform will have the ability to lock the project-specific visits to prevent any modification of data once the project is closed. Once this option is activated, every user will have Read-Only access to the data.

15.4- Data handling and record keeping

The PIs are responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. Data reported in the eCRF derived from source documents should be consistent with the source documents and discrepancies should be explained.

15.5- Confidentiality

The EDC software and RedCAP databases and patient data reside on Georgetown University IRB-approved servers. Physical and software access to the servers and security is provided by GUMC investigators.

15.6- Retention of Records

Research records will be retained in accordance with site IRB policies.

15.7- Publications

The PIs will be responsible for publications of results from this trial. Responsibilities will include the following:

- Analyze and interpret data gathered in this study, and write publications from these data.
- Submit manuscripts to selected journals and address peer reviewers' comments.
- Submit abstracts to selected meetings and present data at the meetings.
- Determine authorship on the basis of the Uniform Requirements for Manuscripts.

16 LITERATURE REFERENCES

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