Nilotinib Effects on Safety, Tolerability, and Biomarkers in Alzheimer's Disease

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Objective: Preclinical evidence with nilotinib, a US Food and Drug Administration (FDA)-approved drug for leukemia, indicates improvement in Alzheimer's disease phenotypes. We investigated whether nilotinib is safe, and detectable in cerebrospinal fluid, and alters biomarkers and clinical decline in Alzheimer's disease.

Methods: This single-center, phase 2, randomized, double-blind, placebo-controlled study investigated the safety, tolerability, and pharmacokinetics of nilotinib, and measured biomarkers in participants with mild to moderate dementia due to Alzheimer's disease. The diagnosis was supported by cerebrospinal fluid or amyloid positron emission tomography biomarkers. Nilotinib 150 mg versus matching placebo was taken orally once daily for 26 weeks followed by nilotinib 300 mg versus placebo for another 26 weeks.

Results: Of the 37 individuals enrolled, 27 were women and the mean (SD) age was 70.7 (6.48) years. Nilotinib was well-tolerated, although more adverse events, particularly mood swings, were noted with the 300 mg dose. In the nilotinib group, central nervous system (CNS) amyloid burden was significantly reduced in the frontal lobe compared to the placebo group. Cerebrospinal fluid Aβ40 was reduced at 6 months and Aβ42 was reduced at 12 months in the nilotinib group compared to the placebo. Hippocampal volume loss was attenuated (-27%) at 12 months and phospho-tau-181 was reduced at 6 months and 12 months in the nilotinib group.

Interpretation: Nilotinib is safe and achieves pharmacologically relevant cerebrospinal fluid concentrations. Biomarkers of disease were altered in response to nilotinib treatment. These data support a larger, longer, multicenter study to determine the safety and efficacy of nilotinib in Alzheimer's disease.

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that impairs cognitive abilities as well as activities of daily living (ADL).[1] Current US Food and Drug Administration (FDA)-approved treatments for AD provide only modest, temporary, palliative, and symptomatic benefits—and have no evidence of disease modification. AD is characterized pathologically by the accumulation of extracellular Aβ/amyloid plaques and intracellular neurofibrillary tangles (NFTs)

consisting primarily of aggregates of the microtubule-associated protein tau (MAPT).[2] The spatial distribution of tau pathology in the brain correlates with cognitive decline in AD, and knockout of the MAPT gene in an AD transgenic mouse model is protective against cognitive deficits.[3] Nilotinib (Tasigna, AMN107; Novartis, Switzerland) is a tyrosine kinase inhibitor that preferentially targets discoidin domain receptors (DDRs)[4–8] and effectively reduces misfolded proteins in animal models of neurodegeneration.[7,9–16] Nilotinib also targets the nonreceptor tyrosine kinase Abelson[4–6] and is FDA-approved for the treatment of Philadelphia chromosome positive chronic myeloid leukemia (CML) at oral doses of 300 mg twice daily.[4–6] Low doses of nilotinib penetrate the blood–brain barrier and promote the degradation of Aβ and tau in animal models of neurodegeneration.[7,9–16] Clinical studies indicate that nilotinib enters the central nervous system (CNS; peak plasma concentration [C_{max}] 2–4 nM in cerebrospinal fluid [CSF]), increases dopamine turnover, and reduces CSF tau, independent of Abelson inhibition[17–19]—further suggesting that nilotinib effects may be mediated by DDR1 inhibition (half-maximal inhibitory concentration [IC_{50}] 1–8 nM).[8,20,21]

The primary objective of this study was to evaluate the safety, tolerability, and pharmacokinetics of nilotinib in participants with mild to moderate dementia due to AD. Secondary objectives included evaluation of the effect of nilotinib on amyloid biomarkers—CSF Aβ42 and Aβ40 and CNS amyloid burden (Florbetaben positron emission tomography [PET]), and markers of tauopathy and neurodegeneration—CSF phospho-tau (ptau-181), total tau, and hippocampal volume (magnetic resonance imaging [MRI]). Exploratory objectives included evaluation of the efficacy of nilotinib in slowing or halting the clinical, cognitive, functional, and behavioral decline of AD, as measured by change from baseline to 6 months (nilotinib, 150 mg vs placebo) and 12 months (nilotinib, 300 mg vs placebo) in the Alzheimer Disease Assessment Scale–cognitive subscale (ADAS-Cog), Alzheimer's Disease Cooperative Study–Instrumental Activities of Daily Living Inventory (ADCS-ADL), Clinical Dementia Rating–Sum of Boxes (CDR-SOB), Neuropsychiatric Inventory (NPI), and Mini-Mental Status Examination (MMSE).

Methods

Participants

Participants had mild to moderate dementia and a biomarker-supported diagnosis of AD according to the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease[22] with MMSE 14 to 24 and either CSF Aβ42 < 1,100 pg/ml or a positive amyloid PET (visual read), or both.

All participants and their study partners provided written informed consent and were stabilized on medications at least 1 to 2 months before enrolling. Participants were stabilized on acetylcholinesterase inhibitors (AChEI)—galantamine (Razadyne), rivastigmine (Exelon), or donepezil (Aricept)—as well as memantine (Namenda) if indicated at least 2 months before screening. Therapeutic doses of selective serotonin reuptake inhibitors (SSRIs), and serotonin-norepinephrine reuptake inhibitors (SNRIs) were allowed. Baseline visits were scheduled 2 to 4 weeks after screening. This study included 16 schedules visits as summarized in Table S1. Male and female participants of all races and nationalities aged 50 to 85 years were recruited with no restriction to geographic boundaries as long as they complied with study visits and procedures. Lumbar punctures (LPs) at baseline, 6 months, and 12 months were mandatory.

Study Design and Objectives

This phase 2, randomized, double-blind, placebo-controlled study evaluated nilotinib effects in mild to moderate AD. The objective was to randomize 42 participants 1:1 into 2 groups receiving a daily oral dose of 150 mg nilotinib for 6 months followed by 300 mg nilotinib for 6 months (12 months total) versus placebo. The primary objective of this study was to evaluate the safety, tolerability, and pharmacokinetics of nilotinib, which was measured in the plasma and CSF of all participants with AD at baseline, 6 months, and 12 months. Secondary objectives included evaluation of nilotinib effects on amyloid biomarkers—CSF Aβ42 and Aβ40 and CNS amyloid burden (Florbetaben PET), and markers of tauopathy and neurodegeneration—CSF ptau-181, total tau, and hippocampal volume (MRI). An exploratory objective included clinical assessments of cognitive and behavioral functions using MMSE, ADAS-Cog, ADCS-ADL, CDR-SOB, and NPI at baseline, 6 months, and 12 months.

Standard Protocol Approvals, Registrations, and Patient Consents

This study was conducted by the Translational Neurotherapeutics Program (TNP) and the Memory Disorders Program at the Georgetown University Medical Center (GUMC) Clinical Research Unit (CRU) of Georgetown-Howard Universities Center for Clinical and Translational Science (GHUCCTS). Participants and study partners were recruited and this study was conducted in accordance with Good Clinical Practice guidelines and approved by the Institutional Review Board (IRB #2016–0351) at GUMC as well as GHUCCTS scientific review board. The study was conducted under FDA Investigational New Drug (IND) #130732, and registered in ClinicalTrials.gov

(NCT02947893). An external independent Data Safety Monitoring Board (DSMB) included a behavioral neurologist, biostatistician, cardiologist, and clinical pharmacologist, as well as an independent study monitor.

Randomization and Blinding

This study used a block randomization using *blockrand* function in R software (version 3.4) to randomize 42 participants into 2 treatment groups. The block size varies between 1 and 7 and the randomization was done within blocks to ensure a balance in sample sizes across group blocks.[23] All site staff, investigators, raters, participants, and caregivers were blinded to the dose and treatment group until study completion. Medications were labeled with a package medical identification number (Med. ID). Each participant was assigned a specific identification number (Pat. ID), which was noted by the investigator on the designated medication package after randomization.

Statistical Data Analysis

Baseline demographic and safety end points were summarized using descriptive statistics as mean ± SD for continuous variables and frequencies for categorical variables by the 2 treatment groups. The proportions of serious adverse events (SAEs) and nonserious adverse events (AEs) among the 2 groups were compared using Fisher's exact test. Exploratory clinical and biomarker end points at baseline, 6 months, and 12 months were summarized by the 2 groups using sample means ± SDs. For within or between treatment group(s) comparison, a paired or unpaired Mann–Whitney U test and its 95% confidence interval (CI) were computed to test whether there is a change in medians of each clinical and biomarker end point, respectively, between baseline and 6 months, baseline and 12 months, and 6 months and 12 months. Statistical significance was determined by a 2-sided $p < 0.05$. For clinical and biomarker end points, false discovery rates (FDRs) using the Benjamini-Hochberg procedure, as reported in Tables S1–S10. All statistical analyses were performed using R version 3.40.

Plasma and CSF Collection

To obtain pharmacological properties and calculate the area under the curve (AUC), blood draw (15 ml) and LP ($\sim$ 15 ml CSF) were performed on all patients approximately at 1, 2, 3, or 4 hours after oral nilotinib administration. Plasma and CSF were isolated immediately after blood draw and LP, aliquoted and stored at −80°C. Freeze/thaw cycles were avoided.

Abelson enzyme-linked immunosorbent assay

Pan tyrosine phospho-Abelson and specific tyrosine (Tyr 412 and 245) phosphorylated Abelson are detected via enzyme-linked immunosorbent assay (ELISA) using PathScan phospho-Abelson solid phase sandwich. ELISA was performed on CSF as we previously described.[17,19]

Tau and Aβ ELISA

A total of 25 μl of soluble protein was incubated overnight at 4°C with 25 μl of a mixed-bead solution containing total tau, and p-tau-181, Aβ40, and Aβ42, 25 μl of detection antibody solution (Cat. #HNABTMAG-68 K, Millipore). After washing, 25 μl of streptavidin-phycoerythrin was added to each well containing suspended beads and incubated at room temperature for 30 minutes. Samples were then washed and suspended in 100 μl of sheath fluid and analyzed on MAGPIX with Xponent software.

Mass Spectrometry

Plasma and CSF samples were extracted in (500 μl) acetonitrile/methanol (50:50) containing the internal standard (5 ng/ml of Nilotinib_13C_2H3) and dialyzed through 25 μm membranes to obtain unbound or free nilotinib. The supernatant containing unbound nilotinib was freeze-dried using speed vacuum and reconstituted in 200 μl of methanol: water (50:50) and processed by mass spectrometry (MS), as we previously described.[17,19]

Volumetric MRI

To assess hippocampal loss, volumetric MRI was performed on a 1.5 T Aera scanner (Siemens, Erlangen, Germany). Sagittal 3D magnetization prepared rapid acquisition of gradient echo (MP-RAGE) sequence was acquired with repetition time 2,400 msec, echo time 2.29 msec, and inversion time 1,000 msec. Field of view was 240 mm × 240 mm with a matrix of 192 × 192, resulting in an in-plane resolution of 1.25 × 1.25 mm. One hundred sixty slices were obtained with a slice thickness of 1.2 mm.

Quantification of Hippocampal Volume

Data preprocessing was performed using the SPM12 software package (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) and the CAT12 toolbox release 12.6, r1450 (http://dbm.neuro.uni-jena.de/cat/) in MATLAB (release 2018b; The MathWorks, Natick, MA). Standard preprocessing procedures (https://www.ncbi.nlm.nih.gov/pubmed/31901790) were used with default parameters, including correction for bias-field inhomogeneities, denoising, skull-stripping, segmentation, and corrections for partial volume estimation. The hippocampal grey matter volume was determined using the default function in CAT12 with the Neuromorphometrics atlas (https://scalablebrainatlas.incf.org/human/NMM1103).

PET Computed Tomography

To determine CNS amyloid level of burden, PET was performed approximately 90 minutes after administration of 300 MBq ± 20% of ^{18}F-Florbetaben and images were acquired in 3D mode for 20 minutes, divided in 4 frames of 5 minutes each. To minimize motion artifacts, participants' heads were immobilized with a head-holder and fixation equipment. PET data obtained were corrected for radioactive decay, dead time, measured attenuation, and scatter. The resulting image data were reconstructed using an iterative algorithm. Reconstructed images were visually inspected by a certified nuclear medicine physician (G.E). Images that were technically suboptimal were excluded from the analysis. Quantitative analysis was performed using an automated software: Cortical Analysis, version VE20A (Siemens Medical Solutions, Molecular Imaging, Hoffman Estates, IL). Each PET image was co-registered with a standard mutual information algorithm and spatially normalized to the reference brain. An automated anatomic labeling template, according to Barthel et al,[24] was then used for standardized, regional brain volume of interest analysis. Volumes of interest were individually defined on both hemispheres (where appropriate) for the frontal cortex, including gyrus rectus and orbitofrontal cortex, temporal cortex, including mesial and lateral temporal cortex, parietal cortex, including the precuneus, occipital cortex, anterior cingulate, posterior cingulate, and cerebellar cortex. Mean standardized uptake values (SUVs) were obtained from each of the automatically defined regional volumes. Regional standardized uptake value ratios (SUVRs) were then obtained for each of the regional volumes of interest, dividing the mean SUVs by that of the reference cerebellar cortex volume mean SUV.

Quantification of Dopamine Metabolites 3,4-Dihydroxyphenylacetic Acid and Homovanillic Acid by Liquid Chromatography-Tandem mass spectrometry

Concentrations of CSF 3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by ultrahigh performance liquid chromatography with electrospray tandem MS (UHPLC-MS/MS) following derivatization with benzoyl chloride as described.[19]

Clinical Assessments

All participants were stable on acetylcholinesterase inhibitors and other AD medications prior to enrollment. Cognitive and behavioral assessments were conducted at baseline, 6 months, and 12 months using MMSE, ADAS-Cog, ADCS-ADL, CDR-SOB, and NPI.

Apolipoprotein Genotyping

EzWay Direct Apolipoprotein (*APOE*) Genotyping Kit was used for *APOE* genotyping from whole blood using the One-step Multiplex PCR system with ApoE primer mixture for E2 (Cys112/Cys158), E3 (Cys112/Arg158), and E4 (Arg112/Arg158) according to the manufacturer's protocol (Komabiotech; catalog no. K0568500).

Results

Patients, Demographics, Enrollment, and Randomization

Of 117 potential participants, 13 declined, 53 were screened, and 37 enrolled; 51 did not meet inclusion/exclusion criteria due to corrected QT (QTc) prolongation or other cardiovascular disease, MMSE score out of range, or a diagnosis of AD not supported by biomarker evidence. This study follows Consolidated Standards of Reporting Trials (CONSORT) reporting guidelines (Fig 1). The study failed to meet the targeted enrollment of 42—enrollment was terminated at 37 (88%; Table 1). Mean (SD) age of the participants was 70.7 years (6.48) and the sample included 10 men (27%) and 27 women (73%). A total of 31 participants (83.78%) completed the study and there were no dropouts due to drug safety or tolerability. The mean (SD) MMSE was 19.2 (3.1) in the placebo group and 19.8 (2.5) in the nilotinib group. The mean (SD) of CSF Aβ42 at screening was 407.9 (219.1) ng/ml in the placebo group and 426.6 (171.9) ng/ml in the nilotinib group. There were no significant differences between the placebo group and the nilotinib group at baseline ($p > 0.05$). The *APOE* genotype in the placebo group was E4/E4 5 (25%), E3/E4 5 (25%), E2/E4 4 (20%), E2/E2 1 (5%), and 5 (25%) inconclusive genotypes. In the nilotinib group, *APOE* was E4/E4 7 (41.2%), E3/E4 1 (5.9%), E2/E4 3 (17.6%), E3/E3 1 (5.9%), E2/E2 1 (5.9%), and 4 (23.5%) inconclusive genotypes.

Adverse Events

Safety and tolerability of nilotinib were evaluated using spontaneously reported AEs, laboratory tests, vital signs, body weight, and physical examinations, including neurological examinations, and electrocardiograms (ECGs). Additional safety assessments included administration of the Columbia-Suicide Severity Rating Scale (C-SSRS) and the Geriatric Depression Scale (GDS) to assess suicidality and worsening depression, respectively. No QTc prolongation was observed in the nilotinib group versus the placebo group (Tables S2 and S3). There was 122 AEs in the nilotinib group and 112 in the placebo group (Table S4). Mood swings were the most commonly reported AEs in the nilotinib group (70.6%) between 6 and 12 months when participants received 300 mg nilotinib compared to placebo ($p = 0.01$), but no difference was observed when participants received 150 mg nilotinib compared to placebo. Mood swings included agitation (52.9%) and irritability (35.2%) in the nilotinib group versus the placebo
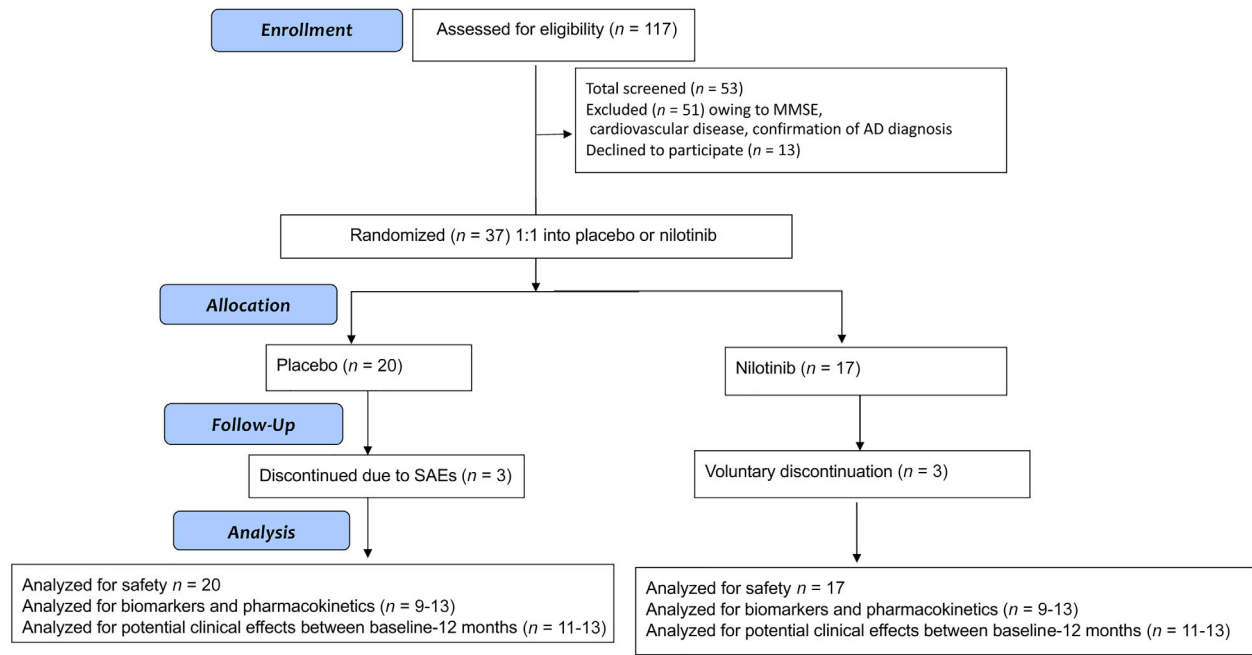
FIGURE 1: CONSORT Flow Diagram. Phase 2, randomized, double-blind, placebo-controlled study to evaluate nilotinib effects on safety, tolerability, pharmacokinetics, biomarkers, and potential clinical outcomes in Alzheimer's disease. [Color figure can be viewed at www.annalsofneurology.org]

group (10% and 15%, respectively). Pain in the nilotinib group (41.2%) versus the placebo group (60%; $p = 0.16$), and diarrhea in the nilotinib group (41.2%) compared to the placebo group (15%; $p = 0.13$). Common AEs also included headache, gastrointestinal, skin, and respiratory disorders. Less common AEs included nervous system, musculoskeletal, urinary, psychiatric, eye, and renal disorders. Rare AEs included hematological, hepatic, and pancreatic disorders.

Serious Adverse Events

The total number of SAEs was 5 (25%) in the placebo group versus none in the nilotinib group (0%; Table 2). Post hoc comparisons show a significant difference in the number of events ($p = 0.05$), but not number of patients ($p = 0.23$), between the placebo and nilotinib groups. One participant was hospitalized with 2 SAEs (rhabdomyolysis and bronchitis) and withdrew from the study. Another participant was hospitalized due to 2 SAEs (orthostatic hypotension and vertigo) and withdrew, and 1 participant was hospitalized with disease progression and psychosis and withdrew from the study.

Pharmacokinetics

Pharmacokinetics was performed to evaluate whether nilotinib enters the CNS. Nilotinib was measurable in the CSF and plasma at the 150 mg daily dosage (C_{max}: 3.46 nM and 1,099 nM, respectively) and the 300 mg daily dosage (C_{max}: 4.7 nM and 1,410 nM, respectively; Table 3).

Biomarkers

To determine whether nilotinib affects brain amyloid burden, Florbetaben PET was performed at baseline and again at 12 months. Standardized regions of interests were displayed on fused PET computed tomography (CT) images and quantified (Fig 2A). The median change from baseline to 12 months of CNS amyloid using SUVR was smaller in nilotinib compared to placebo in the frontal (-0.19; 95% CI, -0.32 to -0.08; $p = 0.01$) and temporal lobes (-0.08; 95% CI, -0.21 to 0.02; $p = 0.09$), respectively (see Fig 2A–E, Tables S5–S7). A nonsignificant difference was observed in whole brain composite (see Fig 2F) SUVR (-0.11; 95% CI, -0.21 to 0.01; $p = 0.1$). Volumetric MRI showed that loss of hippocampal volume (cm^3) in the placebo (-0.14; 95% CI, -0.36 to -0.06; $p = 0.01$) was comparable to the hippocampal volume loss in the nilotinib group (-0.14; 95% CI, -0.22 to -0.06; $p < 0.001$) at 12 months (Fig 3A, Tables S5–S7).

The effects of nilotinib on CSF Aβ42/40 biomarkers and cell death markers, including CSF total tau and ptau-181 were determined. In the nilotinib versus placebo groups, CSF Aβ40 was reduced at 6 months (566 ng/ml; 95% CI, 32 to 1,145; $p = 0.03$) compared to baseline (see Fig 3B). Aβ42 was reduced at 12 months (73.9 ng/ml; 95% CI, 1.6 to 145.0; $p = 0.05$) compared to baseline (see Fig 3C). The ratio of Aβ42/Aβ40 was increased between 6 and 12 months (0.005; 95% CI, 0 to 0.01; $p = 0.02$) in the placebo group, whereas this ratio did not change in the nilotinib group (-0.00; 95% CI, -0.01 to

TABLE 1. Demographics and Enrollment Summary		
	Placebo	Nilotinib
No. enrolled	20	17
No. at end of treatment (%)	17 (85)	14 (82.3)
No. of dropouts (%)	3 (15)	3 (17.6)
Average age, yr ± SD	69.2 ± 6.06	72.2 ± 6.9
Weight, kg ± SD	75.8 ± 16.4	68.3 ± 20.2
Height, cm ± SD	167.4 ± 10.0	159.8 ± 7.66
BMI ± SD	26.7 ± 4.7	26.6 ± 6.83
Male (%)	7 (35)	3 (17.6)
Female (%)	13 (65)	14 (82.3)
Race (%)	18 Whites (90) 1 Asian (5) 1 Black (5)	15 Whites (88.2) 2 Asians (11.7)
MMSE at screening Mean ± SD	19.2 ± 3.1	19.8 ± 2.5
CSF Aβ42 at screening, pg/ml, mean ± SD	407.9 ± 219.1	426.6 ± 171.9
APOE genotype No. (%)	E4/E4 5 (25) E3/E4 5 (25) E2/E4 4 (20) E3/E3 0 (0) E2/E2 1 (5) Inconclusive 5 (25)	E4/E4 7 (41.2) E3/E4 1 (5.9) E2/E4 3 (17.6) E3/E3 1 (5.9) E2/E2 1 (5.9) Inconclusive 4 (23.5)

Aβ42 = amyloid beta peptide 42; APOE = apolipoprotein E; BMI = body mass index; CSF = cerebrospinal fluid; MMSE = Mini-Mental Status Examination.

0; $p = 0.12$). This ratio was reduced between baseline and 12 months (−0.01; 95% CI, −0.01 to 0; $p = 0.04$) in the nilotinib group compared to the placebo group (see Fig 3D). Aβ40 was also reduced at 6 months (−505.5; 95% CI, −817.0 to −126.5; $p = 0.02$) and 12 months (−442; 95% CI, −765.5 to −127.0; $p = 0.04$) within the nilotinib group. Aβ42 was also reduced at 6 months (−54.7; 95% CI, −120.5 to −11.1; $p = 0.02$) and 12 months (−68.6; 95% CI, −94.7 to −18.4; $p = 0.02$) within the nilotinib group.

There was no difference in CSF total tau ($p = 0.89$; see Fig 3E) and ptau-181 ($p = 0.51$; see Fig 3F) between the placebo and nilotinib-treated groups at 12 months. There was a decrease in ptau-181 at 6 months in the nilotinib group (−3.07; 95% CI, −6.64 to −0.51; $p = 0.03$) but not in the placebo group (−2.13; 95% CI, −7.01 to 2.96; $p = 0.37$). There was a decrease in ptau-181 at 12 months in the nilotinib group (−4.75; 95% CI, −8.82 to −1.0; $p = 0.01$), whereas the change was not

significant in the placebo group (−2.87; 95% CI, −8.17 to 1.90; $p = 0.26$). No changes were observed in the ratio of ptau-181/total tau (see Fig 3G) between the groups at 6 months ($p = 1.0$) and 12 months ($p = 0.93$).

Nilotinib treatment leads to an increase in CNS dopamine levels in animals[10,17,19] and individuals with Parkinson's disease (PD), so the levels of CSF dopamine metabolites HVA and DOPAC were measured. HVA was reduced (see Fig 3H) at 6 months (−61.5 nM; 95% CI, −115.6 to −19.1; $p = 0.01$) and nonsignificantly reduced at 12 months (−37.1 nM; 95% CI, −113.9 to 19.2; $p = 0.11$) in the nilotinib group compared to the placebo group. In the nilotinib group, HVA was reduced at 6 months (−71.9 nM; 95% CI, −124.0 to −29.4; $p < 0.001$) and 12 months (−47.4 nM; 95% CI, −103.6 to −1.9; $p = 0.05$) and DOPAC was reduced at 12 months (−0.3 nM; 95% CI, −0.6 to −0.06; $p = 0.02$), suggesting reduced catabolism of CNS dopamine (see Fig 3I). Nilotinib at 300 mg twice daily inhibits Abelson in

TABLE 2. Summary of All SAEs Reported According to Systems/Preferred Organs in All Treatment Groups

Systems preferred organ	Placebo (n = 20) No. of events (%)	Nilotinib (n = 17) No. of events (%)
Musculoskeletal and connective tissue disorders Rhabdomyolysis	Rhabdomyolysis 1 (5)	0
Nervous system disorders Vertigo	Vertigo 1 (5)	0
Psychiatric disorders Psychosis	Psychosis 1 (5)	0
Respiratory, thoracic, and mediastinal disorders Bronchitis	Bronchitis 1 (5)	0
Cardiovascular disorders Hypotension	Hypotension 1 (5)	0
No. of SAEs/no. of patients (%)	5/3 (25)	0

SAE = serious adverse event.

CML.[4–6] Abelson activity (via phosphorylation) was measured to determine nilotinib effects on AD biomarkers. No CSF Abelson inhibition was observed in nilotinib versus placebo groups via phosphorylation at tyrosine residue 412 (see Fig 3J), pan-tyrosine (see Fig 3K), or the ratio of pan-tyrosine/tyrosine 412 (see Fig 3L).

Clinical Outcomes

Measurement of the severity and progression of cognitive impairment using MMSE scoring (Fig 4A) showed a significant annual decline in the placebo (−3.5 patients; 95% CI, −5.50 to −2.50; $p < 0.001$) and the nilotinib-treated groups (−2.50 patients; 95% CI, −4.5 to −1; $p = 0.01$). To assess the severity of cognitive symptoms and dementia using ADAS-Cog, a significant decline (see Fig 4B, Tables S8–S10) was observed in the placebo (4.67 patients; 95% CI, 1.66 to 6.84; $p < 0.001$) and in the nilotinib-treated groups (6.83 patients; 95% CI, 2.16 to 10.83; $p = 0.01$) at 12 months. Executive functioning as measured on subscale 13 of the ADAS-Cog (maze) showed (see Fig 4C) a significant increase in time to complete the maze at 6 months (7.5 seconds; 95% CI, 3.5 to 15.0; $p < 0.001$) and 12 months (6 seconds; 95% CI, 0.36 to 32.98; $p = 0.03$) in the placebo group. However, there was a significant difference in time to complete the maze between placebo and nilotinib-treated groups (4.7 seconds; 95% CI, 0.42 to 12.85; $p = 0.03$) at 6 months.

To determine staging of cognitive and functional performance, CDR-SOB scoring (see Fig 4D) showed that the placebo group declined between 6 months and 12 months (1.75 points; 95% CI, 0.75 to 4; $p < 0.001$) and at 12 months (2 points; 95% CI, 1.25 to 3.5; $p < 0.001$). The nilotinib-treated group declined at 6 months (1.5 points; 95% CI, 0.5 to 2.5; $p = 0.02$) and 12 months (2.5 points; 95% CI, 1.5 to 4; $p < 0.001$). Assessment of instrumental ADL using ADCS-ADL scoring (see Fig 4E), showed that the placebo group declined between 6 months and 12 months (−3 points; 95% CI, −6 to −1; $p = 0.02$) and at 12 months (−7.5 points; 95% CI, −13 to −2; $p = 0.02$), whereas the nilotinib group declined at 12 months (−4.5 points; 95% CI, −10 to −0.5; $p = 0.04$). Assessment of the severity and frequency of behavioral symptoms using NPI (see Fig 4F), showed no difference in the total NPI and in caregiver distress between the placebo and nilotinib groups, but significantly more agitation and aggression ($p = 0.03$) were reported with the 300 mg nilotinib-treated group (35.5%) compared to placebo (5%), and the 300 mg nilotinib group reported more ($p < 0.001$) irritability (17.6%) between 6 and 12 months.

TABLE 3. Pharmacokinetics of Nilotinib in Individuals with Alzheimer's Disease

Nilotinib concentration	Placebo	Nilotinib 150 mg [ng/ml]. Mean ± SD	T_{max} (h)	C_{max} (nM)	AUC (ng/ml*h)	Nilotinib 300 mg [ng/ml]. Mean ± SD	T_{max} (h)	C_{max} (nM)	AUC (ng/ml*h)
CSF, nM	0	1.2 ± 0.74	3	3.46	7.59	1.5 ± 0.62	2	4.7	11.27
Plasma, nM	0	410.6 ± 161.7	4	1099	2507	566 ± 384.4	2	1410	3381

AUC = area under the curve; C_{max} = peak plasma concentration; CSF = cerebrospinal fluid; T_{max} = time to peak plasma concentration.
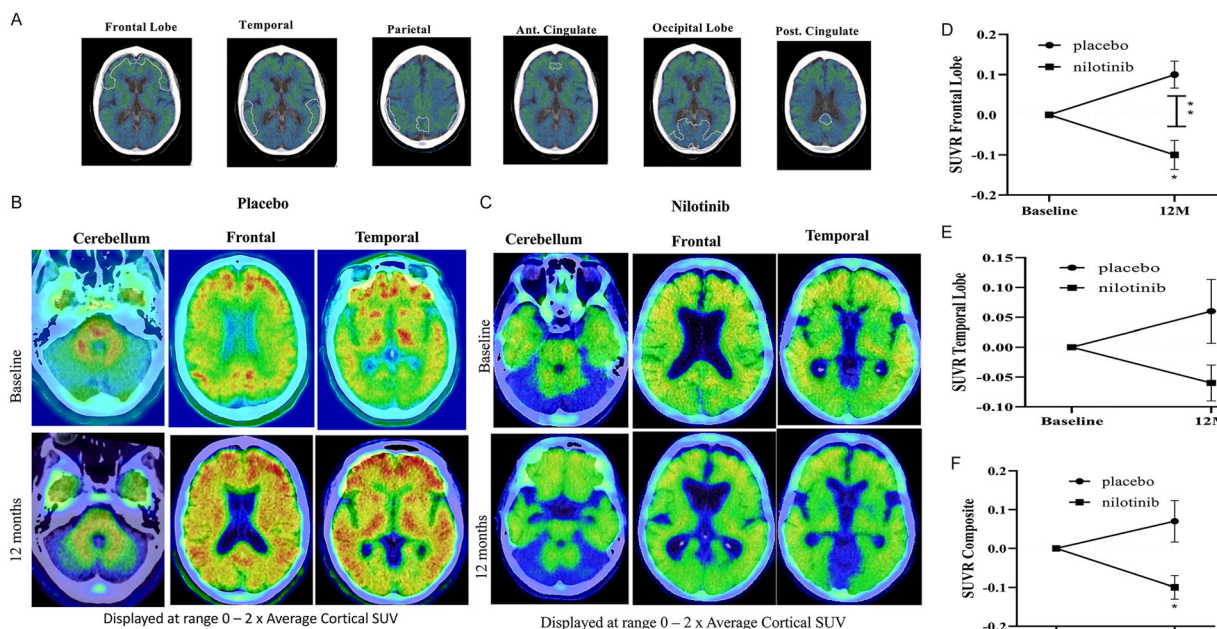
FIGURE 2: Quantitative Regional Analysis of (A) standardized regions of interests displayed on fused positron emission tomography (PET) computed tomography (CT) images. Representative PET CT images at baseline and at 12 months of amyloid deposition in the reference cerebellum, frontal, and temporal lobes in participants who (B) received placebo or (C) nilotinib. The graph shows reduction in standardized uptake value ratio (SUVR) from baseline to 12 months in: (D) frontal lobe, (E) temporal lobe, and (F) whole brain (composite) in nilotinib versus placebo groups. *$p < 0.05$, **$p < 0.01$ within the group or as indicated between groups.
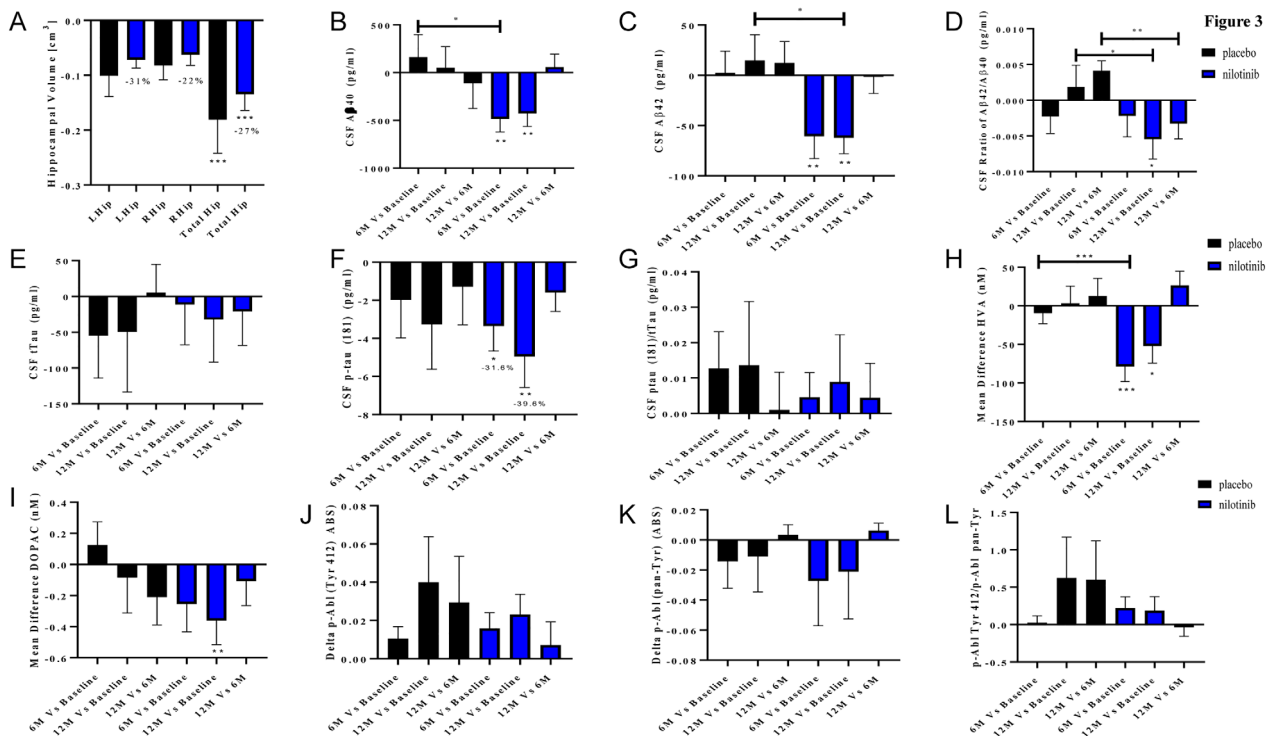
FIGURE 3: Graphs represent, (A) volumetric hippocampal volume and CSF levels of (B) Aβ40, (C) Aβ42, (D) Aβ42/Aβ40 ratio, (E) total tau, (F) phospho-tau-181, and (G) ratio of ptau-181/total tau. The level of dopamine metabolites (H) homovanillic acid (HVA) and (I) 3,4-hydroxyphenylacetic acid (DOPAC). Graphs represent the mean difference in (J) Abelson phosphorylation at tyrosine 412, (K) pan-tyrosine phosphorylation of Abelson, and (L) the ratio of Abelson phosphorylation (activation) at tyrosine 412/pan-tyrosine in the cerebrospinal fluid (CSF) of patients with Alzheimer's disease (AD) treated with nilotinib versus placebo. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ within the group or as indicated between groups (% change is included only with nonsignificant differences).
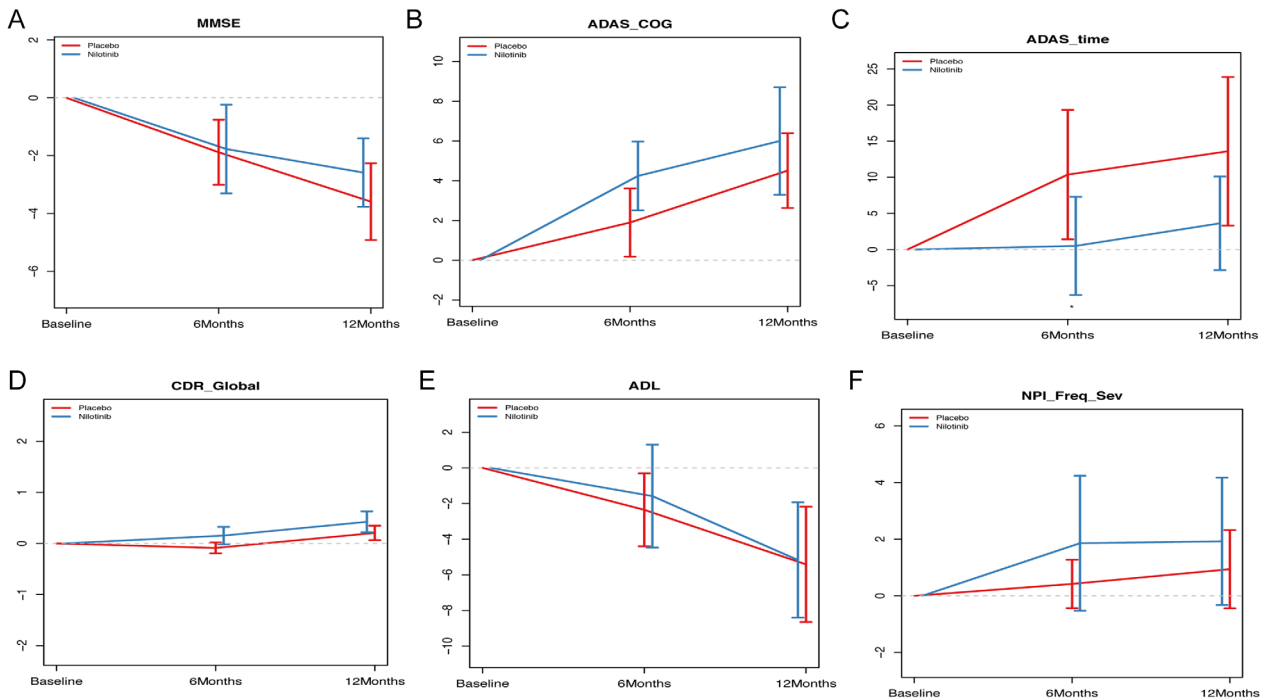
FIGURE 4: Graphs represent exploratory clinical outcomes and their 95% confidence interval between the placebo and nilotinib-treated groups in (A) Mini-Mental Status Examination (MMSE), (B) Alzheimer Disease Assessment Scale–cognitive subscale (ADAS-Cog), (C) ADAS-Cog subscale 13 (Maze) or time to complete task, (D) Global Clinical Dementia Rating–Sum of Boxes (CDR-SOB), (E) Alzheimer's Disease Cooperative Study–Instrumental Activities of Daily Living Inventory (ADCS-ADL), and (F) severity and frequency of behavioral symptoms as measured by Neuropsychiatric Inventory (NPI). *$p < 0.05$.

Discussion

Nilotinib is safe and well-tolerated in participants with mild to moderate AD, although significantly more mood swings were observed in the high-dose nilotinib group compared to the placebo group. Overall, there was no difference in the total number of AEs between groups. However, some of the AEs observed in this study may be related to nilotinib. Mood swings were significantly increased between 6 and 12 months when participants were treated with daily 300 mg nilotinib, and caregiver reports using the NPI indicate that mood swings include agitation and irritability. Our previous studies suggest a dose-dependent increase of CNS dopamine when individuals with PD were treated with nilotinib,[17–19] and these effects on dopamine are observed in this study, suggesting that increases in CNS dopamine (owing to reduced catabolism) with 300 mg nilotinib-treated patients with AD may lead to deleterious behavioral changes.[25] No mood swings or behavioral changes were reported between baseline and 6 months when patients were treated with 150 mg nilotinib, suggesting that this dosage may be preferable for future studies to avoid behavioral adverse effects. There were more reports of diarrhea in the nilotinib group; although gastrointestinal symptoms are common in patients with AD treated with acetylcholinesterase inhibitors, nilotinib may interact with AD drugs to promote gastrointestinal symptoms. Transient elevations of pancreatic and liver enzymes as well as borderline anemia, leukopenia, and thrombocytopenia in the nilotinib group were rarely observed, did not require medical intervention, and resolved spontaneously. There were significantly more SAEs in the placebo group and 3 participants withdrew from the study due to SAEs. No SAEs were observed in the nilotinib group and there were no dropouts due to intolerability. Two participants voluntarily withdrew from the study in the nilotinib group due to travel burden, and one participant declined participation following a baseline visit. Nilotinib is FDA-approved for CML at 300 mg dose twice daily and carries a black-box warning of sudden death due to QTc prolongation, but no hypokalemia, hypomagnesemia, or long QT syndrome were observed in this study. Abelson inhibition via nilotinib may lead to cardiac and hepatic disorders and myelosuppression in patients with CML, but evidence in patients with PD treated with 150 mg and 300 mg nilotinib showed that nilotinib did not inhibit plasma Abelson, suggesting that a low dose (≤ 300 mg nilotinib once daily) is safe.[19] According to nilotinib prescription information, sudden death was reported in 0.3% of patients with CML treated with nilotinib in studies of 5,661 patients. The small sample size in this study does not preclude a similar risk in patients with AD.

Our previous clinical studies indicate that treatment with 150 mg or 300 mg nilotinib daily results in 2 to 4 nM CSF levels of nilotinib.[17–19] This CSF concentration reduces p-tau, independent of Abelson inhibition.[17–19] Nilotinib inhibits Abelson ($IC_{50} > 20$ nM)[4–6] and is FDA-approved for Philadelphia chromosome CML that results from *Abl* mutations at oral doses of 300 mg twice daily.[4–6] However, nilotinib more potently inhibits DDR1 ($IC_{50} = 1–8$ nM).[4–8] The concentration of CSF nilotinib in 150 mg and 300 mg nilotinib-treated groups (3.46 nM and 4.7 nM, respectively) exceeds the IC_{50} required to inhibit DDR1. Therefore, nilotinib achieves sufficient CSF levels to what would potentially inhibit DDR1, and alter disease biomarkers. We demonstrated that DDR1 and DDR2 are increased in post mortem AD and PD brains,[21] whereas DDR knockdown with *shRNA* in vivo and in vitro[21] and pharmacological inhibitors of DDR (including nilotinib)[7] reduce CNS Aβ, tau, and alpha-synuclein in animal models. Collectively, these data suggest that nilotinib may engage its CNS target via inhibition of DDRs. CSF Abelson was not inhibited, but evidence of Abelson inhibition by nilotinib in vitro (cell culture) assays and animal models indicate Abelson inhibition in total tissue lysates,[10,16,26–28] suggesting that CNS Abelson inhibition cannot be ruled out. Nilotinib concentration in the plasma of participants with AD who received 150 mg nilotinib (1,099 nM) and 300 mg nilotinib (1,410 nM) was higher (~ 50%) than previously reported plasma levels of nilotinib in patients with PD,[17–19] suggesting differential drug absorption between PD and AD, perhaps due to specific disease pathology (gut) or concomitant medications.

Nilotinib reduced the level of CSF Aβ42, Aβ40, and p-tau, lowered CNS amyloid burden, and attenuated hippocampal volume loss in patients with AD compared with placebo. These results are consistent with preclinical data showing that nilotinib effectively reduces Aβ42, Aβ40, lowers plaque burden, and clears p-tau via promotion of autophagy in animal models of neurodegeneration.[7–16,20,21] The effects of nilotinib on AD biomarkers are consistent with a previous study showing reduction of CSF oligomeric alpha-synuclein and p-tau in patients with PD.[19] Tau deletion impairs autophagic clearance of toxic intraneuronal Aβ42 in gene transfer animal models, but restoration of tau expression and nilotinib treatment improve autophagic clearance of intraneuronal Aβ42 and p-tau and result in less plaque deposition.[15] Autophagy clears intraneuronal Aβ42 and Aβ40, thus reducing plaque deposition, accumulation of p-tau, and neuronal death.[7,14–16,21,26,29,30] These findings are consistent with our data showing that both plaque deposition and CSF Aβ42 and Aβ40 were concurrently reduced, suggesting that clearance of intraneuronal Aβ via autophagy leads to less secretion of the peptide and reduced plaque deposition and CSF levels. Autophagy clearance of Aβ42, Aβ40, and p-tau is also concurrent with improved astrocytic activity and neurotransmitter balance.[10,11,13,14,31] Collectively, these findings indicate that nilotinib lowers the levels of AD biomarkers via promotion of autophagic clearance. Both 150 mg and 300 mg nilotinib doses reduce CSF Aβ42, Aβ40, and p-tau compared to placebo. Amyloid PET and MRI were only performed at baseline and 12 months so no comparison may be made between nilotinib doses. Taken together, these results indicate that nilotinib may engage the A/T/N biomarker system, as β-amyloid PET and CSF Aβ42 (A); CSF phospho-tau-181 (T), and hippocampal volume (N)[32] are changed in the nilotinib-treated group. Accumulating evidence that reduced CSF Aβ may be associated with white matter lesions,[33] therefore, our future studies will also examine white matter changes and vascular dysfunction in early stages of AD.[34]

This phase 2 trial was underpowered (as designed) to detect differences in clinical and cognitive outcomes and focused on evidence of nilotinib effects on safety and biomarkers, hence the incongruity between biomarker and clinical effects. Nevertheless, exploratory outcomes included efficacy of nilotinib versus placebo on the change from baseline to 6 months and 12 months. As expected, no differences were observed between the placebo and nilotinib groups on clinical, cognitive, functional, and behavioral outcomes, suggesting that a larger multicenter phase 3 study must be adequately powered to examine potential efficacy. The exploratory clinical outcomes in this phase 2 study will guide the design of an adequately powered larger and longer study to evaluate the safety and efficacy of nilotinib in AD. An attenuation of one point in the MMSE score was observed in the nilotinib group compared to the placebo group (albeit nonsignificant). The difference in ADAS-Cog subscale 13 (maze) was significant at 6 months between the placebo and nilotinib groups. The differences of 10 to 13 seconds to finish the task, with more errors in the placebo group compared to no change in the nilotinib group, may be clinically meaningful owing to the impact of increased CNS dopamine levels on executive functioning. There was an increase in CDR and a decrease in ADL in both groups, suggesting equal progression of disease; however, intervention at an earlier disease stage (MMSE 22–30), or in individuals with mild cognitive impairment (MCI), and longer treatment duration may offer a better opportunity to impact clinical decline. Furthermore, the study failed to meet the targeted enrollment of 42 individual owing to potential subjects choosing to enroll in competing phase 3 trials with optional LP. Failure to fully enroll combined with dropouts adversely affected our ability to detect significant differences between groups.

In conclusion, nilotinib is safe and well-tolerated in individuals with mild to moderate dementia due to AD. Nilotinib penetrates the blood–brain barrier to achieve

adequate levels to inhibit DDR1, but not Abelson tyrosine kinase. AD biomarkers, including Florbetaben PET, CSF Aβ, and phospho-tau-181 and hippocampal volume were altered by nilotinib treatment. A lower dose of 150 mg daily may be preferable in future studies to avoid behavioral adverse effects. These data will guide the design of a larger, longer, multicenter phase 3 study to evaluate the safety and efficacy of nilotinib in subjects with MCI or AD.

Author Contributions

R.S.T. and C.M. contributed to study concept and design and drafted the text. A.L., E.L.M., N.Y., J.N.S., M.A., Y.T.-Y., X.L., F.P., J.A., F.B., G.E., M.L.H., W.S., S.M., D.F., and X.J. contributed to data acquisition and analysis. J.A. and M.L.H. contributed to figure and table preparation.

Potential Conflicts of Interest

C.M. is an inventor on several US and international Georgetown University patents to use nilotinib and other tyrosine kinase inhibitors as a treatment for neurodegenerative diseases. C.M., his laboratory, and Georgetown University previously received some income from licensing of the technology to Axovant Science. Georgetown University spun out the technology (April 2020) to a start-up company (KeifeRX LLC), from which C.M. receives consulting fees and Georgetown University, C.M., and F.L.P. receive equities and Y.T.-Y. is an advisor. An individual and institutional conflict management plan (CMP) determines that C.M. can design a study as the (inventor) person who discovered the potential effects of nilotinib in neurodegenerative diseases (thus conceived of the study), can serve as a corresponding principal investigator (PI) and senior author but cannot serve as a clinical PI of any nilotinib clinical study. C.M. must not determine participants' eligibility and must not consent participants. C.M. does not perform primary clinical data analysis and all primary CSF biomarkers analyzed in C.M. laboratory and Georgetown University by blinded investigators must be validated by an external and independent organization using comparable technologies and methods (see Acknowledgments). All investigators must be blinded to treatments until all data are analyzed per group and unblinded by an externally independent DSMB (see Acknowledgments). An external independent study monitor follows study progress and adherence to the protocol. Study visits, scheduled events, and adherence to study protocol are verified by independent nurses and staff of the CRU (see Acknowledgments), which is a Translational Science Awards Program (CTSA). No other authors have any related conflicts of interest. C.M. discovered the potential use of nilotinib in preclinical models and conceived of this phase 2 clinical study and contributed with R.S.T. to the protocol and study design based on his understanding of nilotinib mechanisms of action in preclinical models, especially in relation to nilotinib effects on CNS biomarkers in AD, hence, he is a corresponding and senior author. In adherence with the CMP, C.M. did not provide any scientific or statistical analysis (J.A., R.S.T., and M.L.H.) of the data and the other authors who performed the experiments (R.S.T., G.E., F.B., and X.L.) have analyzed the data with other members (M.L.H., X.J., S.M., M.A., M.S., and S.M.) of the study team who managed the data (S.M., D.F., W.S., M.L.H., M.A., M.S.) and prepared the figures and performed statistical analysis (J.A. and M.L.H.). C.M. was not the clinical principal investigator (PI) who (R.S.T.) executed the protocol and monitored patients (N.J.S., A.L., N.Y., A.L.M., M.A., and Y.T.-Y.). All safety, biomarkers, and clinical data were unblinded by the DMSB and analyzed by S.M., D.F., M.A., M.L.H., W.S., and S.M., and figures and tables were prepared by M.L.H. and

J.A. by the study team. C.M. contributed to manuscript drafting and all authors contributed to the interpretation of the data and approved the final version of manuscript.

Data and Materials Availability

The final data, study protocol, and analyses will be available to the scientific and nonscientific community after publication and presentation. Investigators adhered to the Privacy Rule under the Health Insurance Portability and Accountability Act (HIPAA).

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