



CLINICAL TRIAL PROTOCOL

A randomized, double blind, placebo-controlled study to evaluate the impact of low doses of Nilotinib treatment on safety, tolerability, pharmacokinetics and biomarkers in early Parkinson's disease

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| MMSE | |
| MoCA | |
| C-SSRS Baseline | |
| C-SSRS Since Last Visit | |
| GSRs | |
| GDS | |
| UPDRS | |

LIST OF ABBREVIATIONS

| | |
|---------|---|
| AE | Adverse Event/Adverse Experience |
| AD | Alzheimer's Disease |
| ADL | Activity of Daily Living |
| Abl | Abelson Tyrosine Kinase |
| BBB | Blood Brain Barrier |
| CIB | Clinical Investigator's Brochure |
| CNS | Central Nervous System |
| CRF | Case Report Form |
| CSF | Cerebrospinal Fluid |
| C-SSRS | Columbia Suicide Severity Rating Scale |
| CRU | Clinical Research Unit |
| CML | Chronic Myeloid Leukemia |
| DSMB | Data Safety Monitoring Board |
| DLB | Dementia with Lewy bodies |
| DMSO | Dimethyl Sulfoxide |
| DaTscan | Dopamine Transport Activity SPECT |
| DA | Dopaminergic |
| EDC | Electronic Data Capture |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| FTLD | Fronto-Temporal Lobar Dementia |
| GUMC | Georgetown University Medical Center |
| HVA | Homovanillic Acid |
| HCG | Human Chorionic Gonadotropin |
| ICH | International Conference on Harmonization |
| IND | Investigational New Drug Application |
| IHC | Immunohistochemistry |
| IRB | Institutional Review Board |
| ITT | Intent to treat |
| I.P. | Intraperitoneal |
| IV | Intravenous |
| LP | Lumbar Puncture |
| LB | Lewy Bodies |
| MoCA | Montreal Cognitive Assessment |
| MMSE | Mini Mental Status Exam |
| N | Number (typically refers to subjects) |
| NMR | Nuclear Magnetic Resonance |
| 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 |
| PI | Principal Investigator |
| PK | Pharmacokinetic |

| | |
|-----------|--|
| PDD | Parkinson's Disease with Dementia |
| PD | Parkinson's disease |
| 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 |
| VEGF | Vascular Endothelial Growth Factor |
| UPDRS | Unified Parkinson's Disease Rating Scale |
| UIS | University Information Service |
| WOCBP | Women of Child Bearing Potential |

1- PROTOCOL SUMMARY AND RATIONALE

1.1- Study Title

A randomized, double blind, placebo-controlled study to evaluate the impact of low doses of Nilotinib (Tasigna®) on safety, tolerability, pharmacokinetics and biomarkers in early Parkinson's disease

1.2- Version Number

Original Protocol

1.3- Study Indication

Parkinson's disease (PD)

1.4- Phase of Development

II

1.5- Rationale for the Study

Parkinson's disease (PD) is the second most common neurodegenerative disorder causing motor and non-motor symptoms. PD is characterized by death of dopaminergic (DA) neurons in the substantia nigra (SN) *pars compacta* and formation of inclusions known as Lewy bodies (LBs) that primarily contain aggregated alpha-Synuclein (1-3). Misfolded alpha-Synuclein accumulates intra-neuronally within LBs and SYN is the highest genetic risk factor for PD followed by the microtubule associated protein Tau (MAPT) (4-8). L-Dopa replacement therapies are effective for symptomatic management in PD. However, a major challenge facing PD is to develop a therapy that can halt DA neurons death and alleviate symptoms. No therapeutic approach exists to alter the levels of alpha-Synuclein aggregates and halt DA death in PD. Therefore, degradation of misfolded alpha-Synuclein is the most desirable therapeutic approach. One mechanism to degrade alpha-Synuclein is autophagy (9-13), which is a process by which the cell can degrade its own contents. There is evidence that autophagy is impaired in neurodegeneration (14-21), leading to failure of degradation of accumulating protein aggregates, including misfolded alpha-Synuclein and Tau. Importantly, autophagy is exploited therapeutically in several diseases, including adult chronic myeloid leukemia (CML). Tyrosine kinase inhibitors (TKIs) induce autophagy (13,22,23), leading to destruction of rapidly dividing tumor cells in CML (22) and degradation of intracellular amyloids, including alpha-Synuclein and hyper-phosphorylated Tau (p-Tau) in PD and Alzheimer's disease (AD) models (13,22-25). Nilotinib (Tasigna®, AMN107, Novartis, Switzerland) is approved by 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 alpha-Synuclein and p-Tau, leading to survival of DA neurons and improvement of motor function in PD models (13,23-26). For these studies, Nilotinib (1-10mg/kg daily) was used at significantly less than the clinically approved dose (up to 1200mg daily) in CML (27-29). Additionally, Nilotinib levels peak in the mouse brain 4hrs after intraperitoneal (I.P) injection and wash out by 8hrs (13,23). This is important because the lower dose and short bio-availability (up to 8hrs) will prevent Nilotinib side effects and induce autophagic degradation of alpha-Synuclein and p-Tau 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 (25,26,30-34), we conducted an open label pilot clinical trial in advanced PD with dementia (PDD) and Dementia with Lewy Body (DLB) (stage 3-4) patients. 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 CSF Abelson (Abl) activity via reduction of phosphorylated Abl in agreement with pre-clinical data (13,35,36). Several studies (see biomarkers section) show that CSF alpha-Synuclein and Abeta42 are decreased and CSF total Tau and p-Tau are increased in PD and DLB (37-39). Our data show attenuation of loss of CSF alpha-Synuclein and Abeta40/42 with 300mg (50% of the CML dose) compared to 150mg Nilotinib after 6 months treatment. CSF homovanillic acid (HVA), which is an end by-product of dopamine, is significantly increased; and CSF total Tau and p-Tau are significantly reduced (N=5, P<0.05) with 300mg Nilotinib between baseline and 6 months treatment. Despite the reduction of L-Dopa replacement therapies in our study, UDPRS I-IV scores 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 months 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. These data are very compelling to evaluate the effects of Nilotinib in a phase II, randomized, double-blind, placebo-controlled trial in patients with early PD.

We hypothesize that Nilotinib will be safe in individuals with early PD. Specifically, we hypothesize that low daily oral doses of Nilotinib will lead to CSF penetration, CSF inhibition of Abl, and stabilization of CSF alpha-Synuclein level. We predict that Nilotinib will increase CSF HVA and improve dopamine transport activity via DaTscan imaging. We also predict that Nilotinib will reduce CSF markers of cell death, including total Tau and p-Tau_{181/231} levels, neuron specific enolase (NSE) and S100B.

1.6- Study Objectives

Primary outcomes, we will evaluate the effects of Nilotinib on:

a- Safety and tolerability

- 1- 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 and myelosuppression as listed in Novartis Investigator Brochure (IB), on which we base our power consideration. These AEs will be tracked specially 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 no likely SAEs over a six months 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 outcomes: We will determine the effects of Nilotinib on primary biomarkers, including **1)** measurement of Nilotinib in the CSF and Abl inhibition to demonstrate direct target engagement and **2)** changes of PD-related CSF and plasma levels of alpha-Synuclein and HVA.

a- For primary biomarkers we will compare Nilotinib effects between baseline and 6 months via:

- measurement of phosphorylated Abl (activity) in CSF as evidence of CNS target engagement
- measurement of CSF and plasma alpha-Synuclein and HVA levels

b- Surrogate Biomarkers: cell death biomarkers, including

- CSF Total Tau and p-Tau, neuron specific enolase (NSE) and neuron and glial marker S100B.
- We will use DaTscan to longitudinally measure and quantify brain Dopamine transporters activity via Datquant (in collaboration with GE Healthcare)

c- Exploratory biomarkers:

- We will use ELISA to measure CSF and plasma neuro-inflammatory/modulatory markers, including interleukins (IL)- 1 α & 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).

Exploratory outcomes will include assessment of:

- a- Motor function using UPDRS and timed-up and go
- b- Cognitive function using the Montreal Cognitive Assessment (MoCA) and MMSE
- c- Autonomic nervous system, including orthostatic and gastrointestinal motility

Based on preclinical and clinical studies (see below), **we hypothesize that Nilotinib will be safe and tolerable in individuals with early PD.** We also predict that Nilotinib will be detected in the CSF and will inhibit Abl activity in a dose and time-dependent manner. Nilotinib will also stabilize our primary CSF biomarker levels alpha-Synuclein and increase HVA and reduce cell death markers, including total Tau, p-Tau, NSE and S100B. We further hypothesize that we may see evidence of change in surrogate and exploratory 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 early PD. 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 (see biomarkers section), including alpha-Synuclein, Abeta42/40, total Tau and p-Tau (181, 231). We obtained preliminary data showing that Nilotinib crosses the BBB and is detected in the CSF, resulting in Abl inhibition and downstream target engagement (alpha-Synuclein, Tau and Abeta) in the CNS (pharmacodynamics) and increased CSF HVA levels as a downstream biomarker of dopamine metabolism. We also measured additional plasma and CSF biomarkers that are indicative of immunological and non-immunological changes. These data provide strong feasibility to test Nilotinib in a larger placebo-controlled, phase II clinical trial to demonstrate safety, tolerability, pharmacokinetics/pharmacodynamics (chart 1) and changes in disease biomarkers in patients with early PD.

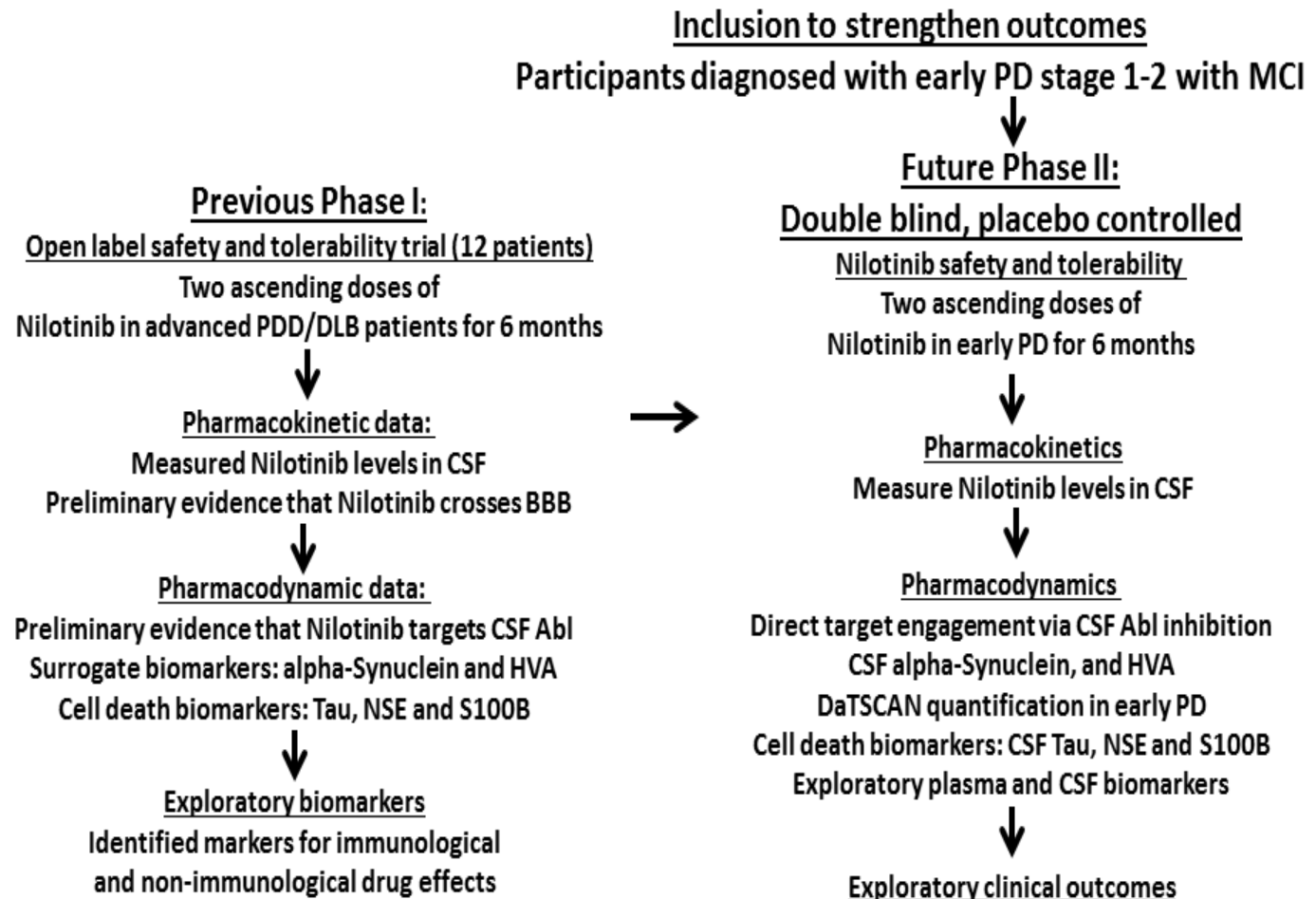


Chart 1- Summary of research activities in phase I Nilotinib clinical trial that provide strong rationale and preliminary evidence supporting development of clinical research into a double-blind, placebo controlled, phase II trial in early PD.

2- Study Design

We propose to perform a randomized, placebo-controlled, phase II clinical trial and aim to evaluate the impact of low doses of Nilotinib treatment on safety, tolerability, pharmacokinetics and biomarkers in patients with early PD (Chart 2). Sixty (60) participants (Hoehn & Yahr <2) will be recruited and randomly assigned 1:1:1 to placebo (group 1), 150mg (group 2) or 300mg (group 3) once daily (taken without a meal) for 6 months and 3 months follow up. Table 2 provides a detailed summary of activities that will be performed to measure the primary outcomes (safety and tolerability) and secondary outcomes (pharmacokinetics/pharmacodynamics). **2.1- Study Objectives and Endpoints**

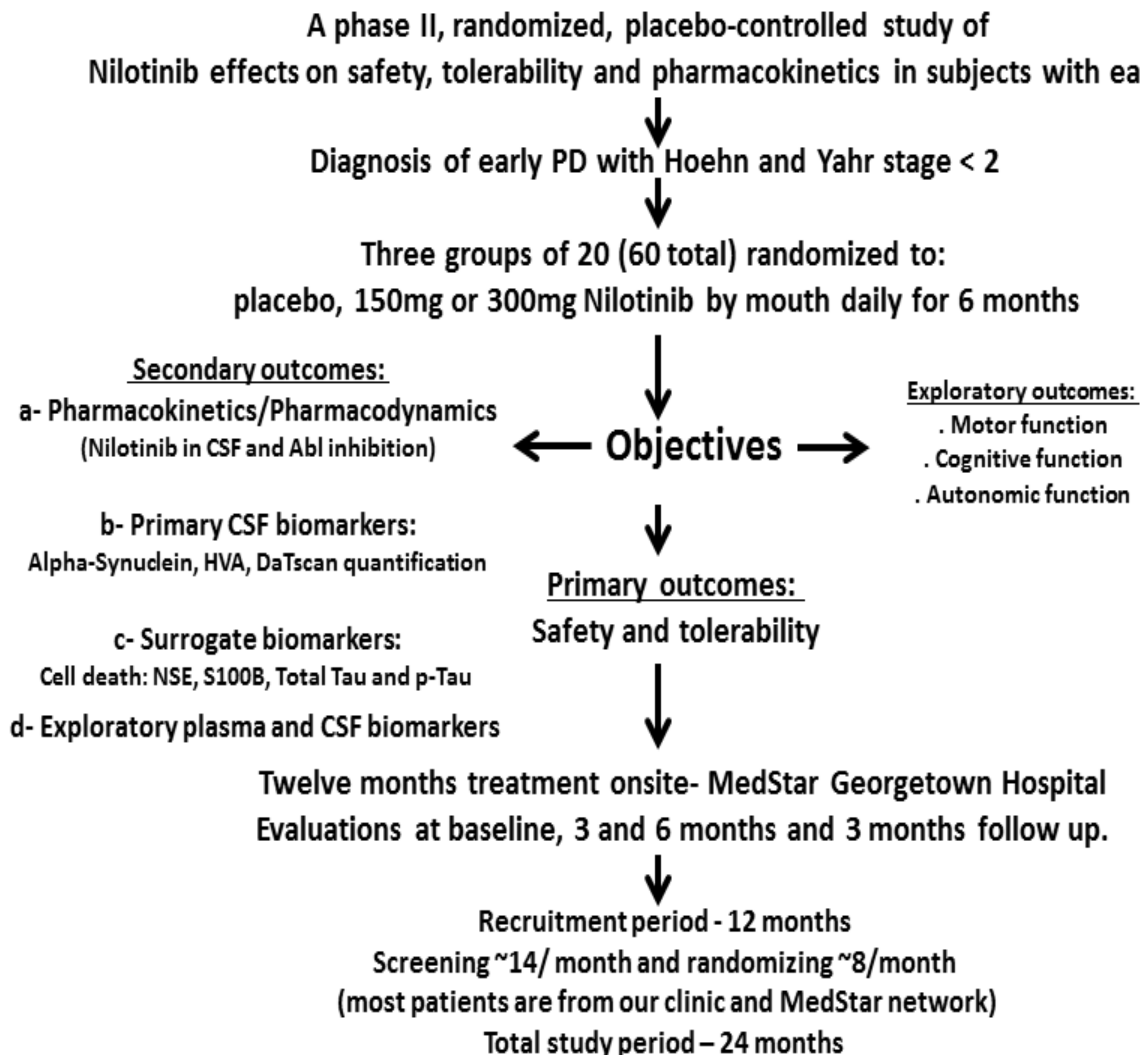


Chart 2- Study design of a randomized, double blind placebo controlled, phase II trial of Nilotinib in early PD.

2.2- 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 (20 placebo, 20 low dose (150mg Nilotinib) and 20 high dose (300mg Nilotinib)).

2.3- Study Population

This study will be conducted in subjects with early PD with Hoehn & Yahr ≤ 2 . Montreal Cognitive Assessment (MoCA) score in the range of 18-26 (inclusive) will be used to identify mild cognitive impairment (MCI) at screening. Eligible participants and study partners must provide written informed consent prior to screening.

Table 1- Summary of primary, secondary and exploratory objectives with the associated endpoints and evaluation criteria:

| Outcomes | Objective | Endpoint |
|--------------------|-------------------------|---|
| Primary | Safety and tolerability | a- Safety - Physical, neurological and laboratory examinations, and electrocardiograms (EKG) b-Tolerability - the ability to complete the study on study drug. A total of up to 25% discontinuations will be considered tolerable. |
| Secondary | Biomarkers | a- Primary biomarkers we will compare Nilotinib effects between baseline and 6 months via: 1- measurement of phosphorylated Abl (activity) in CSF as evidence of direct target engagement 2- measurement of CSF and plasma alpha-Synuclein and HVA levels b- Surrogate biomarkers: cell death biomarkers, including 1- CSF Total Tau and p-Tau (181/231), neuron specific enolase (NSE) and neuron and glial marker S100B. 2- We will use DaTscan to longitudinally measure and quantify brain Dopamine transporters activity via Datquant c- Exploratory biomarkers: We will measure CSF and plasma neuro-inflammatory/modulatory markers. |
| Exploratory | a- Motor function | UPDRS, timed-up and go |
| | b- Cognitive function | MMSE, MoCA |
| | c- Autonomic function | Orthostatic and bowel movement |

3-ACTIVITIES AND VISITS

Screening. This visit will determine study eligibility. Potential participants and their study partners must review and sign an informed consent form prior to any study-related procedures. Information regarding demographics, concurrent medications, and medical history will be gathered from the participant and study partner. Prior to any study-related activities, subjects will be thoroughly informed on all aspects of the study and will be requested to sign an informed consent form. 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.

Vital signs will be recorded with comprehensive physical and neurological examinations. Hoehn and Yahr and EKG 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, glycated urea, uric acid. 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.

All tests will be repeated as detailed in Table-2.

Autonomic nervous system assessments including orthostatic examination will be performed every visit and other autonomic functions will be tested via administration of the Gastrointestinal Symptoms Rating Scale (GSRS).

| Visits/month | Screening | Baseline | 0.5 | 1 | 1.5 | 2 | 3 | 4 | 5 | 6 | 9 |
|--------------|-----------|----------|-----|---|-----|---|---|---|---|---|---|
| Consent | X | | | | | | | | | | |
| Demographics | X | | | | | | | | | | |
| Vitals | X | X | X | X | X | X | X | X | X | X | X |
| H&P | X | X | X | X | X | X | X | X | X | X | X |
| Neuro Exam | X | X | X | X | X | X | X | X | X | X | X |
| Orthostatic | X | X | X | X | X | X | X | X | X | X | X |
| EKG | X | X | X | X | X | X | X | X | X | X | X |
| Blood draw | X | X | X | X | X | X | X | X | X | X | X |
| C-SSRS | X | X | X | X | X | X | X | X | X | X | X |
| GSRS | X | X | X | X | X | X | X | X | X | X | X |
| GDS | X | X | X | X | X | X | X | X | X | X | X |
| Hoehn & Yahr | X | X | | | | | X | | | X | X |
| MoCA | X | X | | | | | X | | | X | X |
| MMSE | X | X | | | | | X | | | X | X |
| Timed-up&GO | X | X | | | | | X | | | X | X |
| UPDRS I-IV | X | X | | | | | X | | | X | X |
| LP | | X | | | | | | | | X | |
| DaTscan | | X | | | | | X | | | X | |

Table 2- A detailed schedule of 11 visits and the assessments done at each visit. History and Physical (H&P), Electrocardiograms (EKG), Columbia Suicide Severity Rating Scale (CSSRS), Gastrointestinal Symptoms Rating Scale (GSRS), Geriatric Depression Scale (GDS), Montreal Cognitive Assessment (MoCA), Mini Mental Status Exam (MMSE), Unified Parkinson's Disease Rating Score (UPDRS), Lumbar Puncture (LP), Dopamine Transporter activity SPECT (DaTscan).

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 baseline procedures may be completed over several visits. CSF collection via lumbar puncture (LP) and blood draw will be performed for biomarker pharmacokinetics, pharmacodynamics and biomarker measurement (CSF alpha-Synuclein, CSF total Tau/p-Tau18, HVA and inflammatory/neurotrophic markers). Blood will also be collected for laboratory tests as above. Vital signs, brief physical and neurological examination along with documentation of concurrent medications and AEs will be performed every visit.

DaTscan will also be performed and quantified via DATQUANT at baseline, 3 and 6 months to determine the effects of Nilotinib on early changes in dopamine transport activity in PD patients.

At baseline, 3, 6 and 9 months - Motor assessments will be performed via standardized tests such as Unified Parkinson Disease Rating Scale (UPDRS) I-IV and Timed-Up and Go. **Cognitive assessments** will be performed via Montreal Cognitive Assessment (MoCA) and Mini Mental Status Examination (MMSE) that can measure progression of cognitive impairment. The Columbia Suicide Severity Rating Scale (C-SSRS) and Geriatric Depression Scale (GDS) will be performed every visit.

4- POPULATION

4.1- Number of Patients & Centers

This is a single center study that will be conducted at Georgetown University Medical Center (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 early PD with MCI (MoCA=18-26) will be enrolled. We anticipate screening 100 individuals to enroll a total of 60 participants over 6-month period. The Movement Disorders Program (MDP) at MedStar Georgetown Hospital (GUH), a National Parkinson's Foundation Center for Excellence, will screen on average 14 patients and enroll 8 per month. There are no competing clinical trials for PD at GUH or the greater Washington-Baltimore area. MDP-GUH follows approximately 3000 PD patients. Screening for 100 patients will be predominately from our program, however 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- Patients between the age of 40-90 years, medically stable
- 4- Diagnosis of PD according to the UK Brain Bank Diagnostic Criteria
- 5- Early PD subjects with MoCA between 18-26, inclusive
- 6- Hoehn and Yahr stage ≤ 2
- 7- Stable treatment (>4 weeks) with mono-amine oxidase (MAO)-B inhibitor (Selegiline up to 10mg/d or rasagiline up to 1mg/d) allowed
- 8- QTc interval 350-460ms, inclusive

- 9- Participants must be willing to undergo LP at baseline and 6 months after treatment
- 10-Participants must be able and willing to safely undergo DaTscan for 15-20 min at baseline, 3 and 6 months

4.3- Exclusion criteria

1. Patients with hypokalemia, hypomagnesaemia, or long QT syndrome- QTc \geq 461ms
2. Concomitant drugs known to prolong the QTc interval and history of any cardiovascular disease, including myocardial infarction 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)
 - d. Anticoagulants, including Coumadin (warfarin), heparin, enoxaparin, dalteparin, xarelto, etc.
 - e. St. John's Wort
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 idiopathic PD, including 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 (e.g. IVig)
16. Contraindication to DaTscan

17. Must not be enrolled as an active participant in another clinical study
18. Diagnosis of DLB

4.4- Randomization and Registration will be performed by an internet based randomization module using interactive voice response system (IVRS). Randomization of the subjects to the 3 treatment groups will be performed in a stratified manner. The chance for randomization to the groups is 1:1:1 for placebo:150mg:300 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.

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 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 Investigator (PI) agrees 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- SAMPLE SIZE AND JUSTIFICATION:

5.1- Sample size determination

Safety: No formal sample-size calculation for 20 participants in each arm was performed. Safety of each dose 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 20 participants exposed to each treatment arm, the study will have a 95% probability of observing at least one instance of any AE with an expected incidence of at least 14% in both arms when the binomial distribution is assumed. For SAEs, this probability of at least one event will decrease to 85% with an expected incidence of at least 9% in both arms.

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 5 or more AEs out of 20 is 13.8% (1-86.2%) given 20 participants in Nilotinib 300mg Arm. If we assume 9% of probability of SAEs occurrence, the probability of discontinuation due to SAEs of this trial, i.e., observing 5 or more severe AEs out of 20 is 2.9% (1-97.1%). We expect that the probabilities of discontinuation due to drug-related AEs or SAEs in the 150mg treatment group will be at most the discontinuation probability in Nilotinib 300mg Arm.

Direct Target Engagement. In our previous trial with 150 mg and 300 mg daily doses of Nilotinib, we detected a maximal concentration of Nilotinib at 2 hrs (T_{max}) and maximal decrease in CSF Abl activity (phosphorylation) between 1 and 3hrs after dose (Figure 5). Therefore, we will collect CSF at baseline (pre dose or time 0), 1, 2 and 3hrs after dosing as time points to determine Nilotinib pharmacokinetics and direct target engagement via CSF Abl inhibition. We observed a total decrease in Abl activity of 20% and 30% among patients treated with 150 mg and 300 mg Nilotinib, respectively (Figure 5), with standard deviations (SD) of 12% and 20%. **To account for large differences between groups at baseline, all estimates used for means and SDs (using educated guess considering budget limitations as well as SD of 6-month changes in other biomarkers) were based on mean of relative changes- not the mean value changes.** Given these estimates, 8 subjects at each treatment arm will have greater than 85% power to detect a reduction in Abl activity at T_{max} in a given dose based on a two-tailed test at $\alpha = 0.05$. We will collect CSF 2 hrs. after Nilotinib dosing from at least 8 patients receiving 150 mg and 8 patients receiving 300 mg and compare changes in phosphorylated Abl at baseline (pre dose) and 6 months after Nilotinib treatment. Table 3 estimates power based on Abl activity when a two-sided significance level of 0.05.

Table 3- Estimated power based on reduction in Abl activity at T_{max}

| Arm | Placebo | Treatment | Difference | SD | Power(N=8) |
|------------------|---------|-----------|------------|-----|------------|
| Arm II vs Arm I | 0% | 20% | 20% | 12% | 92% |
| Arm III vs Arm I | 0% | 30% | 30% | 20% | 85% |

Primary biomarkers. Given sample size of 20 per arm, we calculate powers to Nilotinib effects in terms of primary biomarkers between baseline and 6 months. Given a primary biomarker, we will test the null hypothesis that the biomarker change in the control group (Arm I) over 6 months equal the biomarker change in the treatment group of Arm II (150mg) or Arm III (300mg) using a two-sided significance level 0.05, respectively.

It is assumed that 10% complete losses and additional 10% partial loss of information due to symptomatic treatment leads to “drop-out” rate of 20%. Considering 20% drop-out, a total of 48 patients (16 patients in each arm) will be considered. Given observed statistics on biomarker changes in the pilot study, we anticipate powers of each biomarker analysis as stated in Table 4.

Table 4- Estimated power based on CSF alpha-Synuclein and HVA biomarkers

| Arm | Biomarker | Placebo | Treatment | Difference | SD | Power(N=20) | Power(N=16) |
|---|-----------------|---------|-----------|------------|-----|-------------|-------------|
| Arm II (150mg) vs Arm I (placebo) | HVA | 0% | 50% | 50% | 50% | 89% | 81% |
| | alpha-Synuclein | -50% | -10% | 40% | 20% | 100% | 100% |
| | total Tau | 0% | 10% | 10% | 6% | 100% | 98% |
| | P-tau231 | 5% | -15% | 20% | 10% | 100% | 100% |
| | S100B | 0% | -25% | 25% | 10% | 100% | 100% |
| | NSE | 0% | -30% | 30% | 30% | 89% | 81% |
| Arm III (300mg) vs Arm I (placebo) | HVA | 0% | 50% | 50% | 40% | 100% | 100% |
| | alpha-Synuclein | -50% | -10% | 40% | 30% | 100% | 100% |
| | total Tau | 0% | 20% | 20% | 6% | 100% | 98% |
| | P-tau231 | 5% | -10% | 15% | 6% | 100% | 100% |
| | S100B | 0% | -15% | 15% | 10% | 100% | 99% |
| | NSE | 0% | 20% | 20% | 20% | 89% | 81% |

We expect that we will have at least 81% power to test our null hypotheses for CSF biomarkers. Due to the small sample size, a non-parametric Mann-Whitney-Wilcoxon test is also used to compute the power given the mean change rate and SD.

Given the same set-up in the Table 5, a number of 20 patients will achieve at least 65% power at a two-sided at 0.05 for testing clinical outcomes (UPDRS-IV).

Table 5- Estimated power based on two types of UPDRS scores

| Arm | Clinical outcomes | Placebo | Treatment | Difference | SD | Power(N=20) | Power(N=16) |
|---------------------|-------------------|---------|-----------|------------|----|-------------|-------------|
| Arm II vs Arm I | UPDRS I-IV | 3 | -7 | 10 | 12 | 75% | 65% |
| Arm III vs Arm I | UPDRS I-IV | 3 | -11 | 14 | 8 | 100% | 100% |

5.1- 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 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 each dose will be estimated with exact confidence intervals.

Target Engagement: The measurements of biomarkers at baseline and the change from baseline to 6 months will be summarized in terms of mean and standard deviation. To compare efficacy of the two doses of Nilotinib, 150 mg and 300 mg per day, 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 two-tailed testing at a p-value of 0.05. Other CSF and plasma exploratory biomarkers, and clinical outcomes will be analyzed by the same model. The relationship between 6-month changes in Abl activity 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.

6-ETHICS/PROTECTION OF HUMAN SUBJECTS

6.1- Institutional Review Board (IRB)

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

6.2- 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.

6.3- 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.

6- STUDY FEASIBILITY AND PRELIMINARY DATA**7.1- Preclinical Evidence**

We previously demonstrated that Nilotinib penetrates the brain, inhibits Abl activity and promotes autophagic degradation of amyloid proteins in transgenic mice (13,23,24,26,40). These findings were also reproduced by several other laboratories (13,35,36). 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 30 μ L 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 (40).

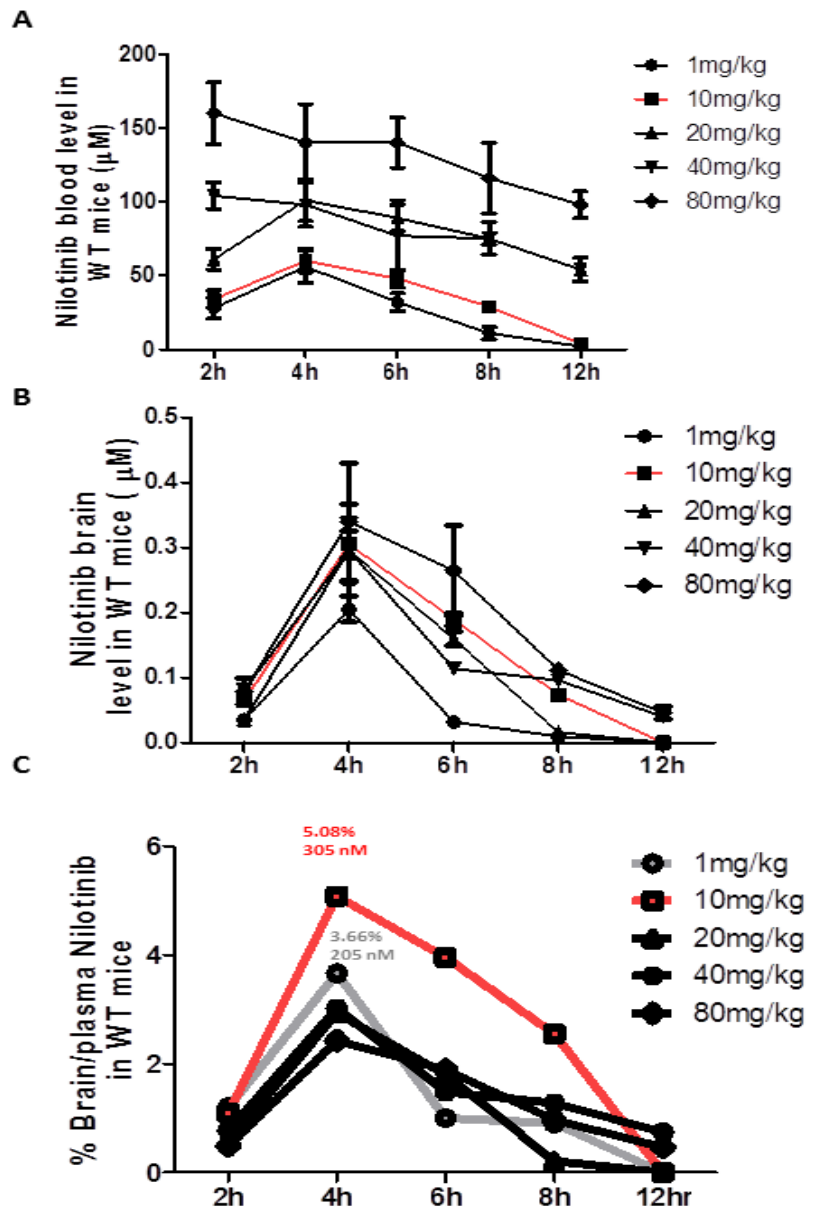


Figure 1. Pharmacokinetics analysis showing Nilotinib concentration in A) plasma, B) brain and C) percentage brain/plasma

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 μ m 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 μ m 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 stereotactically injected with 1×10^4 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

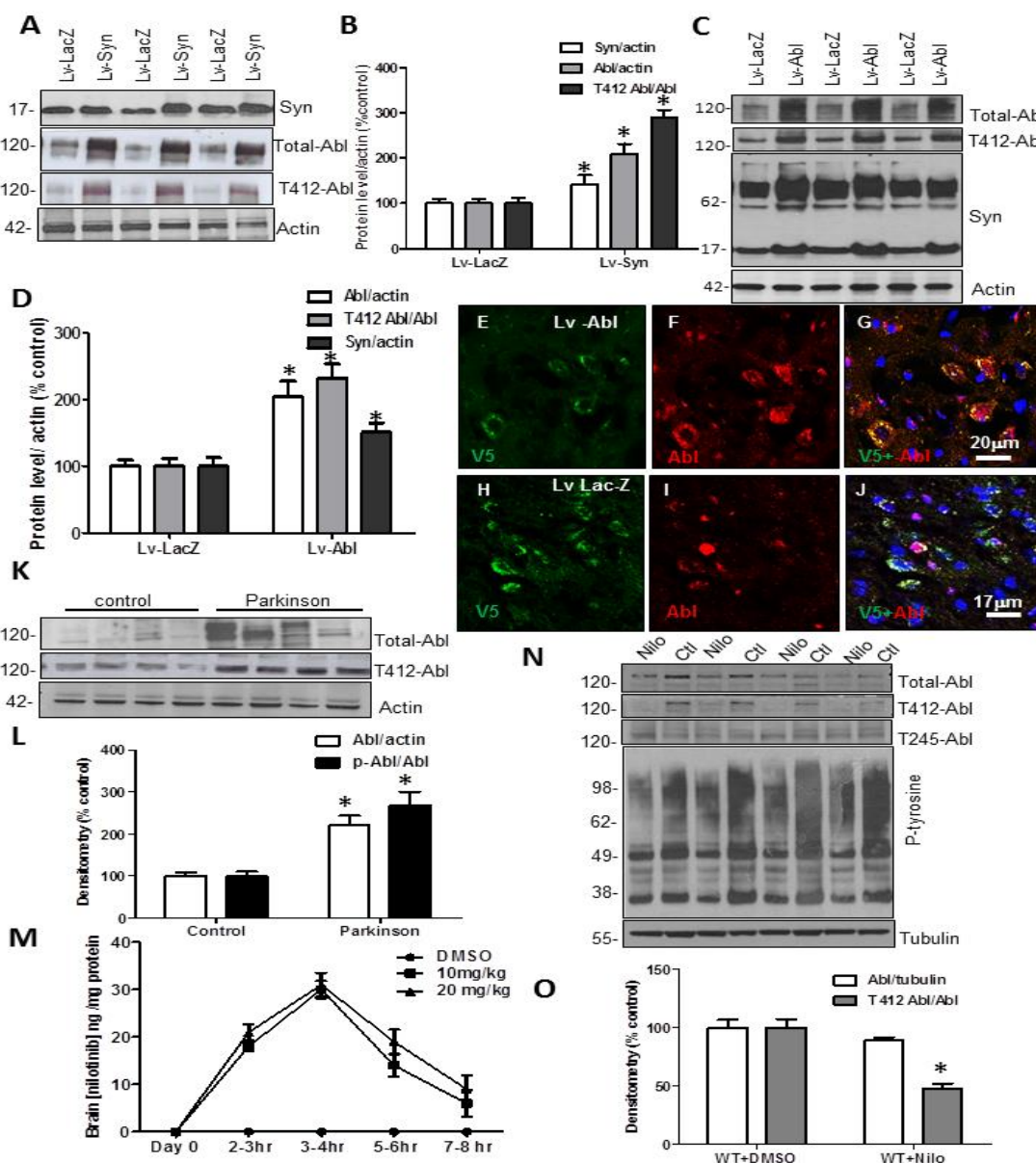


Fig. 2- Abl activation is associated with accumulation of alpha-Synuclein. Lentiviral alpha-Synuclein expression (1st blot), total Abl (2nd blot) and tyrosine 412 (T412) phosphorylated Abl (3rd blot) relative to actin (N=9), and **B)** graphs represent densitometry analysis. **C)** Total Abl (1st blot) 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 μ m 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 μ m 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 (12)) 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

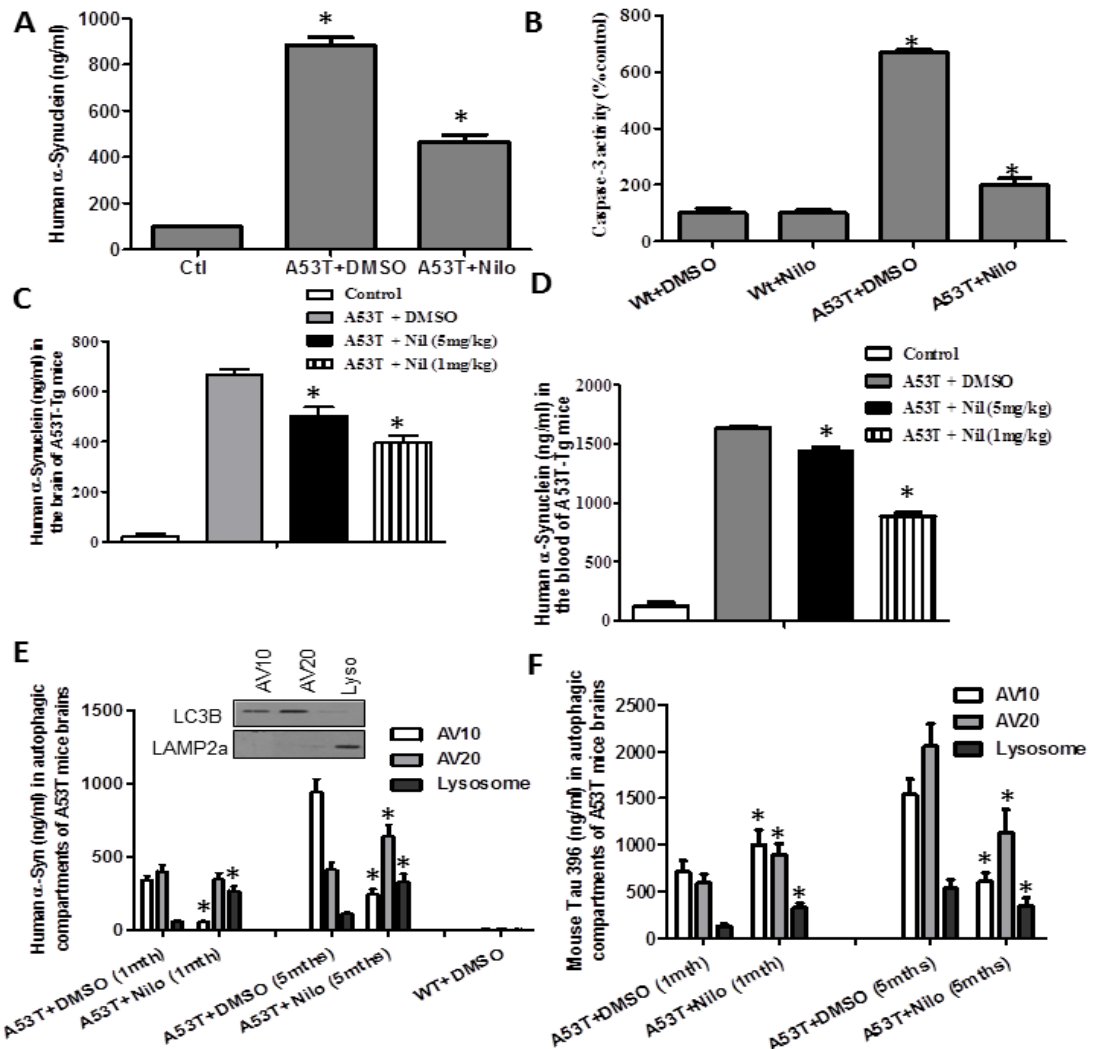


Fig. 3- Abl inhibition via Nilotinib promotes autophagic degradation. ELISA measurement of A) human alpha-Synuclein (N=14) and B) caspase-3 activity (N=64) in 6-8 months old transgenic A53T mice and wild type age-matched controls injected daily IP with 10mg/kg Nilotinib for 3 weeks. Graphs represent 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.

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 changes were detected in T245 Abl relative to actin or total Abl (Fig. 2N, 3rd blot). These data were confirmed by other groups (13,35,36).

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 (41). 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

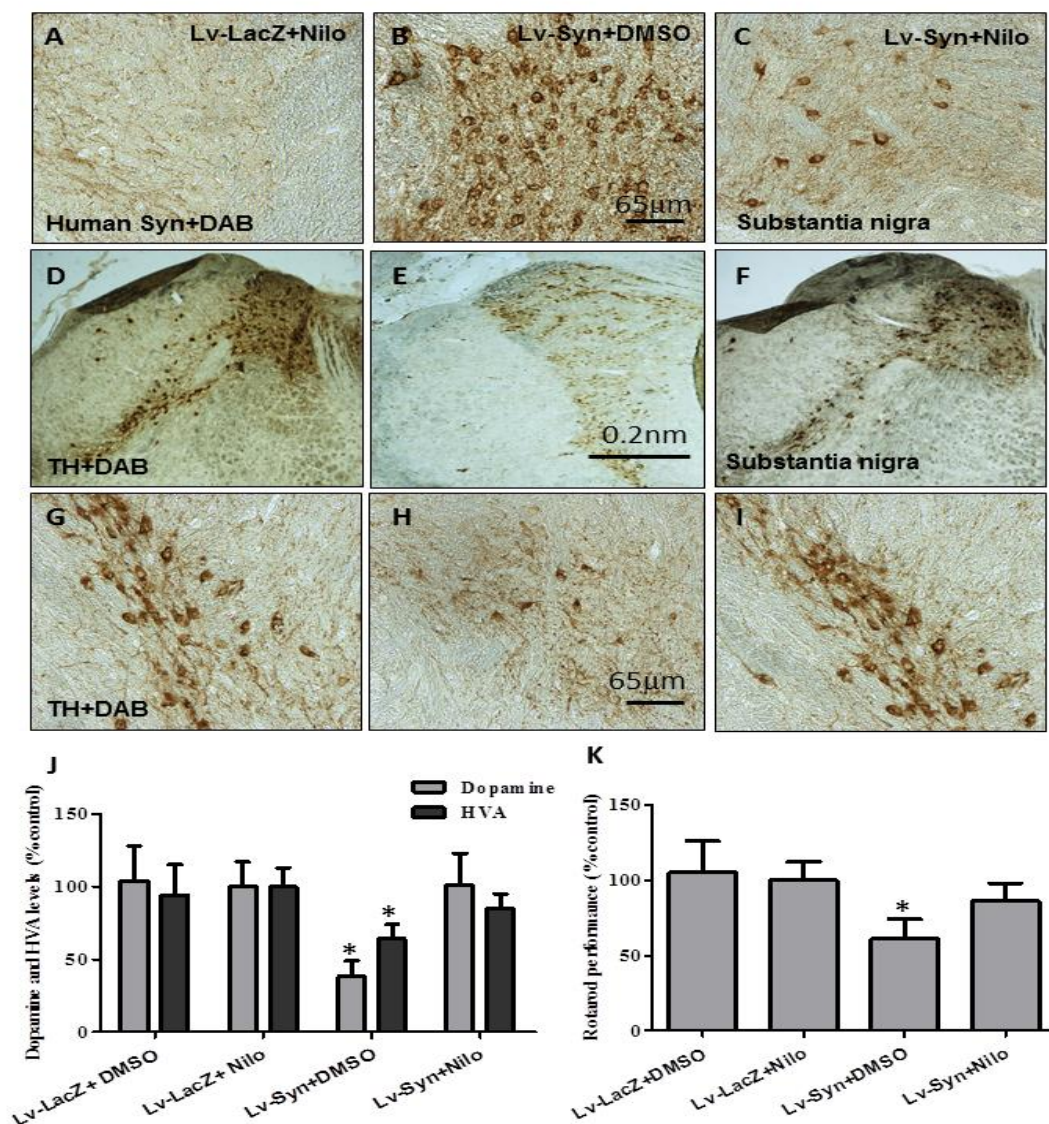


Fig. 4- Nilotinib protects dopamine neurons and improves motor. Staining of 20µm thick brain sections shows human alpha-Synuclein in the SN of A) lentiviral LacZ injected mice treated with 10mg/kg Nilotinib, B) lentiviral alpha-Synuclein treated with DMSO and C) lentiviral alpha-Synuclein treated with 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.

reversed this increase to 201% compared to wild type age-matched controls with and without Nilotinib (N=64). The 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 (13) 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 (42) 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 Homovanillic 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).

7.2- 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-

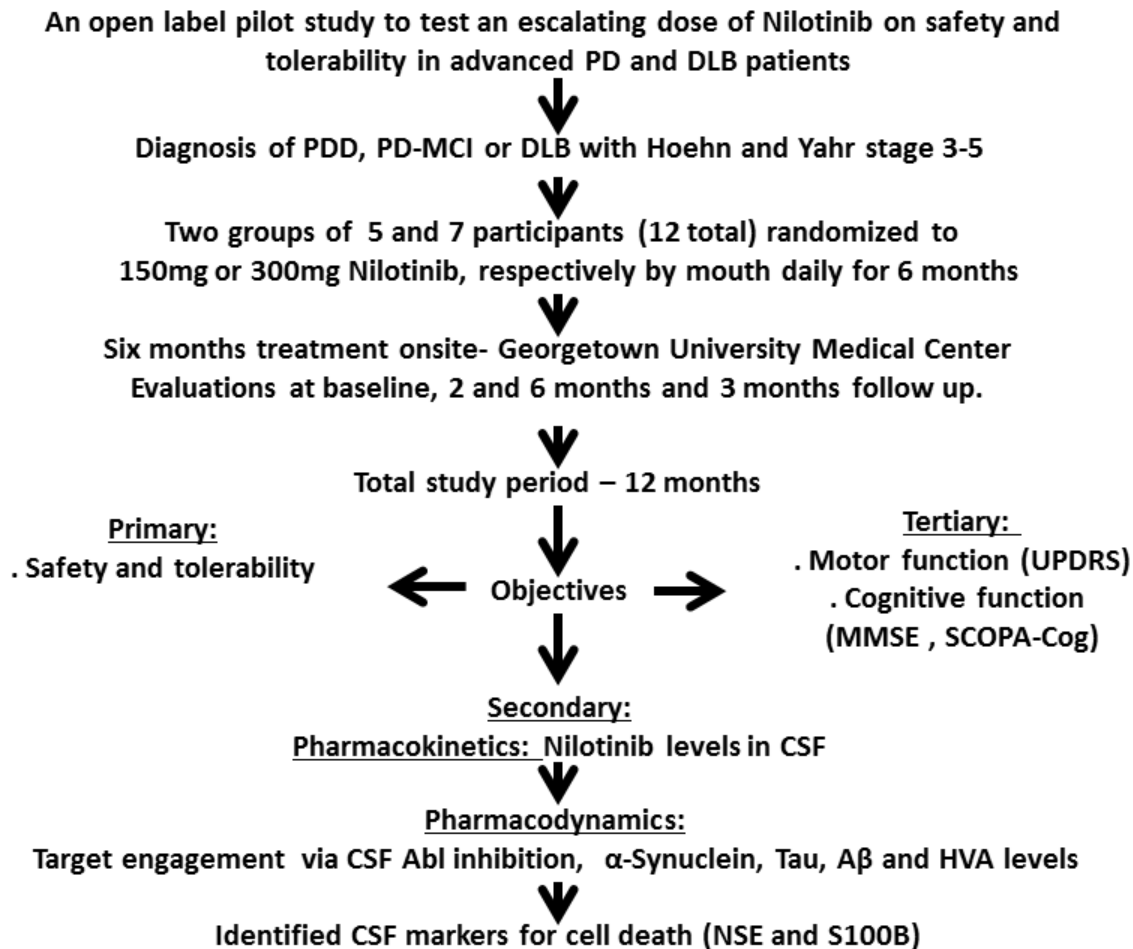


Chart 3- description of pilot trial for PD and DLB

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 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, A β 42 and A β 40. 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

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 Assessment.

| | | | | | | | | | | | | |
|--|-----------|-----------|-----------|-----------|-----------|---------|---------|-----------|-----------|-----------|-----------|---------|
| 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 |

dementia were evaluated using the MMSE and SCOPA-Cog.

7.2.a- Pharmacokinetic studies

Blood and CSF were collected from twelve patients at baseline prior to treatment (N=12, control) and 2 months into the 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 was staggered at 0, 1, 2, 3, 4, 5, 6 and 7hrs after oral administration of Nilotinib. The ratio of CSF:plasma (%) Nilotinib (Fig. 5A)

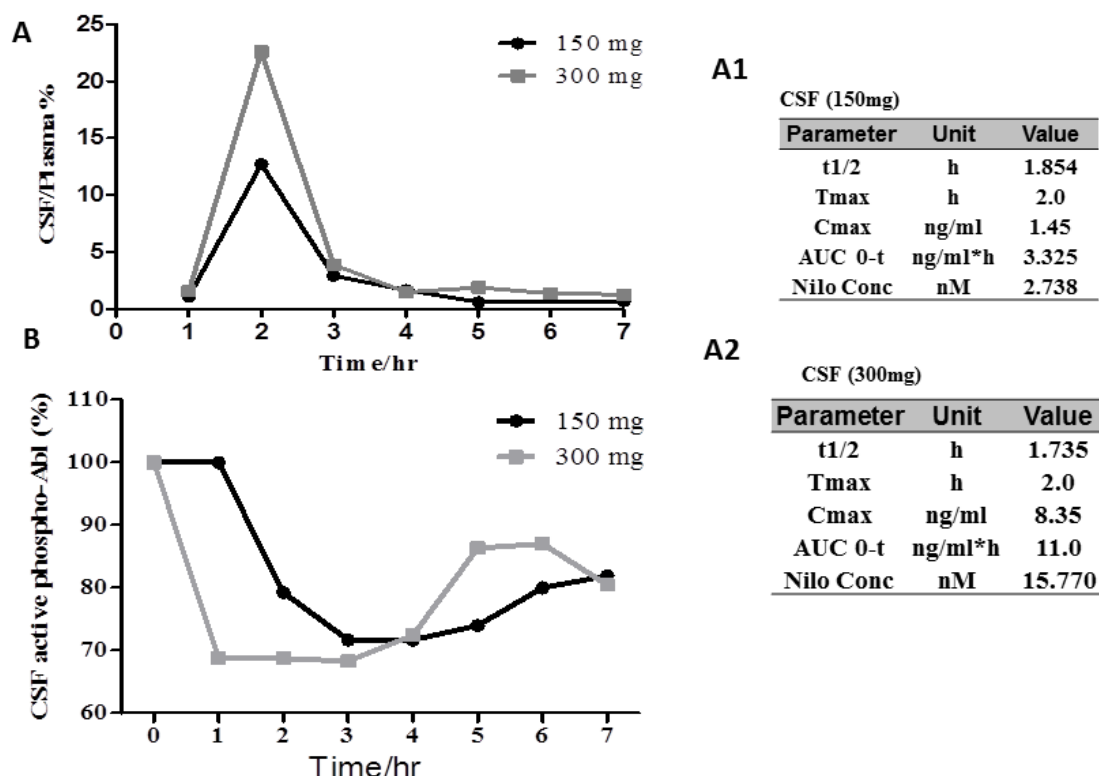


Fig. 5- Pharmacokinetic analysis showing A) Nilotinib concentration (T_{max} =2hrs) in human brain. Pharmacological parameters after treatment with A1) 150 mg and A2) 300 mg Nilotinib. Pharmacodynamics of Nilotinib B) showing direct target engagement via inhibition of CSF Abl.

peaked after 2hrs (T_{max}) at 22% with 300mg Nilotinib and 12% with 150mg Nilotinib (Fig. 5A1 and A2). Nilotinib was quantified in reference to ^{13}C Nilotinib standard, and CSF Nilotinib (C_{max}) was calculated at 1.45ng/ml (Fig. 5A1) and 8.35ng/ml (Fig. 5A2) at 150mg and 300mg, respectively. CSF concentration of Nilotinib was measured at 2.7nM with 150mg (Fig. 5A1) and 15.8nM with 300mg (Fig. 5A2). Area under the curve (AUC) was 3.325 with 150mg and 11 with 300mg.

7.2.b- Pharmacodynamics. To demonstrate whether Nilotinib inhibits Abl via dephosphorylation, 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.

7.3- Safety

7.3.1- 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

| 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 | 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 | |

7.3.2- 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 dysphagia | | | 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 | |

7.3.3- 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 |

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 |

7.3.4- 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.

7.4- 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.

Table 11- Concentration of CSF biomarkers at baseline and after 6-months Nilotinib treatment. Data are summarized as Mean \pm 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 ($A\beta$), 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 \pm 608.08 | 1633.25 \pm 531.72 | 0.0319* | 1263.70 \pm 584.80 | 1464.85 \pm 699.64 | 0.2958 |
| HVA | 2.15 \pm 2.16 | 3.62 \pm 3.07 | 0.2414 | 0.8583 \pm 0.23 | 1.69 \pm 0.88 | 0.0012** |
| Total tau (Invitrogen) | 302.63 \pm 156.29 | 261 \pm 135.49 | 0.0291* | 209.7 \pm 91.29 | 203 \pm 51.99 | 0.3744 |
| p-tau (181) (Invitrogen) | 22.96 \pm 6.34 | 26 \pm 3.12 | 0.0834 | 20.87 \pm 3.13 | 25.57 \pm 3.30 | 0.0051** |
| p-tau (181)/Total tau (Invitrogen) | 0.076 \pm 0.041 | 0.996 \pm 0.024 | 0.0671 | 0.0995 \pm 0.034 | 0.126 \pm 0.063 | 0.0225* |
| Total tau (millipore) | 1.1475 \pm 0.63 | 0.99 \pm 0.60 | 0.0207* | 0.764 \pm .33 | 0.688 \pm 0.14 | 0.234 |
| p-tau (231) (millipore) | 2.97 \pm 1.74 | 2.59 \pm 1.58 | 0.0445* | 2.30 \pm 1.23 | 2.01 \pm 1.00 | 0.0324* |
| p-tau (231)/Total tau (millipore) | 2.59 \pm 2.76 | 2.61 \pm 2.63 | 0.1873 | 3.01 \pm 3.73 | 2.992 \pm 7.14 | 0.3068 |
| $A\beta$ 42 | 860.75 \pm 562 | 726.08 \pm 525.51 | 0.0579 | 811.96 \pm 356.38 | 808.04 \pm 429.68 | 0.4857 |
| $A\beta$ 40 | 2332.6 \pm 1228.78 | 1875.85 \pm 1029.95 | 0.0437* | 1817.9 \pm 765.70 | 1951.789 \pm 892.16 | 0.3964 |
| $A\beta$ 42/ $A\beta$ 40 | 0.369 \pm 0.457 | 0.387 \pm 0.510 | 0.1971 | 0.447 \pm 0.465 | 0.414 \pm 0.482 | 0.2347 |
| S100B | 1531.84 \pm 749.65 | 968.88 \pm 202.18 | 0.1509 | 1062.80 \pm 291.61 | 912.21 \pm 164.07 | 0.0294* |
| NSE | 96.35 \pm 25.48 | 72.23 \pm 36.57 | 0.0197* | 67.20 \pm 20.30 | 74.22 \pm 10.82 | 0.1703 |

CSF $A\beta$ 40 concentration was reduced at six months compared to baseline with 150mg (Table 11, N=4), but $A\beta$ 40 was stable with 300mg Nilotinib. There was no significant change in CSF $A\beta$ 42 (Table 11) and the ratio of CSF $A\beta$ 42: $A\beta$ 40 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.

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 A β 40 did not change after Nilotinib treatment in both 150mg and 300mg groups.

Table 12- Concentration of plasma biomarkers at baseline and after 6-months Nilotinib treatment
Data are summarized as Mean \pm 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 (A β).

| PLASMA | | | | | | |
|---------------|-------------------------|-------------------------|-----------|-------------------------|-------------------------|----------|
| Analytes | 0 mths: | 6 mths (150mg Nilo): | p value : | 0 mths : | 6 mths (300mg Nilo): | p value: |
| α -syn | 30463.05 \pm 18802.60 | 76103.18 \pm 21826.26 | 0.965 | 29637.77 \pm 16476.64 | 29886.46 \pm 21826.26 | 0.491 |
| HVA | 3.73 \pm 1.19 | 6.89 \pm 3.41 | 0.0758 | 5.55 \pm 4.43 | 7.70 \pm 3.46 | 0.0812 |
| A β 40 | 38.92 \pm 18.69 | 33.91 \pm 13.95 | 0.0998 | 29.48 \pm 16.54 | 27.91 \pm 15.19 | 0.26 |

7.5- Nilotinib ameliorates motor 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

| UPDRS-III 150mg | | | | | | | | | | | |
|------------------|-----------|--------|--------|---------|---------|---------|---------|---------|-----------|-------|--|
| | Screening | Week-4 | Week-8 | Week-12 | Week-16 | Week-20 | Week-24 | Week-36 | Diagnosis | Stage | |
| NIL- 01 | 38 | 29 | 32 | 32 | 33 | 29 | 23 | 27 | DLB | 3 | |
| NIL-05 | 52 | 55 | 56 | 59 | 59 | 60 | 60 | 64 | DLB | 5 | |
| NIL-08 | 42 | 4 | 39 | 40 | 43 | 42 | 42 | 54 | DLB | 5 | |
| NIL-11 | 33 | 33 | 28 | 30 | 33 | 31 | 29 | 38 | PD-MCI | 3 | |
| NIL-14 | 24 | 26 | 23 | 22 | 22 | 19 | 18 | 26 | DLB | 3 | |
| AVG | 37.8 | 29.4 | 35.6 | 36.6 | 38 | 36.2 | 34.4 | 41.8 | | | |
| UPDRS-I-IV 150mg | | | | | | | | | | | |
| | Screening | Week-4 | Week-8 | Week-12 | Week-16 | Week-20 | Week-24 | Week-36 | Diagnosis | Stage | |
| NIL- 01 | 65 | 54 | 57 | 56 | 58 | 53 | 44 | 58 | LBD | 3 | |
| NIL-05 | 100 | 103 | 104 | 107 | 107 | 108 | 108 | 124 | LBD | 5 | |
| NIL-08 | 79 | 40 | 76 | 77 | 80 | 83 | 83 | 95 | LBD | 5 | |
| NIL-11 | 71 | 71 | 63 | 60 | 63 | 57 | 53 | 74 | PD-MCI | 3 | |
| NIL-14 | 48 | 50 | 45 | 47 | 43 | 38 | 40 | 45 | DLB | 3 | |
| AVG | 72.60 | 63.60 | 69.00 | 69.40 | 70.20 | 67.80 | 65.60 | 79.2 | | | |
| UPDRS-III 300mg | | | | | | | | | | | |
| | 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 |

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. In the 300mg Nilotinib group, UPDRSI-IV

significantly decreased ($p=0.0098$) 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 in the follow up visit off drug at thirty six weeks.

7.6- 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

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 | | | | | | | SCOPA-Cog (**/43) 300 mg | | | | | | |
|-----------------------------|-----------|--------|---------|---------|-----------|-------|-----------------------------|-----------|--------|---------|---------|-----------|-------|
| | Screening | Week-8 | Week-20 | Week-24 | Diagnosis | Stage | | Screening | Week-8 | Week-20 | Week-24 | Diagnosis | Stage |
| NIL-01 | 5 | 5 | 3 | 4 | LBD | 3 | NIL-02 | 15 | 22 | 21 | 22 | PD-MCI | 3 |
| NIL-05 | 0 | 0 | 1 | 0 | LBD | 5 | NIL-03 | 28 | 30 | 30 | 31 | PD | 3 |
| NIL-08 | 1 | 4 | 0 | out | LBD | 5 | NIL-06 | 13 | 11 | 19 | 10 | PDD | 3 |
| NIL-11 | 22 | 23 | 26 | 23 | PD-MCI | 3 | NIL-12 | 6 | 9 | 10 | 10 | PD-MCI | 3 |
| NIL-14 | 19 | 18 | 18 | 18 | LBD | 3 | NIL-13 | 0 | 0 | 0 | 0 | DLB | 5 |
| AVG | 9.4 | 10 | 9.6 | 11.25 | | | NIL-15 | 0 | 1 | 0 | 1 | PDD | |
| | | | | | | | AVG | 10.33 | 12.17 | 13.3 | 12.33 | | |

compared to 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.

8- TREATMENT ADMINSTERED

8.1- Name of the drug and all active ingredients

One hard white to yellowish powder in red opaque hard gelatin (Tasigna) capsule contains 150 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 (27-29,43). TKIs such as Imatinib (Glevec) are effective in many patients with CML in chronic phase (44,45). Nilotinib (AMN107) is a second generation selective Bcr-Abl inhibitor, which is effective following Imatinib resistance and intolerance (44). Nilotinib was approved by the FDA in 2007 for CML treatment (up to 1200 mg orally daily) (27-29).

8.2- Pharmacological Class of the Drug

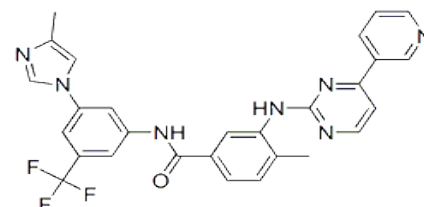
Nilotinib belongs to the pharmacotherapeutic group: Antineoplastic agents, protein kinase inhibitors, ATC code: L01XE08

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.

8.3- 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:



8.4- Formulation of the dosage form to be used

One hard white to yellowish powder in red opaque hard gelatin (Tasigna) capsule contains 150 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.

8.5- Route of Administration

One or two 150 mg capsules of Tasigna will be administered orally once a day. Key elements will be included in the educational brochure a special warnings and pre-cautions, including (Package Insert):

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

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 Tasigna may cause fluid retention, cardiac failure and pulmonary edema
- That Tasigna is metabolized by CYP3A4 and that strong inhibitors or inducers of this enzyme may significantly affect exposure to Tasigna.
- 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 (Tasigna)
- Not to eat within two hours before and one hour after taking Nilotinib (Tasigna)
- The need to avoid foods such as grapefruit juice which inhibit CYP3A4 enzymes

8.6- Planned exposure (e.g. Duration of the study administration)

We anticipate this project to be completed in 2 years, including 12 months for pre-screening and enrolment, 6-12 months for participant treatment, neurological and safety examinations and 3-6 months for post treatment evaluation as well as sample and statistical data analysis.

8.7- 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 ($\geq 10\%$) 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 $\geq 5\%$) 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 ($\geq 10\%$) 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 $\geq 5\%$) 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 ($\geq 1/10$) or common ($\geq 1/100$ to $<1/10$). Within each frequency grouping, adverse reactions are presented in order of decreasing seriousness.

8.8- Status of Drug in Other Countries

Tasigna is available in the United States, Europe and other countries of the world and requires a prescription. Novartis Pharma GmbH, Roonstraße 25, D-90429 Nuremberg, Germany is the manufacturer responsible for batch release. 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.

8.9- 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 (27). 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 (46). 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 (47). 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 (48-50). 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 (13,23), 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.

9- 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.

9.1- Prohibited Medications and Contraindications

Prohibited Medications

Prohibited medications for study subjects are as follows:

- Anti-coagulant medications, including Coumadin, heparin, enoxaparin, fondaparinaux, 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.

10-RATIONALE TO USE NILETINIB 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 (51), 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 (52), 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 (53-55). Microglia, the brain resident immune cells may also phagocytose and destroy toxic proteins (56).

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 (51), 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 (57). Leukemia and many other cancer treatments have been revolutionized by manipulation of autophagy, which leads to bulk degradation of unwanted or toxic molecules (58). 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

(59,60). 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 (59,60). Cell division and apoptosis are mediated by signaling mechanisms via the endosomal (early and recycling) system (53). Tyrosine kinases are activated via auto phosphorylation, triggering various signaling mechanisms that mediate cell division and/or apoptosis (61,62). Tyrosine kinase inhibition via de-phosphorylation leads to signaling via the late endosomal-lysosomal pathway, thus increasing autophagic degradation (53-55) and tumor growth.

TKIs have significantly improved the life quality and expectancies in many cancers, including CML (63,64). 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 (33)) for new indications. Abl is activated in neurodegeneration (24,32,59,60). 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 (25,26,30-34). 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 (25,26,30-34). There was also significant reversal of neurotransmitter alterations, including dopamine and glutamate in several models of neurodegeneration. As a modulator of myeloid cells (65), 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.

11-CLINICAL ASSESSMENTS AND OUTCOME MEASURES

11-1. 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.

11.2- 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.

11.3- 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, glycated urea, uric acid. 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.

11.4- 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)

11.5- 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

11.6- 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).

11.7- 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

11.8- MMSE is a brief 30-point questionnaire test that is used to screen for cognitive impairment. It is commonly used to screen for dementia. It is also used to estimate the severity of cognitive impairment at a given point in time and to follow the course of cognitive changes in an individual over time, thus making it an effective way to document an individual's response to treatment. In the time span of about 10 minutes it samples various functions including arithmetic, memory and orientation. It was introduced by Folstein et al in 1975. Any score greater than or equal to 25 points (out of 30) is effectively normal (intact). Below this, scores can indicate severe (≤ 9 points), Moderate (10-20 points) or mild (21-24 points).

11.9- 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.

11.10- 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/>

11.11- GDS. The FDA recommends that clinical trial participants should be tested for depression during each visit. The GDS tests geriatric depression, which is a mental and emotional disorder affecting senior citizens. Lasting depression is not only a typical part of aging but may also be a result of neurodegenerative diseases or use of medication. Subsyndromal depression doesn't meet the full criteria for major depression. The National Institute for Mental Health estimates that as many as five million elderly people in the U.S. suffer from subsyndromal depression, which can lead to major depression if left untreated and may increase the risk for suicide. The scale is a 15 item test that will examine participants' depression.

11.12- GSRS evaluates gastrointestinal symptoms, including diarrhea and other possible AEs associated with Nilotinib administration. GSRS is an interview-based rating scale consisting of 15 items for assessment of gastrointestinal symptoms, including dyspepsia, ulcer, diarrhea, irritable bowel syndrome, constipation, nausea, etc., that may be developed during the course of Nilotinib trials. The scale is easy to apply and proved to be useful in comparing the effectiveness of different modes of treatment in clinical trials.

11.13- 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.

12-BIOMARKERS

12.-1- Rationale for CSF, plasma and imaging PD 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 pathology.

Several studies indicate that reduction of CSF alpha-Synuclein is associated with alpha-Synuclein pathology in the CNS (66-68). CSF alpha-Synuclein, which probably comes from a neuronal source, has a lower level than the highly abundant presence of peripheral alpha-Synuclein (69). However, only a few studies have investigated CSF alpha-Synuclein levels in patients with neuropathologically confirmed alpha-Synucleinopathies (37,70). CSF alpha-Synuclein is reduced in de novo PD patients compared with healthy individuals (71), and it is lower in patients with advanced alpha-Synucleinopathies compared to other neurological disorders (70). 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 (72). Several studies also demonstrate increased levels of CSF total Tau and p-Tau181 levels (37-39) in PD patients, but another study report slightly decreased or normal levels of CSF total Tau and p-Tau (73). The CSF variability 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.

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 (74,75), 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.

12.2- CSF, plasma and imaging PD biomarkers: Blood draw (15ml) and lumbar puncture (LPs) to obtain CSF (~15ml) will be performed on all patients at baseline and 6-months after treatment. Blood will be drawn 0.5hr before LP at least 2 hrs. after meal. Plasma will be isolated immediately after blood draw and will be aliquoted and stored at -80°C. CSF will be aliquoted and stored at -80°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°C. The supernatant is freeze-dried using speed vacuum and reconstituted in 200µL of Methanol: Water (50:50) and processed by Mass Spectrometry.

12.3- 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 be 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- $^{13}C_2H_3$.

12.3- Phospho Abl (Pan-tyrosine) ELISA. PathScan® Phospho-c-Abl (panTyr) solid phase sandwich ELISA will be performed on human CSF and plasma. A c-Abl rabbit antibody will be coated on the microwells. 100 μ l of CSF or plasma will be added to designated wells. After sample incubation for 2 hrs at 37°C, Bcr-Abl and c-Abl protein (phospho and nonphospho) will be captured by the coated antibody. Following extensive washing, a phospho-tyrosine (pan-tyrosine) detection antibody will be added to each well to detect captured tyrosine-phosphorylated Bcr-Abl and c-Abl protein. Samples will be incubated with detection antibody for 1 hr at 37°C. Following extensive washing, anti-mouse IgG, HRP-linked antibody will be added and incubated for 10 minutes at 37°C to recognize the bound detection antibody. HRP substrate, TMB will be added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of tyrosine-phosphorylated Bcr-Abl and c-Abl protein in the samples.

12.4- 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 (65), 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 markers. Therefore, we will perform unbiased multiplex ELISA (Millipore) to profile a panel of 44 plasma and CSF markers, including interleukins (IL)- 1 α & 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.

12.5- DaTscan. Is a radioactive drug (loflupane I 123 Injection, GE Healthcare) that is injected into the bloodstream to increase the contrast or help take pictures in the brain using a special camera (SPECT). Adults with parkinsonian syndromes may decide to take this imaging test using DaTscan contrast along with other medical tests to help decide if movement difficulties are due to changes in dopamine metabolism. DaTscan is the only FDA-approved imaging drug available that provides an image of the brain to determine –in combination with other tests- if movement difficulties are due to alteration of DA metabolism in the striatum. DaTscan is performed in the nuclear medicine department at GUH and DA transport activities is quantified via a special analytical program called DatQuant (GE Healthcare).

12.6- 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°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°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 be 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.

12.7- Homovanillic Acid ELISA. A 100µl CSF or plasma samples will be incubated with 100µl HRP-conjugate reagent and incubated for 1hr at 37°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°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.

12.8- 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 and 100µl 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.

12.9- 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°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.

13-SAFETY AND ADVERSE EVENTS

The AE definitions and reporting procedures provided in this protocol comply with all applicable regulations and ICH guidelines. 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. 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.

13.1- Adverse Event and Suspected Adverse Drug Reactions

An adverse event (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).

13.2- 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.

13.3- 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.

13.4- 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

13.5- 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)

13.7- Recording of Adverse Events

All clinical AEs are recorded in the Adverse Event (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 MEDUSA 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.

14-SAFETY MONITORING

14.1- 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.

14.2- Data Safety Monitoring Board (DSMB) will be formed. No investigator involved in the trial will be a member of the DSMB. The DSMB will be to 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 (biomarker data are blinded). 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 on a quarterly basis or as necessary.

14.3- 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 at every study center. 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 be 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.

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 MEDUSA 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.

15.1- 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 MEDUA databases and patient data reside on servers located in the Partners Healthcare Systems (Partners) server farm and WAS. Physical and software access to the servers and security is provided by GUMC investigators and Partners IT department.

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.

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17- APPENDIX

- MMSE
- MoCA
- C-SSRS baseline
- C-SSRS since last visit
- GSRS
- GDS
- UPDRS

MINI MENTAL STATE EXAMINATION (MMSE)

| Orientation. | Score | Max. Score |
|--|--|------------------------------------|
| What is the (date) (day) (month) (year) (season)? | () | 5 |
| Where are we: (country) (province) (town) (hospital) (floor) | () | 5 |
| Registration. Name 3 common object eg. (mango) (table) (coin) Take 1 second to say each, then ask the patient to repeat all 3 After you have said them. Give 1 point for each correct answer. Then repeat them until he/she learns all 3. count trails and record trails | () | 3 |
| Attention and Calculation Spell “world” backward. The score is the number of latters in correct order (D L R O W) | () | 5 |
| Recall Ask for the 3 object repeated above. Give 1 point for each correct answer [note : recall cannot be tested if all 3 object were not remembered during regestration] | () | 3 |
| Language. Name a “pencil” and “watch” Repeat the following. “No ifs, ands, or buts” Follow a 3-stage command : “Take a paper in your hand, fold it in half, and put in on the floor” Do the following : Close your eyes, Write a sentence, Copy the following design, | () () () () () () | 2 1 3 1 1 1 |
| Total score : Score of 27 – 30 : Normal Score of < 27 : Impaired cognitive function | () | |

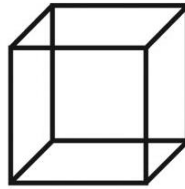
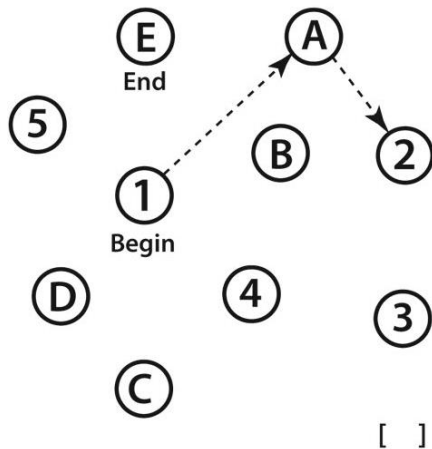


MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.1 Original Version

NAME :
Education :
Sex :

Date of birth :
DATE :

VISUOSPATIAL / EXECUTIVE



Copy
cube

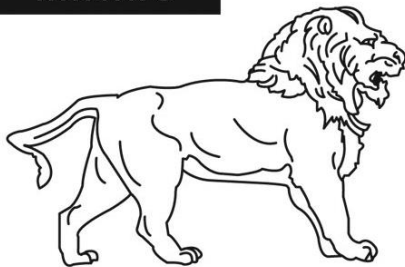
Draw CLOCK (Ten past eleven)
(3 points)

POINTS

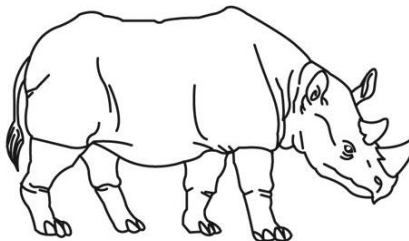
[] [] []
Contour Numbers Hands

___/5

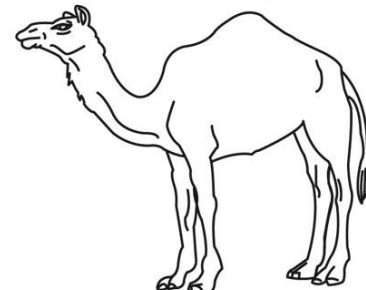
NAMING



[]



[]



[]

___/3

MEMORY

Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

| | FACE | VELVET | CHURCH | DAISY | RED |
|-----------|------|--------|--------|-------|-----|
| 1st trial | | | | | |
| 2nd trial | | | | | |

No
points

ATTENTION

Read list of digits (1 digit/ sec.).

Subject has to repeat them in the forward order

[] 2 1 8 5 4

Subject has to repeat them in the backward order

[] 7 4 2

___/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

___/1

Serial 7 subtraction starting at 100

[] 93

[] 86

[] 79

[] 72

[] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

___/3

LANGUAGE

Repeat : I only know that John is the one to help today. []

The cat always hid under the couch when dogs were in the room. []

___/2

Fluency / Name maximum number of words in one minute that begin with the letter F

[] _____ (N ≥ 11 words)

___/1

ABSTRACTION

Similarity between e.g. banana - orange = fruit

[] train - bicycle

[] watch - ruler

___/2

DELAYED RECALL

Has to recall words
WITH NO CUE

FACE
[]

VELVET
[]

CHURCH
[]

DAISY
[]

RED
[]

Points for
UNCUED
recall only

___/5

Optional

Category cue

Multiple choice cue

ORIENTATION

[] Date

[] Month

[] Year

[] Day

[] Place

[] City

___/6

COLUMBIA-SUICIDE SEVERITY RATING SCALE - BASELINE

**COLUMBIA-SUICIDE SEVERITY
RATING SCALE
(C-SSRS)**

Baseline
Version 1/14/09

**Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.;
Burke, A.; Oquendo, M.; Mann, J.**

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

*Definitions of behavioral suicidal events in this scale are based on those used in **The Columbia Suicide History Form**, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.] Standardized Evaluation in Clinical Practice, pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

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| SUICIDAL IDEATION | |
|--|--|
| <p>Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.</p> | <p>Lifetime: Time He/She Felt Most Suicidal</p> |
| <p>1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. Have you wished you were dead or wished you could go to sleep and not wake up? If yes, describe:</p> | <p>Yes No <input type="checkbox"/> <input type="checkbox"/></p> |

| | |
|--|--|
| <p>2. Non-Specific Active Suicidal Thoughts General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., "I've thought about killing myself") without thoughts of ways to kill oneself/associated methods, intent, or plan. Have you actually had any thoughts of killing yourself? If yes, describe:</p> | <p>Yes No <input type="checkbox"/> <input type="checkbox"/></p> |
| <p>3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, "I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it...and I would never go through with it." Have you been thinking about how you might do this? If yes, describe:</p> | <p>Yes No <input type="checkbox"/> <input type="checkbox"/></p> |
| <p>4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having <u>some intent to act on such thoughts</u>, as opposed to "I have the thoughts but I definitely will not do anything about them." Have you had these thoughts and had some intention of acting on them? If yes, describe:</p> | <p>Yes No <input type="checkbox"/> <input type="checkbox"/></p> |
| <p>5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan? If yes, describe:</p> | <p>Yes No <input type="checkbox"/> <input type="checkbox"/></p> |
| <p>INTENSITY OF IDEATION</p> | |
| <p>The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal.</p> <p>Most Severe Ideation: _____</p> <p>_____</p> <p style="text-align: right;">Type # (1-5)</p> <p>Description of Ideation</p> | <p>Most Severe</p> |
| <p>Frequency How many times have you had these thoughts? (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day</p> | <p>_____</p> |

| | |
|--|-------|
| Duration When you have the thoughts, how long do they last? (1) Fleeting - few seconds or minutes day (2) Less than 1 hour/some of the time hours/persistent or continuous (3) 1-4 hours/a lot of time (4) 4-8 hours/most of (5) More than 8 | _____ |
| Controllability Could/can you stop thinking about killing yourself or wanting to die if you want to? (1) Easily able to control thoughts thoughts with a lot of difficulty (2) Can control thoughts with little difficulty thoughts (3) Can control thoughts with some difficulty to control thoughts (4) Can control (5) Unable to control (0) Does not attempt | _____ |
| Deterrents Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide? (1) Deterrents definitely stopped you from attempting suicide likely did not stop you (2) Deterrents probably stopped you definitely did not stop you (3) Uncertain that deterrents stopped you (4) Deterrents most (5) Deterrents (0) Does not apply | _____ |
| Reasons for Ideation What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both? (1) Completely to get attention, revenge or a reaction from others stop the pain (you couldn't go on (2) Mostly to get attention, revenge or a reaction from others pain or how you were feeling) (3) Equally to get attention, revenge or a reaction from others end or stop the pain (you couldn't go on and to end/stop the pain. pain or how you were feeling) (4) Mostly to end or living with the (5) Completely to living with the (0) Does not apply | _____ |

| | |
|---|-----------------|
| SUICIDAL BEHAVIOR <i>(Check all that apply, so long as these are separate events; must ask about all types)</i> | Lifetime |
|---|-----------------|

| | |
|---|--|
| <p>Actual Attempt: A potentially self-injurious act committed with at least some wish to die, <i>as a result of act</i>. Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is any intent/desire to die associated with the act, then it can be considered an actual suicide attempt. <i>There does not have to be any injury or harm</i>, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. Have you made a suicide attempt? Have you done anything to harm yourself? Have you done anything dangerous where you could have died? What did you do? Did you _____ as a way to end your life? Did you want to die (even a little) when you _____? Were you trying to end your life when you _____? Or did you think it was possible you could have died from _____? Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)? (Self-Injurious Behavior without suicidal intent) If yes, describe:</p> | <p>Yes No <input type="checkbox"/> <input type="checkbox"/></p> <p>Total # of Attempts _____</p> <p>Yes No <input type="checkbox"/> <input type="checkbox"/></p> |
| <p>Has subject engaged in Non-Suicidal Self-Injurious Behavior? Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act (<i>if not for that, actual attempt would have occurred</i>). Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything? If yes, describe:</p> | <p>Yes No <input type="checkbox"/> <input type="checkbox"/></p> <p>Total # of interrupted _____</p> |
| <p>Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything? If yes, describe:</p> | <p>Yes No <input type="checkbox"/> <input type="checkbox"/></p> <p>Total # of aborted _____</p> |

| | | | | |
|---|--|--|--|---------------------------|
| | | | | |
| Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)? If yes, describe: | | | Yes No <input type="checkbox"/> <input type="checkbox"/> | |
| Suicidal Behavior: Suicidal behavior was present during the assessment period? | | | Yes No <input type="checkbox"/> <input type="checkbox"/> | |
| Answer for Actual Attempts Only | | | Most Recent Attempt Date: | Most Lethal Attempt Date: |
| Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; <i>medical</i> hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; <i>medical</i> hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death | | | Enter Code _____ | Enter Code _____ |
| Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death | | | Enter Code _____ | Enter Code _____ |

2 = Behavior likely to result in death despite available medical care

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1/14/09)

C-SSRS—Baseline (Version

COLUMBIA-SUICIDE SEVERITY RATING SCALE – *SINCE LAST VISIT*

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS) Since Last Visit Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

*This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale. Definitions of behavioral suicidal events in this scale are based on those used in **The Columbia Suicide History Form**, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.] Standardized Evaluation in Clinical Practice, pp. 103 -130, 2003.) For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu*

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SUICIDAL IDEATION

Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.

Since Last
Visit

1. Wish to be Dead

Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up.

Have you wished you were dead or wished you could go to sleep and not wake up?

If yes, describe:

Yes No
☐ ☐

| | | | | | |
|---|--|-----|----|--------------------------|--------------------------|
| 2. Non-Specific Active Suicidal Thoughts General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., "I've thought about killing myself") without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. Have you actually had any thoughts of killing yourself? If yes, describe: | <table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </table> | Yes | No | <input type="checkbox"/> | <input type="checkbox"/> |
| Yes | No | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | | | | |
| 3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, "I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it...and I would never go through with it." Have you been thinking about how you might do this? If yes, describe: | <table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </table> | Yes | No | <input type="checkbox"/> | <input type="checkbox"/> |
| Yes | No | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | | | | |
| 4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having <u>some intent to act on such thoughts</u> , as opposed to "I have the thoughts but I definitely will not do anything about them." Have you had these thoughts and had some intention of acting on them? If yes, describe: | <table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </table> | Yes | No | <input type="checkbox"/> | <input type="checkbox"/> |
| Yes | No | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | | | | |
| 5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan? If yes, describe: | <table border="1"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </table> | Yes | No | <input type="checkbox"/> | <input type="checkbox"/> |
| Yes | No | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | | | | |
| INTENSITY OF IDEATION | | | | | |
| The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Most Severe Ideation: _____ <div style="display: flex; justify-content: space-between;"> Type # (1-5) Description of Ideation </div> | Most Severe | | | | |
| Frequency How many times have you had these thoughts? (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day | _____ | | | | |
| Duration When you have the thoughts, how long do they last? (1) Fleeting - few seconds or minutes (4) 4-8 hours/most of day (2) Less than 1 hour/some of the time (5) More than 8 hours/persistent or continuous (3) 1-4 hours/a lot of time | _____ | | | | |
| Controllability Could/can you stop thinking about killing yourself or wanting to die if you want to? (1) Easily able to control thoughts (4) Can control thoughts with a lot of difficulty (2) Can control thoughts with little difficulty (5) Unable to control thoughts (3) Can control thoughts with some difficulty (0) Does not attempt to control thoughts | _____ | | | | |
| Deterrents Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide? (1) Deterrents definitely stopped you from attempting suicide (4) Deterrents most likely did not stop you (2) Deterrents probably stopped you (5) Deterrents definitely did not stop you (3) Uncertain that deterrents stopped you (0) Does not apply | _____ | | | | |
| Reasons for Ideation What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both? (1) Completely to get attention, revenge or a reaction from others (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (2) Mostly to get attention, revenge or a reaction from others (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (0) Does not apply | _____ | | | | |

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| Answer for Actual Attempts Only | | Most Lethal Attempt Date: |
|---|-------------------------|---------------------------|
| Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; <i>medical</i> hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; <i>medical</i> hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death | Enter Code _____ | |
| Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care | Enter Code _____ | |

Gastrointestinal Symptoms Rating Scale (GSRS)

| Symptom | Number of Symptom Days | Duration or Number of Episodes (if applicable); Sum: duration/episode score | Highest Level of Urgency (if applicable) | Severity; Sum severity score | Interference with Activities; Sum interference score | Total Sum of Scores by Symptoms (Across Domains) |
|----------------|------------------------|---|--|---|---|--|
| Diarrhea | 2 | 13 episodes; sum=5 | Extremely urgent; sum=7 | Severe; sum=8 | Completely; sum=7 | Sum=27 |
| Bloating | 2 | >5 hours; sum=4 | N/A | Moderate; sum=4 | Moderate; sum=3 | Sum=11 |
| Abdominal Pain | 1 | 2 hours; sum=2 | N/A | Moderate; sum=3 | Moderate; sum=2 | Sum=7 |
| Headache | 1 | 2 hours; sum=2 | N/A | Mild; sum=2 | A little bit; sum=1 | Sum=5 |
| Tiredness | 1 | 8 hours; sum=4 | N/A | Moderate; sum=3 | Moderate; sum=2 | Sum=9 |
| Nausea | 1 | 1 hour; sum=2 | N/A | Mild; sum=2 | A little bit; sum=1 | Sum=5 |
| Total | 8 Symptom Days | Duration/Episodes Category Sum=19 | Urgency Category Sum=7 | 2 Severe; 3 moderate; 2 mild; 1 very mild. Severity Category Sum=22 | 1 Complete; 1 Very much, 3 moderate; 3 A little bit. Interference Category Sum=16 | Total Sum=64 |

Screening Tool: Geriatric Depression Scale (GDS)

| | | |
|---|----------------|---------------|
| 1. Are you basically satisfied with your life? | Yes | No (1) |
| 2. Have you dropped many of your activities and interests? | Yes (1) | No |
| 3. Do you feel that your life is empty? | Yes (1) | No |
| 4. Do you often get bored? | Yes (1) | No |
| 5. Are you in good spirits most of the time? | Yes | No (1) |
| 6. Are you afraid that something bad is going to happen to you? | Yes (1) | No |
| 7. Do you feel happy most of the time? | Yes | No (1) |
| 8. Do you often feel helpless? | Yes (1) | No |
| 9. Do you prefer to stay at home, rather than going out and doing new things? | Yes (1) | No |
| 10. Do you feel you have more problems with memory than most? | Yes (1) | No |
| 11. Do you think it is wonderful to be alive now? | Yes | No (1) |
| 12. Do you feel pretty worthless the way you are now? | Yes (1) | No |
| 13. Do you feel full of energy? | Yes | No (1) |
| 14. Do you feel that your situation is hopeless? | Yes (1) | No |
| 15. Do you think that most people are better off than you are? | Yes (1) | No |

Sum all bolded answers (worth one point each) for a total score: _____

A score >5 points is suggestive of depression and should warrant follow-up intervention.

A score >10 points is almost always depression.

Unified Parkinson's Disease Rating Scale

I. Mentation, Behavior and Mood

1. Intellectual Impairment

- 0 = None.
- 1 = Mild. Consistent forgetfulness with partial recollection of events and no other difficulties.
- 2 = Moderate memory loss, with disorientation and moderate difficulty handling complex problems. Mild but definite impairment of function at home with need of occasional prompting.
- 3 = Severe memory loss with disorientation for time and often to place. Severe impairment in handling problems.
- 4 = Severe memory loss with orientation preserved to person only. Unable to make judgements or solve problems. Requires much help with personal care. Cannot be left alone at all.

2. Thought Disorder (Due to dementia or drug intoxication)

- 0 = None.
- 1 = Vivid dreaming.
- 2 = "Benign" hallucinations with insight retained.
- 3 = Occasional to frequent hallucinations or delusions; without insight; could interfere with daily activities.
- 4 = Persistent hallucinations, delusions, or florid psychosis. Not able to care for self.

3. Depression

- 1 = Periods of sadness or guilt greater than normal, never sustained for days or weeks.
- 2 = Sustained depression (1 week or more).
- 3 = Sustained depression with vegetative symptoms (insomnia, anorexia, weight loss, loss of interest).
- 4 = Sustained depression with vegetative symptoms and suicidal thoughts or intent.

4. Motivation/Initiative

- 0 = Normal.
- 1 = Less assertive than usual; more passive.
- 2 = Loss of initiative or disinterest in elective (nonroutine) activities.
- 3 = Loss of initiative or disinterest in day to day (routine) activities.
- 4 = Withdrawn, complete loss of motivation.

II. Activities of Daily Living (for both "on" and "off")

5. Speech

- 0 = Normal.
- 1 = Mildly affected. No difficulty being understood.
- 2 = Moderately affected. Sometimes asked to repeat statements.
- 3 = Severely affected. Frequently asked to repeat statements.
- 4 = Unintelligible most of the time.

6. Salivation

- 0 = Normal.
- 1 = Slight but definite excess of saliva in mouth; may have nighttime drooling.
- 2 = Moderately excessive saliva; may have minimal drooling.
- 3 = Marked excess of saliva with some drooling.
- 4 = Marked drooling, requires constant tissue or handkerchief.

7. Swallowing

- 0 = Normal.
- 1 = Rare choking.
- 2 = Occasional choking.
- 3 = Requires soft food.
- 4 = Requires NG tube or gastrostomy feeding.

Unified Parkinson's Disease Rating Scale

8. Handwriting

- 0 = Normal.
- 1 = Slightly slow or small.
- 2 = Moderately slow or small; all words are legible.
- 3 = Severely affected; not all words are legible.
- 4 = The majority of words are not legible.

9. Cutting Food and Handling Utensils

- 0 = Normal.
- 1 = Somewhat slow and clumsy, but no help needed.
- 2 = Can cut most foods, although clumsy and slow; some help needed.
- 3 = Food must be cut by someone, but can still feed slowly.
- 4 = Needs to be fed.

10. Dressing

- 0 = Normal.
- 1 = Somewhat slow, but no help needed.
- 2 = Occasional assistance with buttoning, getting arms in sleeves.
- 3 = Considerable help required, but can do some things alone.
- 4 = Helpless.

11. Hygiene

- 0 = Normal.
- 1 = Somewhat slow, but no help needed.
- 2 = Needs help to shower or bathe; or very slow in hygienic care.
- 3 = Requires assistance for washing, brushing teeth, combing hair, going to bathroom.
- 4 = Foley catheter or other mechanical aids.

12. Turning in Bed and Adjusting Bed Clothes

- 0 = Normal.
- 1 = Somewhat slow and clumsy, but no help needed.
- 2 = Can turn alone or adjust sheets, but with great difficulty.
- 3 = Can initiate, but not turn or adjust sheets alone.
- 4 = Helpless.

13. Falling (Unrelated to Freezing)

- 0 = None.
- 1 = Rare falling.
- 2 = Occasionally falls, less than once per day.
- 3 = Falls an average of once daily.
- 4 = Falls more than once daily.

14. Freezing when Walking

- 0 = None.
- 1 = Rare freezing when walking; may have start hesitation.
- 2 = Occasional freezing when walking.
- 3 = Frequent freezing. Occasionally falls from freezing.
- 4 = Frequent falls from freezing.

15. Walking

- 0 = Normal.
- 1 = Mild difficulty. May not swing arms or may tend to drag leg.
- 2 = Moderate difficulty, but requires little or no assistance.
- 3 = Severe disturbance of walking, requiring assistance.
- 4 = Cannot walk at all, even with assistance.

16. Tremor (Symptomatic complaint of tremor in any part of body.)

- 0 = Absent.
- 1 = Slight and infrequently present.
- 2 = Moderate; bothersome to patient.
- 3 = Severe; interferes with many activities.
- 4 = Marked; interferes with most activities.

17. Sensory Complaints Related to Parkinsonism

- 0 = None.
- 1 = Occasionally has numbness, tingling, or mild aching.
- 2 = Frequently has numbness, tingling, or aching; not distressing.
- 3 = Frequent painful sensations.
- 4 = Excruciating pain.

III. Motor Examination

18. Speech

- 0 = Normal.
- 1 = Slight loss of expression, diction and/or volume.
- 2 = Monotone, slurred but understandable; moderately impaired.
- 3 = Marked impairment, difficult to understand.
- 4 = Unintelligible.

19. Facial Expression

- 0 = Normal.
- 1 = Minimal hypomimia, could be normal "Poker Face."
- 2 = Slight but definitely abnormal diminution of facial expression
- 3 = Moderate hypomimia; lips parted some of the time.
- 4 = Masked or fixed facies with severe or complete loss of facial expression; lips parted $\frac{1}{4}$ inch or more.

20. Tremor at Rest (head, upper and lower extremities)

- 0 = Absent.
- 1 = Slight and infrequently present.
- 2 = Mild in amplitude and persistent. Or moderate in amplitude, but only intermittently present.
- 3 = Moderate in amplitude and present most of the time.
- 4 = Marked in amplitude and present most of the time.

21. Action or Postural Tremor of Hands

- 0 = Absent.
- 1 = Slight; present with action.
- 2 = Moderate in amplitude, present with action.
- 3 = Moderate in amplitude with posture holding as well as action.
- 4 = Marked in amplitude; interferes with feeding.

22. Rigidity (Judged on passive movement of major joints with patient relaxed in sitting position. Cogwheeling to be ignored.)

- 0 = Absent.
- 1 = Slight or detectable only when activated by mirror or other movements.
- 2 = Mild to moderate.
- 3 = Marked, but full range of motion easily achieved.
- 4 = Severe, range of motion achieved with difficulty.

23. Finger Taps (Patient taps thumb with index finger in rapid succession.)

- 0 = Normal.
- 1 = Mild slowing and/or reduction in amplitude.
- 2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
- 3 = Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
- 4 = Can barely perform the task.

24. Hand Movements (Patient opens and closes hands in rapid succession.)

- 0 = Normal.
- 1 = Mild slowing and/or reduction in amplitude.
- 2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
- 3 = Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
- 4 = Can barely perform the task.

25. Rapid Alternating Movements of Hands

(Pronation-supination movements of hands, vertically and horizontally, with as large an amplitude as possible, both hands simultaneously.)

- 0 = Normal.
- 1 = Mild slowing and/or reduction in amplitude.
- 2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
- 3 = Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
- 4 = Can barely perform the task.

Unified Parkinson's Disease Rating Scale

26. Leg Agility (Patient taps heel on the ground in rapid succession picking up entire leg. Amplitude should be at least 3 inches.)

0 = Normal.

1 = Mild slowing and/or reduction in amplitude.

2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.

3 = Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.

4 = Can barely perform the task.

27. Arising from Chair (Patient attempts to rise from a straightbacked chair, with arms folded across chest.)

0 = Normal.

1 = Slow; or may need more than one attempt.

2 = Pushes self up from arms of seat.

3 = Tends to fall back and may have to try more than one time, but can get up without help.

4 = Unable to arise without help.

28. Posture

0 = Normal erect.

1 = Not quite erect, slightly stooped posture; could be normal for older person.

2 = Moderately stooped posture, definitely abnormal; can be slightly leaning to one side.

3 = Severely stooped posture with kyphosis; can be moderately leaning to one side.

4 = Marked flexion with extreme abnormality of posture.

29. Gait

0 = Normal.

1 = Walks slowly, may shuffle with short steps, but no festination (hastening steps) or propulsion.

2 = Walks with difficulty, but requires little or no assistance; may have some festination, short steps, or propulsion.

3 = Severe disturbance of gait, requiring assistance.

4 = Cannot walk at all, even with assistance.

30. Postural Stability (Response to sudden, strong posterior displacement produced by pull on shoulders while patient erect with eyes open and feet slightly apart. Patient is prepared.)

0 = Normal.

1 = Retropulsion, but recovers unaided.

2 = Absence of postural response; would fall if not caught by examiner.

3 = Very unstable, tends to lose balance spontaneously.

4 = Unable to stand without assistance.

31. Body Bradykinesia and Hypokinesia (Combining slowness, hesitancy, decreased arm swing, small amplitude, and poverty of movement in general.)

0 = None.

1 = Minimal slowness, giving movement a deliberate character; could be normal for some persons. Possibly reduced amplitude.

2 = Mild degree of slowness and poverty of movement which is definitely abnormal. Alternatively, some reduced amplitude.

3 = Moderate slowness, poverty or small amplitude of movement.

4 = Marked slowness, poverty or small amplitude of movement.

Unified Parkinson's Disease Rating Scale

IV. Complications of Therapy

(In the past week)

A. Dyskinesias

32. Duration: What proportion of the waking day are dyskinesias present? (Historical information.)

- 0 = None
- 1 = 1–25% of day.
- 2 = 26–50% of day.
- 3 = 51–75% of day.
- 4 = 76–100% of day.

33. Disability: How disabling are the dyskinesias?

(Historical information; may be modified by office examination.)

- 0 = Not disabling.
- 1 = Mildly disabling.
- 2 = Moderately disabling.
- 3 = Severely disabling.
- 4 = Completely disabled.

34. Painful Dyskinesias: How painful are the dyskinesias?

- 0 = No painful dyskinesias.
- 1 = Slight.
- 2 = Moderate.
- 3 = Severe.
- 4 = Marked.

35. Presence of Early Morning Dystonia

(Historical information.)

- 0 = No
- 1 = Yes

B. Clinical Fluctuations

36. Are “off” periods predictable?

- 0 = No
- 1 = Yes

37. Are “off” periods unpredictable?

- 0 = No
- 1 = Yes

38. Do “off” periods come on suddenly, within a few seconds?

- 0 = No
- 1 = Yes

39. What proportion of the waking day is the patient “off” on average?

- 0 = None
- 1 = 1–25% of day.
- 2 = 26–50% of day.
- 3 = 51–75% of day.
- 4 = 76–100% of day.

C. Other Complications

40. Does the patient have anorexia, nausea, or vomiting?

- 0 = No
- 1 = Yes

41. Any sleep disturbances, such as insomnia or hypersomnolence?

- 0 = No
- 1 = Yes

42. Does the patient have symptomatic orthostasis?

(Record the patient's blood pressure, height and weight on the scoring form)

- 0 = No
- 1 = Yes

V. Modified Hoehn and Yahr Staging

STAGE 0 = No signs of disease.

STAGE 1 = Unilateral disease.

STAGE 1.5 = Unilateral plus axial involvement.

STAGE 2 = Bilateral disease, without impairment of balance.

STAGE 2.5 = Mild bilateral disease, with recovery on pull test.

STAGE 3 = Mild to moderate bilateral disease; some postural instability; physically independent.

STAGE 4 = Severe disability; still able to walk or stand unassisted.

STAGE 5 = Wheelchair bound or bedridden unless aided.

VI. Schwab and England Activities of Daily Living Scale

100% = Completely independent. Able to do all chores without slowness, difficulty or impairment. Essentially normal. Unaware of any difficulty.

90% = Completely independent. Able to do all chores with some degree of slowness, difficulty and impairment. Might take twice as long. Beginning to be aware of difficulty.

80% = Completely independent in most chores. Takes twice as long. Conscious of difficulty and slowness.

70% = Not completely independent. More difficulty with some chores. Three to four times as long in some. Must spend a large part of the day with chores.

60% = Some dependency. Can do most chores, but exceedingly slowly and with much effort. Errors; some impossible.

50% = More dependent. Help with half, slower, etc. Difficulty with everything.

40% = Very dependent. Can assist with all chores, but few alone.

30% = With effort, now and then does a few chores alone or begins alone. Much help needed.

20% = Nothing alone. Can be a slight help with some chores. Severe invalid.

10% = Totally dependent, helpless. Complete invalid.

0% = Vegetative functions such as swallowing, bladder and bowel functions are not functioning. Bedridden.

Unified Parkinson's Disease Data Form

Name _____

Unit Number _____

| Date | | | | | | | | | | | | | | | | | |
|---------------------------------------|----------------|----|-----|----|-----|----|-----|----|-----|----|-----|----|-----|----|-----|----|-----|
| DOPA mg/day | hrs DOPA lasts | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | | ON | OFF | ON | OFF | ON | OFF | ON | OFF | ON | OFF | ON | OFF | ON | OFF | ON | OFF |
| 1. Mentation | | | | | | | | | | | | | | | | | |
| 2. Thought Disorder | | | | | | | | | | | | | | | | | |
| 3. Depression | | | | | | | | | | | | | | | | | |
| 4. Motivation/Initiative | | | | | | | | | | | | | | | | | |
| Subtotal 1–4 (maximum = 16) | | | | | | | | | | | | | | | | | |
| 5. Speech | | | | | | | | | | | | | | | | | |
| 6. Salivation | | | | | | | | | | | | | | | | | |
| 7. Swallowing | | | | | | | | | | | | | | | | | |
| 8. Handwriting | | | | | | | | | | | | | | | | | |
| 9. Cutting food | | | | | | | | | | | | | | | | | |
| 10. Dressing | | | | | | | | | | | | | | | | | |
| 11. Hygiene | | | | | | | | | | | | | | | | | |
| 12. Turning in bed | | | | | | | | | | | | | | | | | |
| 13. Falling | | | | | | | | | | | | | | | | | |
| 14. Freezing | | | | | | | | | | | | | | | | | |
| 15. Walking | | | | | | | | | | | | | | | | | |
| 16. Tremor | | | | | | | | | | | | | | | | | |
| 17. Sensory symptoms | | | | | | | | | | | | | | | | | |
| Subtotal 5 – 17 (maximum = 52) | | | | | | | | | | | | | | | | | |
| 18 Speech | | | | | | | | | | | | | | | | | |
| 19. Facial expression | | | | | | | | | | | | | | | | | |
| 20. Tremor at rest: face,lips,chin | | | | | | | | | | | | | | | | | |
| Hands: right | | | | | | | | | | | | | | | | | |
| left | | | | | | | | | | | | | | | | | |
| Feet: right | | | | | | | | | | | | | | | | | |
| left | | | | | | | | | | | | | | | | | |
| 21. Action tremor: right | | | | | | | | | | | | | | | | | |
| left | | | | | | | | | | | | | | | | | |
| 22. Rigidity: neck | | | | | | | | | | | | | | | | | |
| Upper extremity: right | | | | | | | | | | | | | | | | | |
| left | | | | | | | | | | | | | | | | | |
| Lower extremity: right | | | | | | | | | | | | | | | | | |
| left | | | | | | | | | | | | | | | | | |

Unified Parkinson's Disease Data Form

| Date | | | | | | | | | | | | | | | | |
|--------------------------------------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|
| | ON | OFF | ON | OFF | ON | OFF | ON | OFF | ON | OFF | ON | OFF | ON | OFF | ON | OFF |
| 23. Finger taps: right | | | | | | | | | | | | | | | | |
| left | | | | | | | | | | | | | | | | |
| 24. Hand grips: right | | | | | | | | | | | | | | | | |
| left | | | | | | | | | | | | | | | | |
| 25. Hand pronate/supinate: right | | | | | | | | | | | | | | | | |
| left | | | | | | | | | | | | | | | | |
| 26. Leg agility: right | | | | | | | | | | | | | | | | |
| left | | | | | | | | | | | | | | | | |
| 27. Arise from chair | | | | | | | | | | | | | | | | |
| 28. Posture | | | | | | | | | | | | | | | | |
| 29. Gait | | | | | | | | | | | | | | | | |
| 30. Postural stability | | | | | | | | | | | | | | | | |
| 31. Body bradykinesia | | | | | | | | | | | | | | | | |
| Sub-total:18–31 (maximum=108) | | | | | | | | | | | | | | | | |
| Total points: 1–31 (max=176) | | | | | | | | | | | | | | | | |
| 32. Dyskinesia (duration) | | | | | | | | | | | | | | | | |
| 33. Dyskinesia (disability) | | | | | | | | | | | | | | | | |
| 34. Dyskinesia (pain) | | | | | | | | | | | | | | | | |
| 35. Early morning dystonia | | | | | | | | | | | | | | | | |
| 36. "Offs" (predictable) | | | | | | | | | | | | | | | | |
| 37. "Offs" (unpredictable) | | | | | | | | | | | | | | | | |
| 38. "Offs" (sudden) | | | | | | | | | | | | | | | | |
| 39. "Offs" (duration) | | | | | | | | | | | | | | | | |
| 40. Anorexia, nausea, vomiting | | | | | | | | | | | | | | | | |
| 41. Sleep disturbance | | | | | | | | | | | | | | | | |
| 42. Symptomatic orthostasis | | | | | | | | | | | | | | | | |
| Blood Pressure: seated | | | | | | | | | | | | | | | | |
| supine | | | | | | | | | | | | | | | | |
| standing | | | | | | | | | | | | | | | | |
| Weight | | | | | | | | | | | | | | | | |
| Pulse: seated | | | | | | | | | | | | | | | | |
| standing | | | | | | | | | | | | | | | | |
| Name of Examiner | | | | | | | | | | | | | | | | |
| | BEST | WORST | BEST | WORST | BEST | WORST | BEST | WORST | BEST | WORST | BEST | WORST | BEST | WORST | BEST | WORST |
| Hoehn & Yahr Stage | | | | | | | | | | | | | | | | |
| % ADL Score (PD) | | | | | | | | | | | | | | | | |
| % ADL (with dyskinesia) | | | | | | | | | | | | | | | | |