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# First-in-human study of neuron regenerative therapy NNI-362 to evaluate the safety, pharmacokinetics, and pharmacodynamics in healthy aged population

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## Abstract

**Background** A placebo-controlled, double-blind Phase 1a trial examined the safety, tolerability, and pharmacokinetics of NNI-362 as well as the pharmacodynamic outcome of plasma phosphorylated tau$^{181}$ (p-tau$^{181}$).

**Methods** Oral NNI-362 and placebo were randomized in healthy, cognitively-unimpaired individuals (ages 50–72) at a 3:1 ratio, with sponsor, principal investigator, and subjects all blinded. Plasma levels of p-tau$^{181}$ were determined in the placebo and the two highest arms of 120 and 240 mg NNI-362. Plasma biomarker was examined for statistical change from baseline.

**Results** NNI-362 treatment was safe and well tolerated in older individuals. NNI-362, at the two highest multiple doses, significantly reduced plasma p-tau$^{181}$ levels compared to pretreatment levels ($P < 0.0012$ to $P < 0.0009$), while no change occurred in placebo groups.

**Conclusions** These findings suggest in older subjects, oral NNI-362 appeared safe, well tolerated and reduced plasma p-tau$^{181}$. Phase 2 studies of NNI-362 are warranted for Alzheimer's disease and age-related degenerative disorders.

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**Keywords** Plasma p-tau$^{181}$, Neuron regeneration, Single ascending dose, Multiple ascending dose, Aging, Alzheimer's disease, Safety, Pharmacokinetics, Pharmacodynamics

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## Background

Alzheimer's disease (AD) is the most common form of dementia, with a prevalence of approximately 6.9 million Americans. Behavioral symptoms normally begin around the sixth decade of life, with aging described as a major risk factor; the prevalence of AD in people between ages 65–74 is 3%, while this rate rises to 32% for those older than 85 [1]. New disease-modifying, FDA-approved therapies address a single hallmark of the disorder, amyloid β (Aβ) plaques, demonstrating a slowing of early disease progression [2]. Another hallmark, tau hyperphosphorylation, is observed in the brain during continued disease progression. While this target has no approved disease-modifying therapies, steady progress has been made in identifying and validating plasma biomarkers to assess changing brain levels of phosphorylated-tau, with the earlier tested tau biomarker being plasma phosphorylated tau$^{181}$ (p-tau$^{181}$) and then a later biomarker p-tau$^{217}$ [3]. Plasma p-tau$^{181}$ can provide an indication of AD severity beginning with age-dependent increases and then directly correlating with progressive cognitive deficits [4, 5]. Both tau biomarkers show strong diagnostic capacity [6]. Phosphorylated tau, rather than total tau, appears associated with brain region–selective neuron degeneration. Therefore, plasma p-tau levels potentially have both diagnostic and prognostic applications [7]. The selective neuron degeneration appears causative of memory and executive functional deficits in AD. The multitude of associated causes of this chronic neuronal degeneration is still not fully elucidated. In aged AD subjects, there are measurable hippocampal volume changes from baseline, as determined by volumetric magnetic resonance imaging [8].

The long-standing assumption that "once neurons degenerate, the aged brain is incapable of reversing course to replace neurons" may not be entirely correct due to experimental therapeutics that could induce selective neuron regeneration. Early neurogenic agents [9] and even exercise [10] appear to promote neuronal progenitor proliferation but may still lack the ability to fully replace mature neurons lost and protect nascent neurons from degeneration. Experimental agents, such as NNI-362, that have preclinically shown the ability to be proliferative as well as to promote maturation and neuroprotection are designed to halt or reverse chronic age-related neurodegenerative disorders. NNI-362 is a novel small molecule shown to stimulate neuron generation and behavioral reversals in both aging and neurodegenerative rodent models [11]. Through a unique mechanism of action, NNI-362 allosterically modulates a kinase central hub, S6 kinase (or p70S6 kinase) [11], to turn on translation and neuroprotection in the central nervous system [12]. Determination of safety and ability to affect brain biomarkers of disease progression in aged individuals would indicate the potential capacity to safely regenerate neurons in aged AD patients in mid- and late-stage trials.

The objective of this Phase 1a first-in-human trial was to determine the safety, tolerability, and pharmacokinetics (PK) and secondarily measure change from baseline p-tau$^{181}$ by oral therapy NNI-362 in a healthy aged population. When safety, tolerance, and pharmacodynamic signal is shown in Phase 1a, the aim is to then assess NNI-362's ability to statistically demonstrate Phase 2 proof of concept in patients with age-related disorders, specifically mild to moderate AD.

## Methods

### Overall clinical design and amendments

This was a Phase 1a, single-center, double-blinded, ascending single and multiple dose study to assess the safety, tolerability, and PK of oral NNI-362 in healthy aged subjects. The study was conducted from July 2019 through November 2021, with a final study report submitted to FDA in February 2024. The study consisted of seven cohorts (A–G) of eight subjects each (six active and two placebo). Cohorts A–D were single ascending dose (SAD), with Cohort D being in a fed state and all others being in a fasted state, and Cohorts E–G were 10-day multiple ascending dose (MAD) of NNI-362 in 1% w/v methylcellulose in sterile water oral suspension. Cohorts A–D each included two Japanese subjects to compare Japanese to Caucasian/non-Japanese individuals in the NNI-362 active subjects. The study protocol was approved by the Institutional Review Board and was designed, executed, and reported according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines for Good Clinical Practice and the ethical principles outlined in the Declaration of Helsinki.

At the conclusion of each cohort dosing, the monitoring committee (i.e., the principal clinical investigator, the clinical monitor, and the sponsor) reviewed the collected data, which included PK, adverse events (AEs), changes in the clinical laboratory test assessments (hematology, coagulation, serum chemistry, and urinalysis), vital sign measurements, 12-lead electrocardiogram (ECG) results, physical examination findings, and number of subjects with suicidality changes from baseline. Based on the results of this review, the committee signed off, and the next cohort (see Table 1 for dose-limiting criteria) proceeded to dosing.

This Phase 1a trial of new chemical entity NNI-362 in liquid formulation utilized a healthy aged population for the first-in-human trial to establish safety and PK. In Cohorts A–D, a Japanese subgroup was used for the purpose of determining potential PK differences (Japanese to all others, 1:3 ratio). The control (placebo) group

**Table 1** Dose-limiting decision table

| Number of Subjects with DLT at a Given Dose Level | Escalation Decision Rule |
|---|---|
| ≤ 1 out of 8 | Enter two subjects at the next dose level (1 placebo: 1 NNI-362). |
| 0 of 2 | Enter six more subjects at this dose level.<br>If ≤ 1 of these six subjects experience DLT, proceed to the next dose level.<br>Enter two subjects at the next dose level. |
| ≥ 2 of 8 | Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered) following de-masking and will be identified as intervention related. |
| ≤ 1 out of 8 | Enter eight subjects for MAD, at next lowest dose. |

Abbreviations: DLT, dose-limiting toxicity; MAD, multiple ascending dose

contained the liquid formulation in the absence of therapeutic. The controls were at a 1:3 ratio to drug groups, as was consistent with other safety trials. For Cohorts A–D, two subjects were dosed prior to the remaining subjects as a sentinel safety measure.

Two amendments changed the original dosing scheme for Cohorts D–G (Protocol Amendments 2 and 3). The planned initial study design was for Cohort D subjects to also receive 60 mg, but in a fed state to evaluate the effect of food on bioavailability (Protocol Amendment 2). However, it was determined that the planned dose of 60 mg for Cohort D was too low to evaluate the effect of food on NNI-362 bioavailability after oral administration, and the dose for Cohort D was changed to a single dose of 120 mg. Following completion of Cohort E showing lack of absorption, Cohorts F and G were changed to SAD plus 7-day MAD using medium-chain triglycerides (MCTs), Kolliphor EL, and beeswax (95/3/2% w/w) lipid-based oral suspension (Protocol Amendment 3). Cohorts F and G consisted of a single dose followed by a minimum waiting period of 5 days and then 7 days of daily dosing.

A schematic of the amended study design is included in Fig. 1 below.

The starting dose selected was based on nonclinical studies. Following single-dose administration of 10, 20, or 60 mg NNI-362, there were no safety or tolerability concerns at any of these dose levels. Following single doses of 10 and 20 mg, little to no NNI-362 was detectable in the plasma of any subject (lower limit of quantitation [LLOQ] = 25.0 pg/mL). Following a single dose of 60 mg, three of six subjects had detectable plasma levels of NNI-362, ranging from 25.2 – 69.0 pg/mL. The average observed maximum concentration ($C_{max}$) for the three subjects was 49.8 pg/mL with a time of observed maximum concentration ($T_{max}$) of 6 h for two of the subjects and 24 h for one subject. Due to inconsistency in PK of NNI-362 administered in the aqueous liquid formulation in Cohorts A–E, Cohorts F and G were revised from MAD to SAD/MAD cohorts to evaluate the lipid liquid formulation (Protocol Amendment 3). Subjects were enrolled under Protocol Amendment 1.

### Selection of study population

The population of healthy aged participants being of 50–72 years of age was chosen according to the National Institute on Aging statement that an aged person may

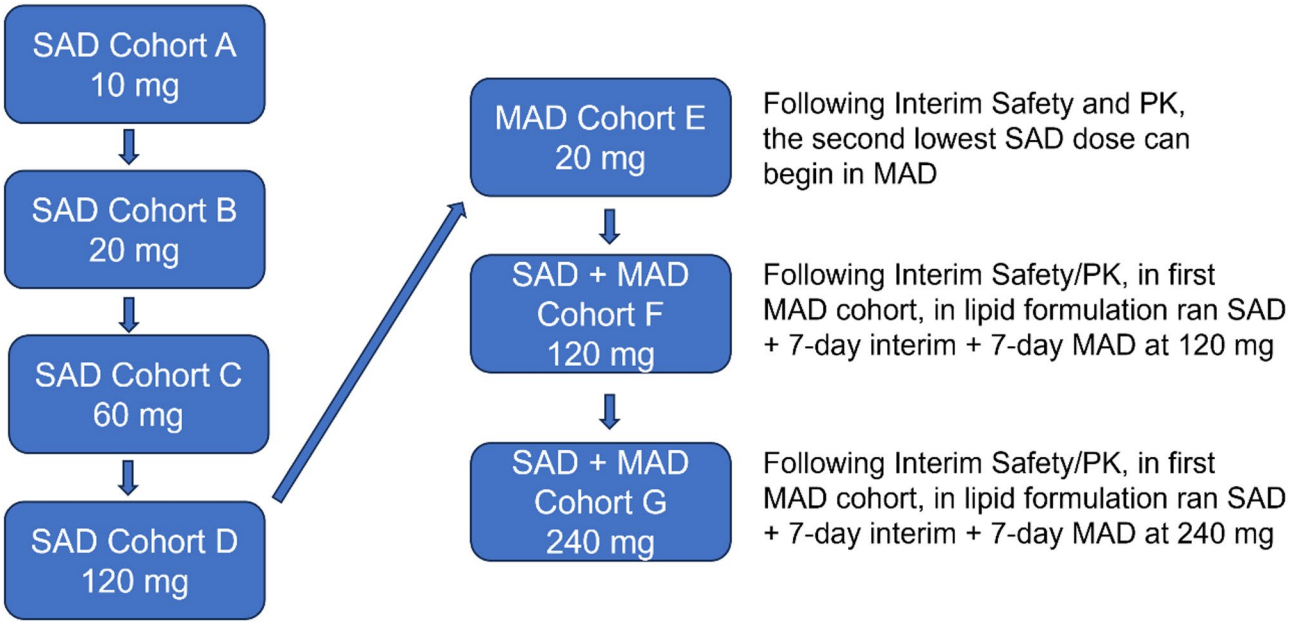


**Fig. 1** Study design. A schematic of the SAD and MAD dose-escalation study design. This first-in-human study of NNI-362 uses placebo control to assess the safety and tolerability in both SAD and MAD studies. MAD, multiple ascending dose; PK, pharmacokinetics; SAD, single ascending dose

start at 50 years of age. The 72-year-old cutoff aimed to ensure the ability to find healthy individuals according to the inclusion and exclusion criteria listed in Sect. 2.2.1. The study also included an ethno-bridging component to compare Japanese to Caucasian/non-Japanese individuals for Cohorts A–D. A single site was chosen for this Phase 1a trial, the California Clinical Trials Medical Group in Glendale, CA, which provided for a diverse population in terms of demographics.

#### *Inclusion criteria*

- Subjects previously dosed with NNI-362 from SAD Cohorts A–D may participate in Cohorts F or G following rescreening.
- Healthy aged volunteers of either sex between the ages of 50–72, inclusive. Subjects must be in reasonably good health as determined by the investigator based on medical history, vital sign measurements, physical examination, screening laboratory results, and ECG. Normal age-related findings as well as well-controlled, chronic, and stable medical conditions (e.g., hypertension, osteoarthritis, non-insulin-dependent diabetes mellitus, osteoporosis, gout, Paget's disease, and hypothyroidism) will not be exclusionary if they are not expected to compromise subject safety, study conduct, or study objectives.
- Noninteracting medications for stable, allowable medical conditions are permitted following review and approval by the medical monitor.
- Adequate understanding of the requirements of the study, provision of written informed consent, and agreement to abide by the study restrictions.
- Negative urine screen for drugs of abuse within 24 h before the administration of the first dose of study drug and, in the multiple-dose study, upon readmission to the clinical unit from outpatient status.
- Body mass index of between 18 and 30 $kg/m^2$, inclusive, and a total body weight greater than 48 kg at screening.

#### *Exclusion criteria*

- Alcohol consumption > 10 units per week or > 2 units per day. One unit will be defined as the amount of alcohol in a 12-ounce glass of beer, a 5-ounce glass of wine, or a 1.5-ounce serving of 80-proof spirits.
- Any psychiatric condition that could jeopardize the subject's safety or the subject's ability to comply with the protocol.
- Participation in any interventional clinical trial within the previous 90 days or 5 half-lives in which the subject received an investigational drug.
- Difficulty swallowing.
- Recipient of an organ transplant (solid or hematopoietic).
- Febrile illness or significant infection within 96 h before administration of the study drug.
- Hepatitis B surface antigen–positive or serologic evidence of infection with hepatitis C virus or human immunodeficiency virus, with the exception of hepatitis B surface antibody.
- History of allergy to any component of the investigational drug formulation or placebo.
- Lifetime history of suicide attempts or suicidal ideation in the last 2 years prior to screen, as determined by the Columbia-Suicide Severity Rating Scale (C-SSRS).
- Women of childbearing potential, defined as premenopausal (unless the potential research subject has previously undergone hysterectomy and/or bilateral salpingo-oophorectomy).
- Pregnant or breastfeeding.
- Any clinically significant hematology, chemistry, coagulation, or urinalysis value at screening and Day − 1. Abnormal liver enzymes (alanine aminotransferase and/or aspartate aminotransferase > 1.5 × upper limit of normal) applies at screening and Day − 1.
- Serum creatinine > upper limit of normal applies at screening and Day − 1.
- Hemoglobin < 13 g/dL for males or < 11.5 g/dL for females, leukocytes < $3.0 \times 10^3/\mu L$, absolute neutrophil count < 1000/μL, or platelets < $150 \times 10^3/\mu L$ applies at screening and Day − 1.
- Current smoker.
- Glomerular filtration rate < 50 mL/min based on Cockcroft-Gault calculation using ideal body weight at screening.
- Active substance abuse.
- Any significant medical illness that could compromise the interpretability of study data or affect subject safety, including but not necessarily limited to:
- Chronic pulmonary disease or sleep apnea.
- Clinically significant cardiac arrhythmia (either at screening or based on history).
- Congestive heart failure, valvular heart disease, or ischemic heart disease.
- Pulmonary hypertension.
- Any disorder of the kidney or urinary tract.
- Active peptic ulcer disease, gastrointestinal bleeding, inflammatory bowel disease, or chronic pancreatitis.
- Liver disease (excluding Gilbert's syndrome).

- Any neurologic disorder other than chronic Bell's palsy.
- History of malignancy that has not been cured or in complete remission for at least 10 years (excluding resected nonmetastatic basal cell carcinoma).
- History of seizure activity other than early childhood.
- Any traumatic brain injury in adulthood.

#### *Removal of subjects from therapy or assessment*

Subjects were free to withdraw from the study at any time and for any reason. The predetermined reasons for removal of subjects from the study as well as the planned follow-up are provided in the protocol.

A subject may be terminated from the study themselves or at the discretion of the study nurse and/or principal clinical investigator for the following reasons:

- AE.
- Violation of the protocol.
- An exclusion criteria occurs or was not immediately identified during the pre-screen.
- Subject withdraws consent.
- Subject does not complete full study.

### Treatment

Subjects received NNI-362 or placebo, as specified in Table 2.

Subjects fasted 8 h prior to NNI-362/placebo administration. While in clinic, the subjects were fed three prepared meals available at regular times. The daily caloric intake was approximately as follows:

*Kcal 2800.*
*Protein % 16%.*
*Carbohydrate % 55%.*
*Fat % 29%.*
*Saturated fat % <12%.*
*Salt < 6 g/day.*

The prepared meals did not contain grapefruit, pomelos, or Seville oranges or foods that interfere with microsomal metabolism and must not be taken from pre-study (14 days minimum) to end of study. Subjects did not smoke or use tobacco/nicotine patches or devices. All subjects agreed to this ban on tobacco/nicotine items and grapefruit.

After the full volume was dosed, the syringe was rinsed a minimum of two times, and the subjects drank the rinsing water. To rinse the syringe, the nurse drew up approximately 4 mL of drinking water and added an additional 1 mL of air space by pulling the plunger down. The syringe was shaken for approximately 15 s, and the rinsing water was administered to the subjects. If there was still residue remaining in the syringe after two rinses, it was rinsed until the full dose was administered.

**Table 2** Treatments

| Cohort | Dose | Formulation |
|---|---|---|
| A (single dose) | 10 mg NNI-362 (1 mL); placebo | 1% (w/v) methylcellulose in sterile water |
| B (single dose) | 20 mg NNI-362 (2 mL); placebo | |
| C (single dose) | 60 mg NNI-362 (6 mL); placebo | |
| D (single dose) | 120 mg NNI-362 (12 mL); placebo | |
| E (multiple dose) | 20 mg NNI-362 (2 mL); placebo | |
| F (single dose; multiple dose) | 120 mg NNI-362 (1 mL); placebo | Medium-chain triglycerides, Kolliphor EL, and beeswax (95/3/2% w/w) |
| G (single dose; multiple dose) | 240 mg NNI-362 (2 mL); placebo | |

### Randomization

After subjects provided written informed consent and were screened, a unique subject identification number was assigned and recorded. A subject was considered enrolled in the study if all of the inclusion criteria and none of the exclusion criteria were met. A subject was considered randomized once the subject had been assigned by the unblinded study personnel to receive either placebo or NNI-362 and given a randomization number. Within each cohort, subjects were randomly assigned to NNI-362 and placebo in a 3:1 ratio.

A randomization program provided by Clinical Trial Data Services (CTDS) was utilized whereby only the pharmacy manager was unblinded in order to provide placebo or intervention to the randomized subjects within each cohort. Allocation of NNI-362 or placebo took place within each cohort separately. CTDS generated the randomization list using the Statistical Analysis System (SAS). At least one set of corresponding replacement randomization numbers was provided. Each cohort consisted of eight subjects: A sentinel dose design was followed, with a dose administered to the first two subjects (1 placebo/1 NNI-362, both Caucasian/non-Japanese), and the final 6 subjects consisted of 4 non-Japanese subjects (randomized to 1 placebo and 3 NNI-362) and two Japanese subjects (active; ethno-bridging) in the SAD study (Cohorts A–D). Placebo subjects were always non-Japanese, while all Japanese subjects received NNI-362.

### Selection of doses in the study

In selecting the human starting dose for the NNI-362 first-in-human trial, it is appropriate to consider the results of both pivotal repeated-dose toxicity studies as well as the human equivalent dose and the calculated plasma protein binding (PB) factor. The no observed adverse effect level in rats in the 14-day study was 150 mg/kg/day. Using this information, the human starting dose is estimated as follows:

150 mg/kg/day × 7 ($K_m$ for 250 g rat) = 1050 mg/m$^2$.

1050 mg/m$^2$ ÷ 10 (safety factor) ÷ 19.8 (PB factor) = 5.3 mg/m$^2$.

Human starting dose (mg/kg basis):

5.3 mg/m$^2$ ÷ 37 kg/m$^2$ = 0.143 mg/kg × 70 kg human = 10 mg.

The rat is the study's most sensitive species. Since the no observed adverse effect level is 150 mg/kg oral in rats and 210 mg/kg (bid) oral in dogs and bioavailability is not greater in larger animals, the higher dose of ~2 mg/kg (120 mg) and ~4 mg/kg (240 mg) is required to reach the human equivalent dose to reach targeted efficacy. This first-in-human study was not aimed at reaching a maximum tolerated dose.

Due to inconsistency in PK of NNI-362 administered in the aqueous liquid formulation in Cohorts A–E, a liquid lipid formulation was utilized for Cohorts F and G.

### Blinding

This Phase 1a was blinded to everyone (subjects, sponsor, principal investigator, and study staff) except the Parexel pharmacist and a nurse, who delivered the investigational products and then returned the spent oral syringes to the pharmacy. Unblinding occurred only following completion of monitoring, database lock, and sign-off by the principal investigator.

### Endpoints and statistics

#### *Safety assessment*

Safety and tolerance as the primary outcome measures were determined using clinical assessments and patient responses. Safety and tolerability endpoints included monitoring and recording of AEs, changes in the clinical laboratory test assessments (hematology, coagulation, serum chemistry, and urinalysis), vital sign measurements, 12-lead ECG results, physical examination findings, and number of subjects with suicidality changes from baseline.

Vital signs included the assessment of body temperature, blood pressure, pulse rate, and respiratory rate when in a supine, relaxed position for 10 min. Orthostatic vitals always followed after supine vital signs and after subject was standing for at least 2 min. Oxygen saturation was assessed through pulse oximetry. All information was reported in the subject's case report form. A repeat assessment was allowed at the clinical principal investigator's discretion.

Physical examination was performed by a physician or designee to examine general appearance, eyes, ears, nose, throat, head, chest/respiratory, heart/cardiovascular, gastrointestinal, liver, musculoskeletal, skin, thyroid/neck, and lymph nodes, as well as provide a neurological/psychiatric assessment. Assessment of suicidality was performed by a trained study team member administering the C-SSRS protocol. Body weight and height were obtained by the study team.

#### *Pharmacokinetic assessments*

Blood and urine samples for analysis of NNI-362 levels were collected from all subjects at the time points specified in the Schedule of Activities. Subjects were dosed in the fasted state (except Cohort D) and confined for intensive PK sample procurement (blood was drawn pre-dose as well as at 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h post-dose) and study monitoring. Urine was collected post-dose at 60 min, >60 min to 4 h, >4 h to 9 h, and >9 h to 24 h. Urine samples were not analyzed for drug levels due to funding limitations.

A validated liquid chromatography-tandem mass spectrometry assay was used for determination of NNI-362 in human plasma. The analytical range was 25.0–25,000 pg/mL, with 0.05 mL sample volumes. Blood samples were prepared in batches by centrifugation by trained personnel and occurred within 30 min of blood collection. Centrifugation was at 1500 g (~2500 rpm) for 10 min at set point 4 °C. When centrifugation was complete, approximately 0.500 mL plasma were aliquoted into prelabeled cryovials using an appropriate pipette. Duplicate samples were obtained, and tubes were labeled as Aliquot 1 and Aliquot 2. Samples were frozen at −80 °C in a sample freezer until shipment to the analytical laboratory. A minimum of 1.50 mL of blood was collected from each patient per time point, with 10 time points per patient (including pre-dose). The $K_3$ EDTA tubes were inverted 2–3 times. The time of blood draw, fasting status upon blood collection, and all relevant data were recorded as per study operating procedure. Blood in $K_3$ EDTA vacutainers can be stored for up to 30 min on ice prior to preparation of plasma. Cold blood samples were transferred to the plasma preparation room in closed biological containers as specified for human biological samples.

For a calibration curve to be considered acceptable, at least 75% of calibration standards must be accurate to within ±15% of the nominal concentration (±20% at the LLOQ). For accuracy and precision experiments, individual QC samples at low, low/mid, mid, and high concentrations were considered acceptable if they were accurate to within ±15% of the nominal concentration (±20% at the LLOQ). The intra- and inter-batch accuracy and precision were acceptable if they were ≤15% (±20% at the LLOQ). Within a run, at least 50% of the QC samples at each concentration must have satisfied the acceptance criterion, with at least 67% of the total QC samples in a run satisfying the acceptance criterion.

PK data analysis was performed on the measurable plasma concentrations of NNI-362. The plasma concentration data were evaluated using Phoenix WinNonlin™ software (version 8.3) to perform noncompartmental

analysis using the linear trapezoidal method. Nominal blood collection times were used in data analysis. The dose administered in the dose group was entered into the program as mg/subject. The following PK parameters were determined for NNI-362 plasma concentrations for each subject: observed maximal plasma concentration ($C_{max}$), observed time to reach $C_{max}$ ($T_{max}$), area under the plasma concentration time curve up to the last blood collection time ($AUC_{last}$) and extrapolated to infinity ($AUC_{inf}$), the percent of $AUC_{inf}$ that was extrapolated (%Extrap $AUC_{inf}$), terminal elimination half-life ($t_{1/2}$), and mean residence time to infinity ($MRT_{inf}$). Terminal $t_{1/2}$, $AUC_{inf}$, and $MRT_{inf}$ could not be calculated in some subjects due to limited data or poor fit ($r < 0.8$) of the terminal phase of the plasma concentration and time curve. Extrapolation of more than 20% of the AUC may result in unreliable values for the terminal parameters.

PK profile ($C_{max}$, $T_{max}$, $AUC_{last}$, $AUC_{inf}$, $t_{1/2}$, and $MRT_{inf}$) following single and multiple dosing was evaluated. Significant differences in selected PK parameters between the SAD and MAD groups of Cohorts F and G were evaluated using the t-test (two-tailed, paired test; $P < 0.05$).

PK analysis dataset was defined by the subject not being included when there was lack of linearity of the terminal phase ($r < 0.8$) or fewer than three data points.

#### *Pharmacodynamic assessment*

Plasma was collected at baseline (pre-dose), Day 15 (12 h after 7-day multiple dosing), and Day 16 (24 h after 7-day multiple dosing) for multiple dose administration for measurement of p-tau$^{181}$ concentrations.

The duplicate frozen plasma sample from the unused PK samples (described in Sect. 2.7.2) were analyzed (blinded to treatment) at Mayo Clinical Laboratories (Rochester, MN) from the 120 and 240 mg SAD/MAD groups (Cohorts F and G). Levels of plasma p-tau$^{181}$ were determined using a Simoa™ HD Analyzer (Lot Number 503008) with an Advantage V2 kit. Calibration curves of p-tau$^{181}$ ranged between 0.0 and 74.6 pg/mL. Plasma biomarkers were examined at pre-dose (baseline), Day 15 (12 h post final dose), and Day 16 (24 h post final dose). Statistical analyses examined a change from the pre-dose level of p-tau$^{181}$. Data was received by NeuroNascent, the blind was broken, and all data was provided for analyses to a clinical statistician, Dr. Chengjie Xiong (Lily Inc. and Washington University in St. Louis, St. Louis, MO), for significance of change from baseline at 120 or 240 mg NNI-362 and significance from placebo change from baseline.

Changes from baseline (i.e., pre-dose) in plasma p-tau$^{181}$ were further analyzed by mixed models for repeated measures, including dose (the between-subject factor), post-baseline time (the within-subject factor), and their interaction as the fixed effects, in addition to the baseline level of ptau$^{181}$. An unstructured covariance matrix was assumed on the repeated measures. These analyses were implemented by SAS PROC MIXED.

**Table 3** Subject disposition and analysis populations

| Status | Number of Placebo Subjects | Number of NNI-362 Subjects | All Subjects |
|---|---|---|---|
| Randomized and Dosed | 14 (100%) | 42 (100%) | 56 |
| Completed Study | 14 | 42 | 56 |
| Prematurely Withdrawn | 0 | 0 | 0 |
| Safety Analysis Population | 14 | 42 | 56 |
| Pharmacokinetic Analysis Population | 14 | 41* | 55 |

*Subject 5008 from Cohort E was not included in the PK analysis, as a plasma sample was not received by SRI International for this subject. This subject was randomized to 20 mg NNI-362 for 10 days. Only subjects from Cohorts F and G were included in the ptau$^{181}$ analysis

## Results

### Disposition of subjects

Subject disposition and analysis populations are summarized in Table 3; Fig. 2. Of the 150 subjects consented and screened, 56 were randomized. The other 94 were not randomized due to not meeting inclusion or exclusion criteria or for other reasons as detailed. There were no discontinuations during the study. The reasons for all screening failures are listed in Fig. 2.

### Demographics and other baseline characteristics

Subject demographics are summarized in Table 4.

### Pharmacokinetic results

Plasma samples using $K_3$ ethylenediaminetetraacetic acid as the anticoagulant were analyzed for NNI-362 using a validated liquid chromatography-tandem mass spectrometry method, and all sample results were acceptable. Subjects receiving placebo or a single dose of 10 mg NNI-362 did not have measurable plasma concentrations of the test article; two subjects receiving a single dose of 20 mg NNI-362 had one plasma sample each that had drug concentrations over the LLOQ (25 pg/mL). A total of 22 samples had detectable levels of NNI-362 in the remainder of 60 and 120 mg SAD or 20 mg MAD cohorts receiving NNI-362 in the liquid aqueous suspension. The lack of quantifiable concentrations in Cohorts A–D preclude any comparison of Japanese subject PK to the rest of the population.

Due to the low exposure to NNI-362 observed in Cohorts A–E, a lipid-based formulation for oral administration of NNI-362 was evaluated for Cohorts F and G only. Table 5 shows the PK parameters for Cohorts F and G, SAD and MAD phases. All subjects except those that received the placebo had at least one sample with plasma

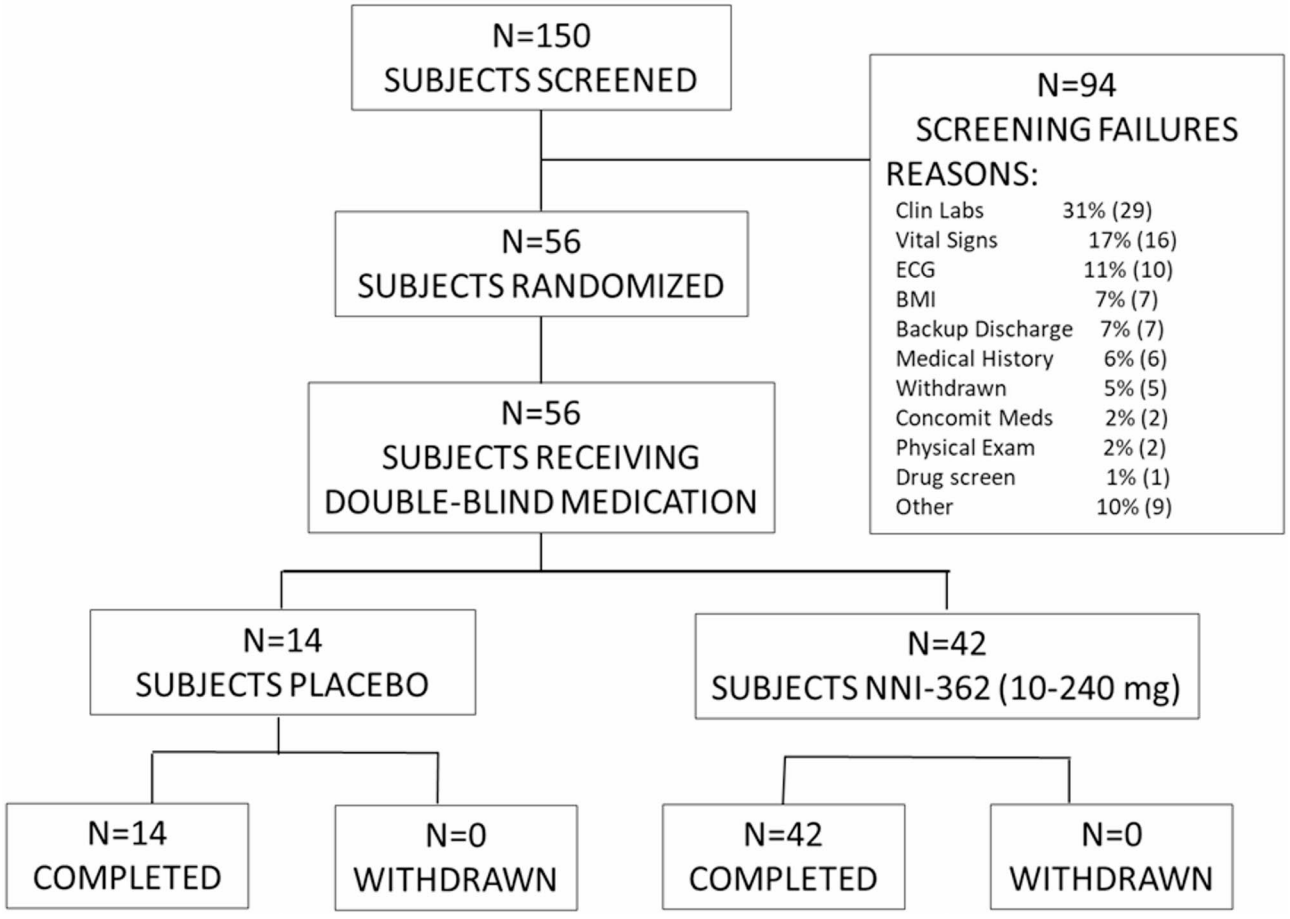


**Fig. 2** Subject disposition. All 56 subjects that were randomized completed the study. All the randomized subjects received study treatment and completed the study. There were no discontinuations during the study. BMI, body mass index; ECG, electrocardiogram

**Table 4** Subject demographics

| Cohort (Dose Group) | Gender | | Ave. Age (years) | Ethnicity | | | Race | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Female | Male | | Hispanic or Latino | Not Hispanic or Latino | Unknown | Asian | Black or African American | White | Other |
| **A (SAD 10 mg)** | 6 | 2 | 61.9 | 1 | 6 | 1 | 2 | 1 | 5 | 0 |
| **B (SAD 20 mg)** | 6 | 2 | 61.2 | 3 | 5 | 0 | 2 | 2 | 4 | 0 |
| **C (SAD 60 mg)** | 4 | 4 | 56.5 | 1 | 7 | 0 | 2 | 1 | 5 | 0 |
| **D (SAD 120 mg)** | 4 | 4 | 56.4 | 1 | 7 | 0 | 3 | 1 | 3 | 1 |
| **E (MAD 20 mg)** | 4 | 4 | 61.0 | 1 | 7 | 0 | 1 | 1 | 6 | 0 |
| **F (SAD + MAD 120 mg)** | 5 | 3 | 60.5 | 1 | 7 | 0 | 2 | 1 | 4 | 1 |
| **G (SAD + MAD 240 mg)** | 4 | 4 | 59.9 | 1 | 7 | 0 | 0 | 1 | 7 | 0 |
| **Total** | 33 | 23 | — | 9 | 46 | 1 | 12 | 8 | 34 | 2 |

concentrations that were greater than the LLOQ. Figure 3A shows that the terminal concentration was 12 h for SAD and 24 h for MAD (120 mg dose). Exposure based on AUC values were higher after multiple doses (MAD) than after a single dose (Fig. 3B). The $T_{max}$ values in Cohorts F and G ranged from 0.5 to 8 h and exhibited similar variability for all four treatment regiments, as shown in Table 5, and although the mean $T_{max}$ values for the MAD treatments were slightly higher than the mean values for the SAD treatments, the difference was not statistically significant. The mean $C_{max}$ values (i.e., the systemic accumulation) were greater with repeated administration across both dose groups (Fig. 3). Systemic exposure (the $AUC_{inf}$ values) indicates that exposure to NNI-362 increases with repeat-dose administration. Additionally, some of the PK variable calculations were based on a very limited number of values (e.g., $t_{1/2}$ of the 120 mg SAD group was only observed in three subjects).

**Table 5** Additional per-subject Pharmacokinetic parameters of NNI-362: cohorts F and G

| Cohort | Subject ID | SRI ID | Number of Samples [a] | Dose (mg) | Phase | $t_{1/2}$ (hr) | $T_{max}$ (hr) | $C_{max}$ (pg/ml) | $AUC_{last}$ (hr•pg/ml) | $AUC_{inf}$ (hr•pg/ml) | Extrap $AUC_{inf}$ (%) | $MRT_{inf}$ (hr) | $C_{min}$ (pg/ml) | $C_{tau}$ (pg/ml) | Accumulation Index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F | 6001 | SRI-F-005 | 6 | 120 | SAD | 4.45 | 1 | 83.4 | 289 | 458 | 36.9 | 7.35 | NA | NA | NA |
| F | 6002 | SRI-F-007 | 6 | 120 | SAD | 6.15 | 4 | 70.1 | 561 | 800 | 29.9 | 10.2 | NA | NA | NA |
| F | 6003 | SRI-F-006 | 5 | 120 | SAD | 8.13 | 0.5 | 43.5 | 182 | 511 | 64.5 | 12.4 | NA | NA | NA |
| F | 6004 | SRI-F-008[b] | 0 | 120 | SAD | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| F | 6005 | SRI-F-004[b] | 0 | 120 | SAD | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| F | 6006 | SRI-F-002 | 4 | 120 | SAD | NC[c] | 6 | 101 | 555 | NC | NC | NC | NA | NA | NA |
| F | 6007 | SRI-F-003 | 7 | 120 | SAD | NC | 8 | 208 | 1210 | NC | NC | NC | NA | NA | NA |
| F | 6008 | SRI-F-001 | 1 | 120 | SAD | NC | 2 | 48.4 | NC | NC | NC | NC | NA | NA | NA |
| | | | | | *Mean* | 6.24 | 3.58 | 92.4 | 559 | 590 | 43.8 | 9.99 | NA | NA | NA |
| | | | | | *SD* | 1.84 | 2.97 | 60.6 | 400 | 184 | 18.3 | 2.55 | | | |
| F | 6001 | SRI-F-005 | 7 | 120 | MAD | 2.46 | 6 | 193 | 1150 | 1280 | 9.78 | 6.68 | 35.2 | 1.18 | 1.00 |
| F | 6002 | SRI-F-007 | 9 | 120 | MAD | 12.2 | 2 | 144 | 2680 | 3740 | 28.4 | 19.3 | 60.4 | 60.4 | 1.34 |
| F | 6003 | SRI-F-006 | 9 | 120 | MAD | 29.5 | 2 | 208 | 3800 | 8950 | 57.5 | 42.9 | 96.8 | 121 | 1.31 |
| F | 6004 | SRI-F-008[b] | 0 | 120 | MAD | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| F | 6005 | SRI-F-004[b] | 0 | 120 | MAD | NA | NA | NA | NA | NA | NA | NA | NA | NA | 2.32 |
| F | 6006 | SRI-F-002 | 7 | 120 | MAD | 5.24 | 2 | 63.8 | 525 | 719 | 26.9 | 9.7 | 25.6 | 5.54 | 1.04 |
| F | 6007 | SRI-F-003 | 9 | 120 | MAD | 11.6 | 8 | 126 | 1770 | 2440 | 27.7 | 17.6 | 40.5 | 40.5 | 1.31 |
| F | 6008 | SRI-F-001 | 3 | 120 | MAD | NC | 6 | 40.4 | 179 | NC | NC | NC | 32.5 | NC | NC |
| | | | | | *Mean* | 12.2 | 4.33 | 129 | 1684 | 3426 | 30.0 | 19.2 | 48.5 | 45.7 | 1.39 |
| | | | | | *SD* | 10.5 | 2.66 | 67.3 | 1369 | 3298 | 17.2 | 14.3 | 26.4 | 48.8 | 0.48 |
| G | 7001 | SRI-G-003[c] | 1 | 240 | SAD | NC | 2 | 52.3 | NC | NC | NC | NC | NA | NA | NA |
| G | 7002 | SRI-G-007[b] | 0 | 240 | SAD | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| G | 7003 | SRI-G-002 | 3 | 240 | SAD | NC | 2 | 31.6 | 103 | NC | NC | NC | NA | NA | NA |
| G | 7004 | SRI-G-005 | 4 | 240 | SAD | 2.8 | 6 | 130 | 677 | 790 | 14.4 | 7.76 | NA | NA | NA |
| G | 7005 | SRI-G-008[b] | 0 | 240 | SAD | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| G | 7006 | SRI-G-001 | 4 | 240 | SAD | 2.6 | 1 | 85.7 | 243 | NC | NC | NC | NA | NA | NA |
| G | 7007 | SRI-G-006 | 7 | 240 | SAD | NC | 2 | 84.6 | 1020 | 2090 | 51.1 | 36.9 | NA | NA | NA |
| G | 7008 | SRI-G-004 | 5 | 240 | SAD | 2.30 | 4 | 210 | 880 | 1090 | 19.3 | 5.56 | NA | NA | NA |
| | | | | | *Mean* | 2.58 | 2.83 | 99.0 | 585 | 1323 | 28.3 | 16.7 | NA | NA | NA |
| | | | | | *SD* | 0.25 | 1.83 | 63.9 | 398 | 681 | 19.9 | 17.5 | | | |
| G | 7001 | SRI-G-003 | 9 | 240 | MAD | 26.2 | 2 | 124 | 1990 | 4520 | 54.8 | 39.5 | 62.7 | 66.9 | 2.13 |
| G | 7002 | SRI-G-007[b] | 0 | 240 | MAD | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| G | 7003 | SRI-G-002 | 5 | 240 | MAD | 4.29 | 2 | 59.0 | 235 | 426 | 43.9 | 7.10 | 28.7 | 1.62 | 1.02 |
| G | 7004 | SRI-G-005 | 9 | 240 | MAD | 5.10 | 6 | 679 | 4000 | 4230 | 5.10 | 9.51 | 31.3 | 31.3 | 1.04 |
| G | 7005 | SRI-G-008[b] | 0 | 240 | MAD | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| G | 7006 | SRI-G-001 | 9 | 240 | MAD | NC | 1 | 221 | 2610 | NC | NC | NC | 51.6 | 51.6 | 1.58 |
| G | 7007 | SRI-G-006 | 9 | 240 | MAD | 13.5 | 6 | 267 | 2850 | 3980 | 27.7 | 18.8 | 57.9 | 57.9 | 1.21 |
| G | 7008 | SRI-G-004 | 8 | 240 | MAD | 9.24 | 2 | 82.2 | 569 | 975 | 40.5 | 13.5 | 30.4 | 11.8 | 1.20 |
| | | | | | *Mean* | 11.7 | 3.17 | 239 | 2042 | 2826 | 34.4 | 17.7 | 43.8 | 36.9 | 1.36 |
| | | | | | *SD* | 8.94 | 2.23 | 230 | 1431 | 1959 | 19.0 | 13.0 | 15.4 | 26.3 | 0.43 |

[a] Number of plasma samples with NNI-362 with concentrations greater than the LLOQ (25 pg/ml)

Abbreviations: $AUC_{inf}$, area under the concentration-time curve to infinity; $AUC_{last}$, area under the plasma concentration curve up to the last blood collection time; $C_{max}$, maximum concentration; $C_{min}$, minimum concentration; $C_{tau}$, drug concentration at the end of a given dosing interval; Extrap $AUC_{inf}$, extrapolated $AUC_{inf}$; MAD, multiple ascending dose; $MRT_{inf}$, mean residence time to infinity; [b]NA, not applicable (subjects received placebo); [c]NC, not calculated (due to lack of linearity of terminal phase [$r < 0.8$] or fewer than three data points); SAD, single ascending dose; $t_{1/2}$, terminal elimination half-life; $T_{max}$, time of observed maximum concentration

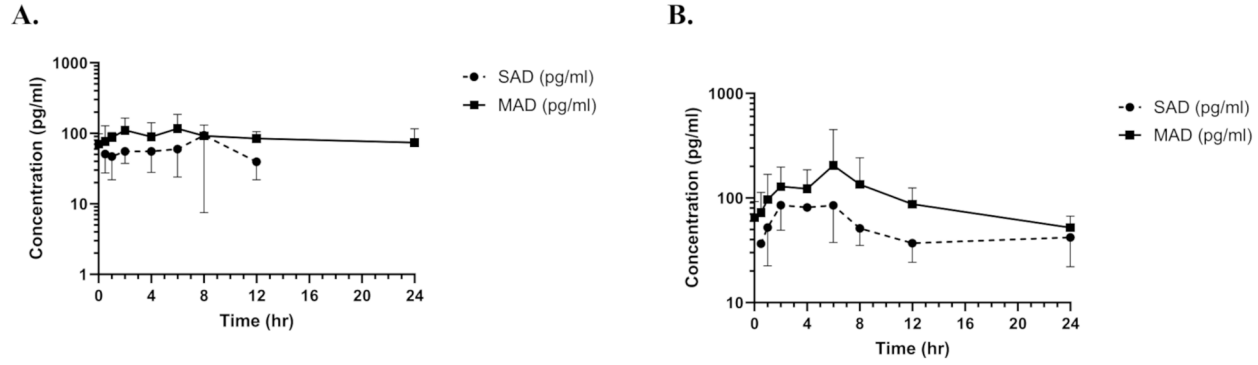


**Fig. 3** Comparison of SAD (dashed line) and MAD (solid line) plasma concentrations for NNI-362 (**A** = 120 mg, Cohort F; **B** = 240 mg, Cohort G). Plasma samples were collected through 24 h from the same 8 individuals for SAD and MAD in each cohort. The lower limit of quantitation was 25.0 pg/mL

As a result, the PK assessment for the SAD values should be regarded with caution. The $t_{1/2}$ values indicate that NNI-362 is not completely cleared within 24 h, resulting in accumulation of NNI-362 and higher AUC values after daily administration compared to a single dose of NNI-362.

Modification of the formulation for NNI-362 increased the plasma concentrations observed in Cohorts F and G, compared to Cohorts A–E. Cohorts D and F were both administered 120 mg NNI-362 but in the two different formulations. Variability among the NNI-362 subjects, even in Cohorts F and G, was substantial, but the majority of subjects had quantifiable plasma samples, unlike

with the original aqueous formulation. Mean $C_{max}$ and $AUC_{last}$ multiple dosing values increased in a less than dose-proportional manner from 120 to 240 mg. Variability was also seen with $C_{tau}$ values being lower than $C_{min}$ in a few subjects, particularly in the 120 mg cohort.

## Safety results

### *Adverse events*

Summary of all AEs and treatment-emergent AEs (TEAEs) are summarized by System Organ Class and Preferred Term in Table 6.

A total of 34 TEAEs (17 in placebo and 17 in NNI-362) were reported in seven subjects; 17.5% of treated subjects had at least one TEAE; six (three in placebo and three in NNI-362) of the TEAEs were reported in the SAD portion of the study. The majority of TEAEs in NNI-362 treatment groups were in Nervous System Disorders (NNI-362: four of 42 subjects, 9.5%; placebo: five of 14 subjects, 35.7%) and in the placebo were in Gastrointestinal Disorders (placebo: six of 14, 42.9%; NNI-362: three of 42, 7.1%). All TEAEs were assessed as mild, grade 1.

None of the 34 TEAEs were assessed as definitely related. Prior to unblinding, 24 TEAEs were assessed by the principal investigator as possibly related, of which 15 were in subjects administered placebo. The remaining 10

**Table 6** Cumulative AEs and TEAEs of subjects by system organ class

| System Organ Class (MedDRA)* Preferred Term | Study Drug Dose | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | SAD 10 mg (*n* = 6) | SAD 20 mg (*n* = 6) | SAD 60 mg (*n* = 6) | SAD 120 mg (*n* = 6) | MAD 20 mg (*n* = 6) | SAD/MAD 120 mg† (*n* = 6) | SAD/MAD 240 mg† (*n* = 6) | Placebo Total (*n* = 14) | NNI-362 Total (*n* = 42) |
| | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) |
| **Eye Disorders** | | | | | | | | | |
| Conjunctivitis | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **1 (7.1)** | 0 (0.0) |
| Ocular hyperaemia | 1 (16.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.4) |
| **Cardiac Disorders** | | | | | | | | | |
| Extrasystoles | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (16.7) | 0 (0.0) | 1 (2.4) |
| **Gastrointestinal Disorders** | | | | | | | | | |
| Gastrointestinal sounds abnormal | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **1 (16.7)** | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.4) |
| Constipation | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **1 (7.1)** | 0 (0.0) |
| Diarrhoea | 0 (0.0) | 1 (16.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.4) |
| Faeces discoloured | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **1 (16.7)** | 0 (0.0) | 1 (2.4) |
| Vomiting | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **3 (21.4)‡** | 0 (0.0) |
| Nausea | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **2 (14.3)** | 0 (0.0) |
| **General Disorders and Administration Site Conditions** | | | | | | | | | |
| Catheter site pain | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (16.7) | 0 (0.0) | 0 (0.0) | 1 (2.4) |
| Feeling of relaxation | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **1 (16.7)** | 0 (0.0) | 0 (0.0) | 1 (2.4) |
| Feeling abnormal | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **1 (16.7)** | 0 (0.0) | 1 (2.4) |
| **Injury, Poisoning, and Procedural Complications** | | | | | | | | | |
| Laceration | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (16.7) | 0 (0.0) | 0 (0.0) | 1 (2.4) |
| Thermal burn | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (16.7) | 0 (0.0) | 0 (0.0) | 1 (2.4) |
| **Musculoskeletal and Connective Tissue Disorders** | | | | | | | | | |
| Back pain | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (16.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.4) |
| **Nervous System Disorders** | | | | | | | | | |
| Dizziness | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **2 (14.2)** | 0 (0.0) |
| Headache | 0 (0.0) | **1 (16.7)** | 0 (0.0) | 0 (0.0) | **1 (16.7)** | **1 (16.7)** | 0 (0.0) | **3 (21.4)** | 3 (7.1) |
| Somnolence | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **1 (16.7)** | 0 (0.0) | 1 (2.4) |
| **Psychiatric Disorders** | | | | | | | | | |
| Abnormal dreams | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **1 (16.7)** | **1 (7.1)** | 1 (2.4) |
| Depressed mood | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **1 (7.1)** | 0 (0.0) |
| Insomnia | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | **1 (7.1)** | 0 (0.0) |
| **Respiratory, Thoracic, and Mediastinal Disorders** | | | | | | | | | |
| Paranasal sinus discomfort | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (16.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.4) |

Abbreviations: AE, adverse event; MAD, multiple ascending dose; SAD, single ascending dose; TEAE, treatment-emergent adverse event

*Values represent the number (and percent) of subjects who had at least one occurrence of the listed TEAE. Values in bold were assessed as possibly related to treatment by the principal investigator prior to unblinding

†SAD/MAD = single oral dose plus seven daily oral doses for MAD study with 7-day interim safety monitoring period between single and seven daily doses

‡Vomiting was assessed as possibly related to test article in two subjects receiving placebo and unrelated in one subject

**Table 7** Adjusted least squares estimates to the mean changes in plasma p-tau$^{181}$ levels at each dose group or as compared between dose groups from baseline (post-baseline minus baseline)

| Dose/Arms | Post-baseline Time | Mean Difference ± SE | *P* Value |
|---|---|---|---|
| 120 mg | 12 h post Day 7 dosing | −0.021 ± 0.185 | 0.91 |
| 120 mg | 24 h post Day 7 dosing | −0.330 ± 0.079 | 0.001 |
| 240 mg | 12 h post Day 7 dosing | −0.525 ± 0.187 | 0.02 |
| 240 mg | 24 h post Day 7 dosing | −0.360 ± 0.082 | < 0.001 |
| Placebo | 12 h post Day 7 dosing | −0.045 ± 0.232 | 0.85 |
| Placebo | 24 h post Day 7 dosing | 0.055 ± 0.109 | 0.63 |
| 120 mg vs. placebo | 12 h post Day 7 dosing | 0.024 ± 0.299 | 0.94 |
| 120 mg vs. placebo | 24 h post Day 7 dosing | −0.384 ± 0.138 | 0.02 |
| 240 mg vs. placebo | 12 h post Day 7 dosing | −0.480 ± 0.302 | 0.14 |
| 240 mg vs. placebo | 24 h post Day 7 dosing | −0.415 ± 0.146 | 0.01 |
| 120 mg vs. 240 mg | 12 h post Day 7 dosing | 0.504 ± 0.262 | 0.08 |
| 120 mg vs. 240 mg | 24 h post Day 7 dosing | 0.030 ± 0.112 | 0.79 |

Abbreviations: SE, standard error

Note: Six subjects per dose group received NNI-362

TEAEs were assessed as unlikely related or unrelated. All TEAEs were of a mild grade.

There were no deaths, serious AEs, or discontinuations recorded in any of the cohorts.

No apparent treatment-related trends were observed in clinical laboratory test results, ECG results, vital sign measurements, or physical examination findings.

Thus, NNI-362 was considered safe and well tolerated by all subjects up to a dose of 240 mg daily.

### Pharmacodynamic results

NNI-362, at the two highest multiple doses, 120 mg and 240 mg, showed a nominal significant reduction of p-tau$^{181}$ levels in study participants compared to pre-dose levels, while no change occurred in subjects receiving placebo using the Mayo Clinic's Simoa™ method.

The adjusted least squares estimates to the mean changes in plasma p-tau$^{181}$ levels from baseline are presented in Table 7. The table also contains the *P* value for testing whether the change is significantly different from zero. The placebo arm showed no significant change from baseline in plasma p-tau$^{181}$. However, both active arms showed significant decline from baseline at Day 16, and the high-dose arm (240 mg) also showed significant decline from baseline at Day 15. Overall, the estimated mean change from baseline across both post-baseline time points showed a dose-dependent trend, in that participants in the 120 mg arm showed less decline (− 0.176, standard error [SE] = 0.117), which is not statistically significant (*P* = 0.159), whereas those in the high dose (240 mg) showed a statistically significant decline (− 0.443, SE = 0.119; *P* = 0.003).

Additionally, Table 7 presents the comparisons between each of the active arms and the placebo on mean p-tau$^{181}$ level change from baseline at both Day 15 and Day 16. Both active arms showed a significantly faster decline in mean change from baseline than the placebo at Day 16. Whereas the two active arms showed no difference at Day 16 on mean change from baseline, the high dose showed a trend toward a faster decline than the lower dose at Day 15 (*P* = 0.08).

## Discussion

A total of 56 subjects received at least one dose of study drug or placebo in this Phase 1a randomized, placebo-controlled study (23 males, 33 females; average age 59.6 ± 5.4 years for placebo and 59.6 ± 5.5 years for NNI-362). Of treated subjects, 42 (75%) received active agent (single dose of 10, 20, 60, or 120 mg; or 10 daily doses of 20 mg; or single dose plus seven daily doses of 120 or 240 mg) and 14 (25%) received placebo.

The plasma concentrations of NNI-362 were evaluated to determine the PK parameters after oral administration of a single dose or multiple doses. Although plasma levels were quantifiable in a limited number of samples in Cohorts A–E, modification of the formulation for Cohorts F and G resulted in higher plasma concentrations of NNI-362. Multiple daily doses and increased doses in the lipid formulation also tended to result in higher exposure to NNI-362 based on $C_{max}$ and AUC values and in longer $t_{1/2}$ and $MRT_{inf}$ values in the latter two cohorts. Variability was also observed in several PK parameters (e.g., $C_{min}$ being greater than $C_{tau}$; Table 5). This observation may be due to metabolic characteristics of the drug via enterohepatic recirculation. Additionally, the observation of the large percentage of the area extrapolated (i.e., ≥ 20%) most likely would be attributed to the small number of available blood samples above the LLOQ.

Modification from 1% w/v methylcellulose in sterile water oral suspension to an MCT, Kolliphor EL, and beeswax (95/3/2% w/w) lipid-based formulation for NNI-362 increased the plasma concentrations observed in Cohorts F and G, compared to Cohorts A–E. Due to change in formulation, funding constraints did not allow the evaluation of a food effect. This lack of food effect evaluation would not affect a Phase 2 study since NNI-362 is administered once daily in the morning, though a food effect may enhance exposure, as observed with the lipid formulation.

In clinically defined groups of cognitively unimpaired, mild cognitive impairment, and AD, the p-tau$^{181}$ level was associated with disease stage, suggesting that plasma

p-tau$^{181}$ is selective for AD [13]. Recent clinical findings concluded that differences in baseline levels of p-tau$^{181}$ in elderly subjects (>70 years of age) was associated with memory deficit [14]. Analyzing data from the Alzheimer's Disease Neuroimaging Initiative database over 5 years suggests that increases from baseline of plasma p-tau$^{181}$ was associated with greater risk of progression from mild cognitive impairment to dementia [14]. Indeed, longitudinal analysis of cognitively unimpaired and cognitively impaired individuals demonstrated that plasma p-tau$^{181}$ levels correlated with cognitive decline and neurodegeneration longitudinally and was selective for AD—i.e., subjects had elevated Aβ [15].

Although the use of plasma p-tau$^{181}$ in clinical trials for prognostication is still in its infancy, plasma collected from this study was used to assess NNI-362's ability to modify brain levels of p-tau$^{181}$. NNI-362, at the two highest multiple doses, 120 mg and 240 mg, was shown to significantly reduce p-tau$^{181}$ levels in aged study participants compared to pre-dose levels, while no change occurred in aged subjects receiving placebo. Given the mechanism of action of NNI-362 to inhibit hyperphosphorylation of tau in human neurons within hours [11], it was not surprising to see results within the SAD plus MAD time frame. It is unknown whether long-term treatment with NNI-362 in AD patients in a Phase 2 trial would continue to reduce p-tau$^{181}$ over that period. It is expected that NNI-362 could halt or reverse disease progression, rather than being preventative, since the mechanism of action promotes neuron regeneration and ameliorates degeneration [11].

These results suggest that p-tau$^{181}$ could be a useful diagnostic—and potentially also prognostic—tool. The use of plasma p-tau$^{181}$ in AD clinical trials of monoclonal antibody to Aβ, aducanumab, appears to demonstrate a correlation between Aβ positron emission tomography change from baseline and reduction of p-tau$^{181}$ from baseline in both Phase 3 trials, EMERGE and ENGAGE [2].

A limitation of the study is that the number of subjects receiving NNI-362 at the highest doses in lipid-liquid formulation was still quite low, and as such, the number of unique plasma samples from baseline to 12 h and 24 h post final dose to determine change in plasma p-tau$^{181}$ was also quite low.

Though this first-in-human study was not aimed at reaching a maximum tolerated dose, the 240 mg dose did reach an exposure level equivalent to the efficacious dose in all nonclinical rodent models. Therefore, it may be unnecessary to go to higher dosing levels in future trials.

At the time of the National Institute on Aging grant submission for support of this Phase 1a first-in-human trial of NNI-362, p-tau$^{181}$ was at least as common a plasma biomarker as p-tau$^{217}$ and was certainly identified as originating in the brain. With the most recent acceptance of Fujirebio's blood test (p-tau$^{217}$/Aβ1–42) for AD detection, this blood test will therefore be used as a diagnostic criterion for the Phase 2 proof-of-concept trial. P-tau$^{181}$ will continue to be utilized, but p-tau$^{217}$ will also be included as a further indicator of the proof of concept of NNI-362 to not only promote new neuron generation but to minimize tau hyperphosphorylation (i.e., modulating S6 kinase; see [11]), as was seen with in vitro human neuron cultures.

For the next development phase, a parallel placebo and single NNI-362 ($N=36$ for each group) dose Phase 2a study is being planned in which AD patients will receive either placebo or NNI-362 as a single oral dose daily for 26 weeks with a washout period of at least 14 days. The primary objective of this study is to establish safety and tolerability of oral NNI-362 in mild to moderate AD patients (determine sex effects) and to compare drug intervention vs. control for efficacy in change from baseline to plasma p-tau$^{181}$ to plasma p-tau$^{181}$ at 6 months. The secondary and exploratory objectives include changes from baseline to Week 26 for Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog-12) and Clinical Dementia Rating-Sum of Boxes (CDR-SB) scores. Additionally, changes from baseline will be evaluated by volumetric magnetic resonance imaging of the hippocampus, the University of Pennsylvania Smell Identification Test, and logical memory as well as plasma Aβ1–42, glial fibrillary acidic protein, neurofilament light chain, and p-tau$^{217}$ biomarkers.

## Conclusions

The results suggest that NNI-362 was considered safe and well tolerated by all subjects in this study to the top dose of 240 mg. There were no deaths, serious AEs, or discontinuations recorded in any of the cohorts. All TEAEs were considered mild in severity, and none were assessed as definitely related to test article. The percentage of subjects with TEAEs were similar across subjects receiving NNI-362 or placebo. No apparent treatment-related trends were observed in clinical laboratory test results, ECG results, vital sign measurements, or physical examination findings. A common clinical biomarker in aging and AD, plasma p-tau$^{181}$ was significantly reduced from baseline following SAD and MAD treatment at the highest NNI-362 doses, with no change in placebo over the same time period.

**Abbreviations**

| | |
|---|---|
| %Extrap $AUC_{inf}$ | Percent of $AUC_{inf}$ that was extrapolated |
| Aβ | Amyloid β |
| AD | Alzheimer's disease |
| ADAS-Cog-12 | Alzheimer's Disease Assessment Scale-Cognitive Subscale |
| AE | Adverse event |
| $AUC_{inf}$ | Area under the concentration-time curve to infinity |

| | |
|---|---|
| $AUC_{last}$ | Area under the plasma concentration curve up to the last blood collection time |
| CDR-SB | Clinical Dementia Rating-Sum of Boxes |
| CTDS | Clinical Trial Data Services |
| $C_{max}$ | Maximum concentration |
| $C_{min}$ | Minimum concentration |
| C-SSRS | Columbia-Suicide Severity Rating Scale |
| $C_{tau}$ | Drug concentration at the end of a given dosing interval |
| DLT | Dose-limiting toxicity |
| ECG | Electrocardiogram |
| Extrap | $AUC_{inf}$ extrapolated $AUC_{inf}$ |
| LLOQ | Lower limit of quantitation |
| MAD | Multiple ascending dose |
| MCT | Medium-chain triglyceride |
| $MRT_{inf}$ | Mean residence time to infinity |
| NA | Not available |
| NC | Not calculated |
| PB | Plasma binding |
| PK | Pharmacokinetics |
| p-tau$^{181}$ | Phosphorylated tau$^{181}$ |
| SAD | Single ascending dose |
| SAS | Statistical Analysis System |
| SE | Standard error |
| $t_{1/2}$ | Terminal elimination half-life |
| TEAE | Treatment-emergent AE |
| $T_{max}$ | Time of observed maximum concentration |

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**Author contributions**
JKA is the study sponsor. EY conducted the study. CG analyzed the samples and provided the PK analysis. CF analyzed the samples and provided the PK analysis. DB provided study monitoring. LPT provided regulatory support. RST provided independent medical monitoring. All authors read and approved the final manuscript.

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**Data availability**
Data is provided within the manuscript.

## Declarations

**Ethics approval and consent to participate**
The study protocol was approved by the Institutional Review Board and was designed, executed, and reported according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines for Good Clinical Practice and the ethical principles outlined in the Declaration of Helsinki. All patients were consented for study participation. Institutional Review Board: WCG IRB, formerly the Western Institutional Review Board (WIRB).

**Consent for publication**
The authors consent to publishing this manuscript.

**Competing interests**
The authors declare no competing interests.

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