Official Study Title: Pilot study to investigate the safety and feasibility of AntiRetroviral Therapy for Alzheimer's Disease (ART-AD)

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Pilot study to investigate the safety and feasibility of **A**nti**R**etroviral **T**herapy for **A**lzheimer's **D**isease (**ART-AD**)

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Funding Sponsors: *William and Ella Owens Medical Research Foundation*
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Study Product: *3TC Tablets (lamivudine, Epivir®)*

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List of Abbreviations

FDA – U.S. Food and Drug Administration
CSF – Cerebrospinal fluid
HPLC/MS/MS – High performance liquid chromatography with tandem mass spectrometry
CDR – Clinical Dementia Rating
WMS-IV – Wechsler Memory Scale
MMSE – Mini-Mental State Evaluation
BHA – Brain Health Assessment
CNS – Central Nervous System
HIV – Human Immunodeficiency Virus
HBV – Hepatitis B Virus
NRTI – Nucleoside Reverse Transcriptase Inhibitor
PI – Principal Investigator
AE – Adverse Event
SAE – Serious Adverse Event
UPIRSO – Unanticipated Problems Involving Risk of Subjects or Others
PACC-5 – Preclinical Alzheimer Cognitive Composite score
NfL – Neurofilament Light chain
GFAP – Glial Fibrillary Acidic Protein
MARC – Medical Arts and Research Center
CBC – Complete Blood Count
CMP – Comprehensive Metabolic Panel
IRB – Institutional Review Board
PT/PTT/INR – Prothrombin Time/Partial Thromboplastin Time/International Normalized
BP – Blood Pressure

Study Summary

Title	**A**nti**R**etroviral **T**herapy for **A**lzheimer's **D**isease (ART-AD)
Protocol #	
Phase	2a
Methodology	Open-label trial
Study Duration	Approximately 28 weeks
Objective	Evaluate 3TC central nervous system (CNS) penetration, target engagement, efficacy based on fluid-based biomarkers of neurodegeneration and neuropsychological assessment, and safety in older adults with early Alzheimer's disease as initial proof-of-concept for a larger phase 2 clinical trial.
Number of Subjects	Screen 60 subjects for 12 completers with early Alzheimer's disease.
Inclusion Criteria	1) Aged 50-99 years 2) Clinical diagnosis of early Alzheimer's disease (Clinical Dementia Rating (CDR) = 0.5, Mini-Mental State Exam (MMSE) = 24-30) 3) If using drugs to treat symptoms related to Alzheimer's disease, doses must be stable for at least eight weeks prior to screening visit 1 4) Labs: Adequate blood cell counts (white blood cells: 4,000-11,000 cells/mcL; absolute neutrophil count: 1,800-8,700 cells/mcL; platelets: 120-500 K/μL; hemoglobin 12.0-17.5 grams/dL); LFT's within 2x normal value; CrCl ≥ 50 mL/min; cholesterol (≤260 mg/dl), triglycerides≤ 400 mg/dl), and glucose control (HbA1c ≤ 8%). Prothrombin time/partial thromboplastin time/international normalized ratio (PT/PTT/INR) within normal limits 5) Body mass index (BMI) within range of 16 - 35 kg/m^2 6) Must have a reliable informant or caregiver 7) Participants must have no plans to travel that interfere with study visits
Exclusion Criteria	1) Any medical or neurologic condition (other than Alzheimer's Disease) that might be a contributing cause of the subject's cognitive impairment 2) Clinically significant unstable psychiatric illness in the past six months 3) Significant hearing, vision, or motor deficits that interfere with participation 4) Alcohol or drug abuse/dependence in the past six months 5) Stroke, transient ischemic attack, or unexplained loss of consciousness in the past six months 6) Unstable angina, myocardial infarction, advanced chronic heart failure, or clinically significant conduction abnormalities within the past six months 7) Relevant brain hemorrhage, bleeding disorder and cerebrovascular abnormalities 8) Diagnosis of HIV infection or AIDS (CD4 count < 200), HIV/HBV co-infection, HBV or human T-cell leukemia virus infection 9) History of impaired renal or liver function 10) Current use of memantine or sorbitol-containing products (Note: participants who are taken off memantine may be eligible for enrollment following a one month washout period). 11) Individuals with HIV, HBV, or who have current use of NRTIs or non-NRTIs 12) Poorly controlled blood pressure (BP) (systolic BP > 160, diastolic BP > 90 mmHg) 13) Uncontrolled diabetes (HbA1c > 8%, or the current use of insulin) 14) Significant systematic illness or infection in the past 30 days 15) Pregnant women 16) Space occupying lesion in brain that contraindicates LP 17) Imaging within one year prior to enrollment that identifies any exclusionary lesions

Study Product, Dose, Route, Regimen, Duration	3TC will be administered once daily via an oral tablet (300 mg) with or without food. Study medication will be administered for 24 weeks.
Statistical Methodology	We seek preliminary evidence of blood brain penetration, target engagement, efficacy, and safety in this early phase 2a trial. Our objective is to determine the pre/post differences in pertinent laboratory values and adverse event reporting. We will report the change in post intervention laboratory values relative to baseline with a 95% confidence interval. Experimental results will be expressed as mean ± SE.

1. Introduction

This document is a protocol for a human research study. This study will be conducted according to Good Clinical Practice guidelines as adopted by the U.S. Food and Drug Administration (FDA), applicable government regulations, and institutional research policies and procedures.

1.1. Background

Transposon biology. Transposable elements, known colloquially as "jumping genes," constitute approximately 45% of the human genome[1]. Transposable elements are a diverse superfamily of genomic DNA species that have the ability to either copy themselves and insert the DNA copy into a new genomic location (retrotransposons) or excise themselves from the genome and insert in a new genomic location (transposons). Retrotransposons outnumber DNA transposons 13 to 1 in the human genome[2]. Over the course of human evolution, most retrotransposons and all DNA transposons have lost the ability to mobilize due to truncation and mutation. Some human retrotransposons, however, retain mobilization potential, including specific long and short interspersed nuclear element (LINE and SINE, respectively) subfamilies[3]. Retrotransposon activation has been identified as a disease mechanism in several human disorders, including cancer[4], Aicardi Goutières Syndrome[5], amyotrophic lateral sclerosis (ALS)[6], frontotemporal dementia[7], Alzheimer's disease and related tauopathies[8–10].

Retrotransposons are structurally akin to retroviruses. Retrotransposition occurs through a copy-and-paste mechanism involving transcription of retrotransposon DNA to RNA, reverse transcription of retrotransposon RNA into a new DNA copy, and reinsertion of the DNA copy into the genome[3] (**Fig. 1**). Retrotransposition is carried out by proteins, including a reverse transcriptase, encoded by retrotransposons.

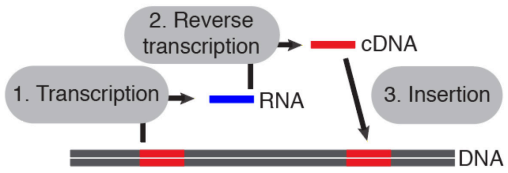

Figure 1 | Retrotransposon life cycle.
1) Retrotransposon is transcribed into RNA
2) Retrotransposon encodes reverse transcriptase, which reverse transcribes the RNA transcript into DNA. Retrotransposon encodes an integrates that inserts the new DNA copy into the genome.

Evidence of retrotransposon activation in Alzheimer's disease and related tauopathy. Alzheimer's disease is a progressive neurodegenerative disorder that affects over 5.8 million Americans and has no curative treatment[11]. Recent evidence suggests that Alzheimer's disease pathophysiology begins decades prior to symptoms[12]. A poor understanding of mediators that drive disease onset and progression during the long prodromal, asymptomatic stages presents a large barrier for developing effective therapeutic strategies. We have reported that pan-neuronal transgenic expression of human tau in *Drosophila* activates retrotransposons by disrupting two arms of retrotransposon control: 1) heterochromatin- and 2) piRNA-mediated silencing[8]. Consistent with the increase in DNA copy number of select retrotransposons in brains of tau transgenic *Drosophila*[9], we demonstrated active retrotransposition as a consequence of human tau in neurons of the adult *Drosophila* brain. We found that genetic manipulation of retrotransposon regulatory machinery modifies tau-induced neurodegeneration, supporting a causal link between retrotransposon dysregulation and neurodegeneration[8].

RNA-seq analysis of brain lysates from post-mortem human controls, Alzheimer's disease and progressive supranuclear palsy, a "primary" tauopathy, revealed elevated levels of specific retrotransposon transcripts, including LINE-1, human endogenous retrovirus (HERV), and SVA, and decreased levels of Alu family members[8]. Consistent with our work, Shulman and colleagues reported a significant association between decreased cognitive performance in the year prior to death and elevation of specific HERV subfamilies in human Alzheimer's disease brain, as well as an association between tau tangle burden and increased transcript levels of select LINE-1 and HERV elements[9]. We now have additional data suggesting that retrotransposon activation occurs early in human Alzheimer's disease, which we describe below. Together with reports of heterochromatin relaxation[13,14] and piRNA dysregulation[15,16] in post-mortem human Alzheimer's disease brain, these studies suggest that tau-induced heterochromatin decondensation, piRNA dysregulation and consequent transposable

element activation is a novel, conserved, pharmacologically targetable driver of neurodegeneration in Alzheimer's disease and related tauopathies.

Use of antiviral reverse transcriptase inhibitors in human diseases associated with retrotransposon activation. Based on the similarities between exogenous retroviruses and retrotransposons, numerous studies have investigated the therapeutic efficacy of antiviral medications, including NRTIs, to prevent retrotransposition[8,17–19]. The Lighthouse trial (NCT02868580), for example, an open label, multi-center study to investigate the safety and tolerability of Triumeq in patients with ALS, has recently completed phase 2a. Triumeq consists of two NRTIs, abacavir and 3TC, and an integrase inhibitor, dolutegravir, which blocks episomal DNA integration into genomic DNA. 35 out of 40 patients completed the 24-week treatment period. Non-serious adverse events that occurred in three or more participants included nausea and rash. While five deaths were expected based on modeling[20], only one death was observed five months after conclusion of the trial. DNA copy number of retrotransposon DNA was significantly reduced by the end of the trial, and the ALS functional rating scale demonstrated a declining trend[21]. The trial is slated for phase 3, in which overall survival and disease progression will be measured in a larger cohort. An additional trial (NCT02437110) for a retroviral combination therapy (darunavir, ritonavir, dolutegravir, and tenofovir alafenamide,) in ALS is currently in phase 1. A recently-completed 12 month clinical trial (NCT02363452) in children with Aicardi-Goutières Syndrome, in which the body mounts a viral response due to activation of retrotransposons, reports that patients administered 3TC, zidovudine, and abacavir have a significantly reduced blood interferon score after treatment[22].

3TC was approved by the FDA for the treatment of HIV-1 and hepatitis B virus in the late 1990's, and continues to be prescribed as both a mono- and combination therapy for retroviral infection. Combination therapies are warranted in HIV, as the virus demonstrates a high mutation rate that can overcome an individual NRTI[23]. It is not clear, however, that retrotransposons have a mutational capacity that requires a combinatorial approach. We aim to reduce the risk of negative health consequences associated with polypharmacy in older patients by using 3TC as a monotherapy at a dose that is cost-effective and currently recommended for treatment of HIV+ patients in the clinic[24]. Of relevance to older patients, who are often subject to polypharmacy, 3TC has few clinically-relevant pharmacological interactions due to its low metabolic clearance, minimal binding to plasma protein, and no detectable effects on liver function[25]. While 3TC is considered to be generally safe and is widely prescribed, no study to date has specifically investigated the use of 3TC in older individuals with cognitive impairment. Outcomes of this 24-week open label phase 2a clinical trial include i) target engagement, ii) CNS penetration, iii) efficacy based on cognitive scores and biomarkers of neurodegeneration, and iv) safety and tolerability of 3TC in patients with early stage Alzheimer's disease.

1.2. Innovation

Traditionally, therapeutic development in Alzheimer's disease has been focused on targeting amyloid beta, pathological forms of tau protein, or strategies to boost neuronal function. Rather than targeting the initiating proteins that have been present in brains of affected individuals for decades prior to symptom onset, we will target a mechanism that is downstream of pathogenic forms of tau that is well-connected to neuronal death.

1.3. Preliminary data

Evidence of transposable element activation in brains of tau transgenic mice. Based on our previous work showing that transposable element mobilization is significantly increased in an age-dependent manner in tau transgenic *Drosophila* and that endogenous retroviruses are aberrantly expressed in late-stage Alzheimer's disease and progressive supranuclear palsy[8], we determined the time course of retrotransposon activation in a mouse model of tauopathy. We analyzed RNA-sequencing data from non-transgenic controls versus JNPL3[26] human tau transgenic mice that harbor the disease-associated tau *P301L* mutation. We find that elevated retrotransposon expression occurs prior to neurofibrillary tangle formation and neuronal loss in this model, suggesting that tau drives aberrant transposable element expression early in disease, considerably before symptom

onset (**Fig. 2a, b**).

Staging of transposable element activation in human Alzheimer's disease. Increased retrotransposon DNA copy number (**Fig. 1**) suggests that a given retrotransposon is activated and mobilization-competent. We have quantified retrotransposon DNA copy number in Braak III and V/VI postmortem human Alzheimer's disease brain relative to control (tissue provided by Dr. Dennis Dickson). Since some retrotransposons reverse transcribe in the cytoplasm and then import the new DNA copy into the nucleus, we isolated DNA from nuclei in order to reduce contribution from episomal cytoplasmic DNA. To quantify DNA copy number, we created a custom NanoString codeset to detect 52 human transposable elements. DNA copy was normalized to ten single-copy, invariant internal control genes, and is presented relative to control brains. We find that 49 out of 52 retrotransposons have significantly increased DNA copy number at Braak III (**Fig. 2c**, retrotransposons in red are significant). Interestingly, Braak V/VI brains do not feature significant elevation of retrotransposon DNA copy number with this relatively small sample size (**Fig. 2d**). As our experiments in *Drosophila* indicate that retrotransposon activation is toxic, we speculate that cells that undergo retrotransposon activation die, and that cells surviving into a Braak V/VI brain are cells in which transposable elements have not been activated. These data, along with data from JNPL3 tau transgenic mice, which we find exhibit a similar early increase and late decrease in retrotransposon expression at the transcript level, serve as rationale for targeting early-stage Alzheimer's disease in our clinical trial.

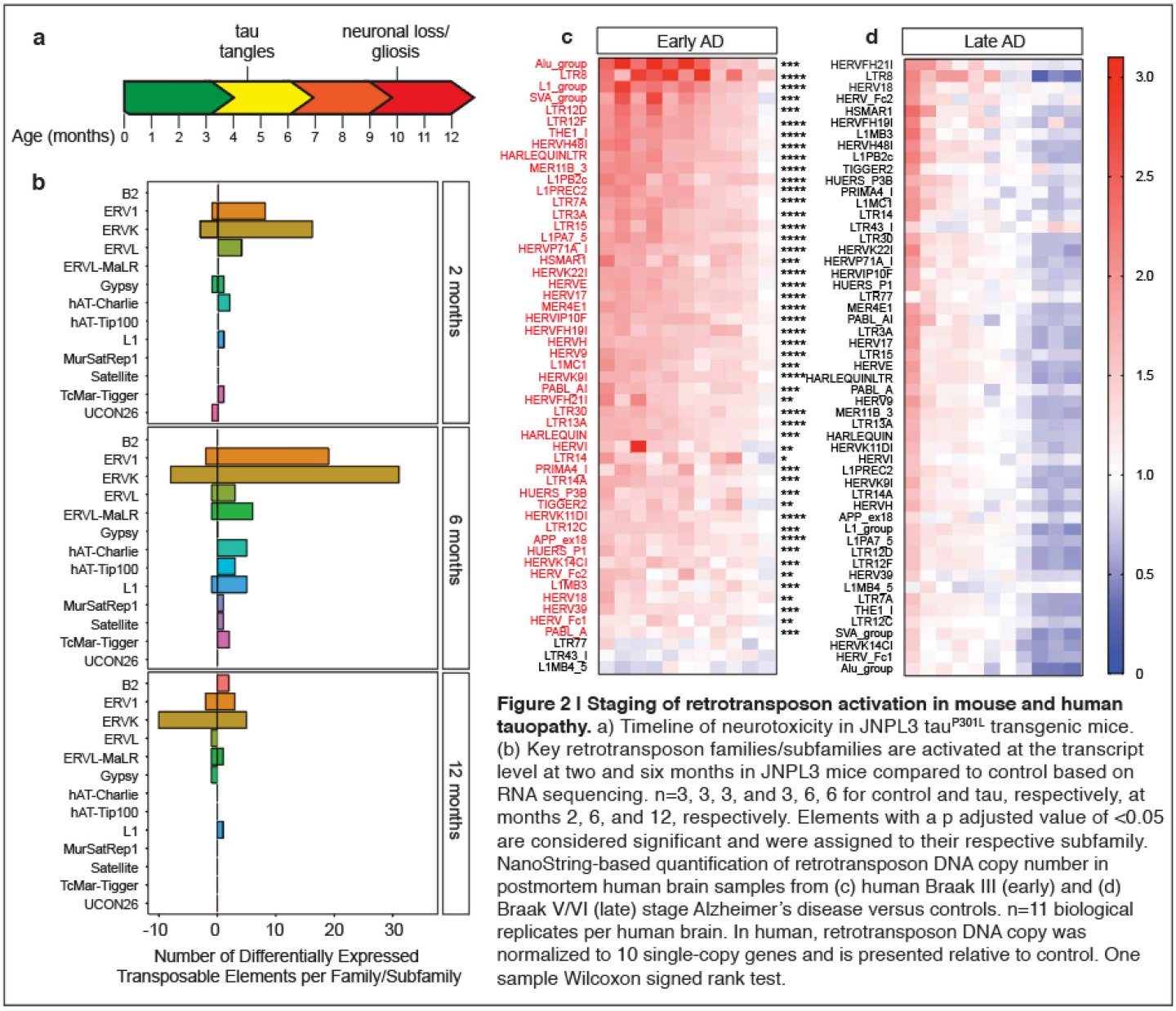

Figure 2 | Staging of retrotransposon activation in mouse and human tauopathy. a) Timeline of neurotoxicity in JNPL3 tau^{P301L} transgenic mice. (b) Key retrotransposon families/subfamilies are activated at the transcript level at two and six months in JNPL3 mice compared to control based on RNA sequencing. n=3, 3, 3, and 3, 6, 6 for control and tau, respectively, at months 2, 6, and 12, respectively. Elements with a p adjusted value of <0.05 are considered significant and were assigned to their respective subfamily. NanoString-based quantification of retrotransposon DNA copy number in postmortem human brain samples from (c) human Braak III (early) and (d) Braak V/VI (late) stage Alzheimer's disease versus controls. n=11 biological replicates per human brain. In human, retrotransposon DNA copy was normalized to 10 single-copy genes and is presented relative to control. One sample Wilcoxon signed rank test.

3TC mechanism of action and pharmacokinetics. 3TC 5'-triphosphate, the active analog of 3TC, competitively inhibits viral reverse transcriptase, resulting in proviral DNA chain extension termination. 3TC is one of two NRTIs used in the most well-tolerated and common antiretroviral therapy, Triumeq, and has mild brain penetration in pediatric patients[25]. Importantly, we[8] and others[19,27,28] have shown that 3TC effectively inhibits activation of retrotransposons, which, like retroviruses, encode a reverse transcriptase.

3TC is absorbed rapidly after oral administration, with an absolute bioavailability of 82% in adults. 3TC 5'-triphosphate peaks in serum within 0.5-1.5 h, with an elimination half-life of 5-7 hours. 2-4 hours following treatment, the concentration of 3TC in cerebrospinal fluid (CSF) is 4-8% of serum concentrations in adults[25]. In collaboration with Dr. Marty Javors, we have successfully detected 3TC in brains of older mice and in CSF of humans who have taken 3TC. For mouse studies, we administered a five day, 50mg/kg dose of 3TC in drinking water, corresponding to the current recommended dosage for HIV adjusted to body area (0.026 and 0.30 m^2/kg of body weight in humans vs. mice, respectively[18]). The quantity of 3TC dissolved into drinking water was determined based on a daily liquid consumption of 5 mL per mouse. Daily liquid intake is not affected by 3TC treatment, and stability of 3TC in drinking water is 90-103% of the initial value over one week[18]. Using HPLC/MS/MS chromatography[29], we measured 3TC levels in plasma (**Fig. 3a)** and brains (**Fig. 3b)** of 24-25

month old 3TC-treated mice, and calculated the brain to plasma ratio (**Fig. 3c**). Similarly, we have used HPLC/MS/MS chromatography to quantify 3TC levels in plasma (**Fig. 3d**) and CSF (**Fig. 3e**) of humans who are actively taking 3TC. With this very limited human sample size, 3TC CNS penetration does not significantly differ between younger individuals and older individuals (**Fig. 3f**, young=average age of 28 years old, old=average age of 65 years old). In mice and in younger and older human individuals, we find that the CNS harbors approximately 20% of the levels of circulating 3TC.

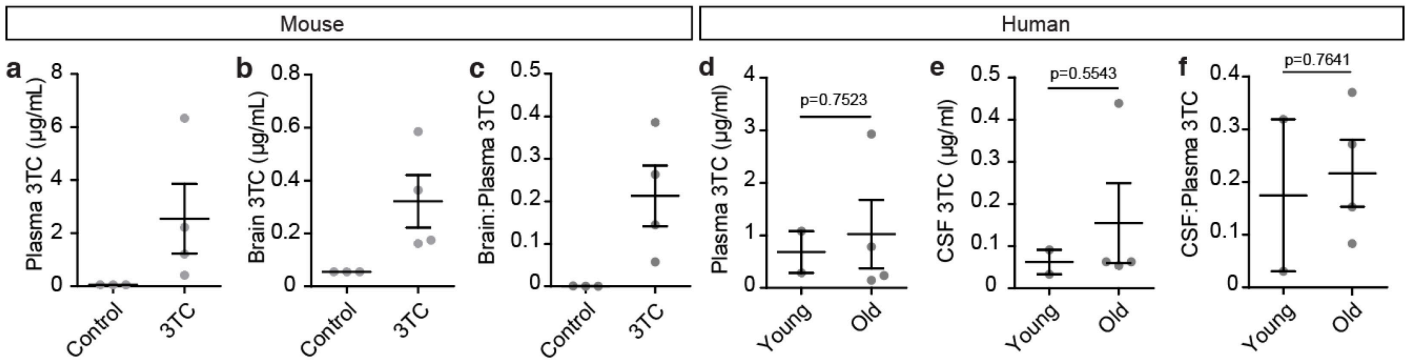

Figure 3 | Orally-administered 3TC penetrates the blood brain barrier of aged mice and humans. Male mice aged 25-26 months administered 2 mg/mL 3TC (or vehicle) in drinking water for 5 days demonstrate 3TC accumulation in blood (a), and brain (b), with a brain penetration ratio average of 0.21 (c). Young and old HIV positive individuals prescribed 300 mg daily oral 3TC have 3TC in plasma (d), and CSF (e), with a brain penetration ratio average of 0.21 (f). Student's t test.

1.4. Dose Rationale and Risk/Benefits

The study will consist of a screening/baseline period of 30 days pre-medication, a 24-week open label treatment period, and a post-intervention visit one month following treatment. During the treatment period, daily 300 mg 3TC will be taken orally with or without food. The selected dosage is what is currently prescribed for HIV-1 patients. 3TC is widely prescribed and well-tolerated. While 3TC has fewer side effects than many other antiretroviral treatments, side effects can include cause vomiting, weakness, diarrhea, headache, and nausea.

2. Study Objective

The objective of the study is to evaluate the efficacy of 3TC to suppress neurodegeneration based on fluid-based biomarkers and neuropsychological assessment, the ability of 3TC to engage its intended target (retrotransposon-derived reverse transcriptase), and the safety, tolerability and CNS penetration of 3TC in patients with early stage Alzheimer's disease. This research, if positive, may provide the initial data on target engagement and Alzheimer's disease-relevant outcomes for future trials.

3. Study Design

3.1. Subjects

The study staff will pre-screen up to 60 potential candidates to identify at least 12 men and women aged 50-99 years with a clinical diagnosis of early Alzheimer's disease based on a CDR of 0.5 and MMSE score between 24 and 30. Refer to Section 4 for Subject Selection, Inclusion and Exclusion criteria.

3.2. General Design

This open label study of 3TC will collect initial proof-of-concept data on 3TC efficacy, target engagement, CNS penetration, and safety in older adults with early stage Alzheimer's disease. If successful, data will be used to

design a larger phase 2 clinical trial. We aim to I) Quantify 3TC CNS penetration and target engagement, II) Determine if 3TC suppresses Alzheimer's disease-relevant outcomes, and III) Assess the safety and tolerability of 3TC in older individuals with early Alzheimer's disease. The study will consist of a screening/baseline period of 30 days pre-treatment, a 24-week open label treatment period, and a follow up visit one month following treatment. Visits to the clinic include a pre-treatment screening visit that includes a comprehensive neuropsychological exam and a tablet-based neuropsychological exam, a lumbar puncture to collect CSF, and a blood draw. Participants will return to clinic on day one of treatment and at weeks 8, 16, and 24 of treatment to complete medication checks, physical examinations, brief cognitive screening, and blood draw (see Table 2 for additional details). At week 24 of treatment, patients will complete a post-treatment comprehensive neuropsychological exam, a lumbar puncture to collect CSF, and a blood draw. One month after the final dose of medication, participants will return to the clinic for a final safety assessment and disenrollment.

3.3. Study Endpoints

Primary outcome:

Quantify 3TC CNS penetration and target engagement.

3TC 5'-triphosphate competitively inhibits viral reverse transcriptase, resulting in proviral DNA chain extension termination. 3TC is absorbed rapidly after oral administration, with an absolute bioavailability of 82% in adults. 3TC 5'-triphosphate peaks in serum within 0.5-1.5 h, with an elimination half-life of 5-7 hours. 2-4 hours following treatment, the concentration of 3TC in CSF is 4-8% of serum concentrations in adults[25]. We will determine the extent of CNS penetration in older patients with Alzheimer's disease by calculating the CSF to plasma ratio of 3TC 5'-triphosphate using HPLC/MS/MS chromatography[31] using plasma and CSF collected during the clinic visit corresponding to the last dose of 3TC (week 24 of treatment).

To quantify target engagement, we will measure reverse transcriptase activity in plasma and CSF collected from participants prior to (day 1) and following treatment (week 24 of treatment) using a modified version of the EnzCheck Reverse Transcriptase Assay. This assay utilizes a PicoGreen-based reagent to detect double stranded DNA or RNA-DNA heteroduplexes formed by reverse transcriptase activity in a biological sample. As samples can be read by a microplate reader, this assay is suitable for rapid, sensitive and large-scale testing of many biological samples.

As an additional indicator of the ability of 3TC to suppress endogenous reverse transcriptase activity, we will compare retrotransposon DNA copy number in PBMCs and CSF prior to (day 1) and following treatment (week 24 of treatment) using the NanoString codeset that we have developed to detect retrotransposon DNA (**Fig. 2c, d**).

Secondary outcomes:

a) **Assess changes in cognition, functional status, and neurodegeneration**.

We will use neuropsychological testing and fluid-based biomarkers of neurodegeneration to assess the degree of neurodegeneration. Following 24 weeks of treatment, we will measure cognitive change from baseline using the Preclinical Alzheimer Cognitive Composite (PACC-5) score. The PACC score was designed to serve as a primary outcome to evaluate subjects with prodromal Alzheimer's disease, and studies have shown that cognitively healthy individuals with pathologic evidence of Alzheimer's disease perform worse on the PACC when compared to healthy controls without pathologic evidence of AD[32,33]. More recent updates to the PACC paradigm include measures of semantic memory. The newly-adopted PACC-5 is thought to demonstrate improved detection of early Alzheimer's disease-related cognitive decline[34]. We therefore

propose a comprehensive neuropsychological assessment that allows for the calculation of a PACC-5 score prior to and following treatment, providing the most robust measure to capture Alzheimer's disease-related cognitive change over time.

In addition to pre- and post-treatment PACC-5 assessment, participants will undergo serial cognitive screening using the tablet-based Brain Health Assessment (BHA) at the initiation of treatment and weeks 8, 16, and 24 of treatment. The BHA is a sensitive, 10-minute, tablet-based neurocognitive screening assessment with excellent psychometrics and neuroanatomical correlates[17]. The BHA includes measures of memory, executive function, visuospatial awareness, and language. Preliminary studies demonstrate that test reliability is not dependent upon familiarity with tablet use – an important caveat when introducing new technology in a cohort of older adults. As such, all subjects will complete the BHA at clinic visits, and cognitive functioning will be captured every 8 weeks over the course of the study.

Using plasma and CSF collected pre- and post-treatment, we will quantify biomarkers of neurodegeneration and neuroinflammation including neurofilament light chain (NfL)[35], glial fibrillary acidic protein (GFAP)[36], and inflammatory cytokines.

b) **Establish the safety and tolerability of 24-week 3TC treatment in older adults with early symptomatic Alzheimer's disease.**

Participants will be assessed at clinic visits every two months during the 24-week treatment period to track adverse events and compliance to study drug regimen. Physical examination, vital signs, cognitive functioning (BHA), and blood draws for laboratory assessments of safety and compliance (complete blood count (CBC), comprehensive metabolic panel (CMP), plasma 3TC levels) will be performed at each visit, and the long-term effects of chronic 3TC treatment will be evaluated a month post-treatment.

3.4. Potential Risks to Subject Safety

a) Drug Administration

3TC is indicated for use in HIV and HBV. In clinical trials of 3,568 HIV-1 infected subjects, the most frequently reported (≥ 15%) adverse events are headache, nausea, malaise, fatigue, nasal signs and symptoms, diarrhea, and cough. Other common adverse reactions are decreased appetite, musculoskeletal pain, and dizziness (**Please refer to Attachment C, Full Prescribing Information**). 3TC treatment may result in neutropenia (absolute neutrophil count < 750/mm^3), elevated amylase (> 2x upper limit of normal) and liver enzymes (aspartate transaminase/alanine transaminase > 5x upper limit of normal). Clinical adverse reactions are similar for subjects receiving 3TC 300 mg once daily or 150 mg twice daily in combination retroviral therapies. Co-administration of 3TC with sorbitol-containing products must be avoided, as sorbitol may decrease the concentration of 3TC in circulation. In addition, caution is advised when combining 3TC and memantine. This combination increases 3TC levels and risk of adverse events, possibly due to inhibition of renal transport of 3TC. Boxed warning and precautions include the most serious side effects: exacerbation of hepatitis B, lactic acidosis and severe hepatomegaly with steatosis, immune reconstitution syndrome, and pancreatitis (more common in pediatric patients). Female sex and obesity are considered risk factors for lactic acidosis and severe hepatomegaly with steatosis in patients treated with antiretrovirals such as 3TC.

b) Blood withdrawal

- Pain, bleeding, bruising, or swelling at the site of the needle stick
- Hematoma
- Nerve damage

- Infection
- Fainting or light-headedness

* To minimize these risks, a qualified phlebotomist will perform venipunctures

c) Cognitive Assessment

- Psychosocial - embarrassment, discomfort or anxiety upon testing memory and thinking
- Patient or study partner-reported outcomes include questionnaires related to Alzheimer's disease symptoms, cognition, and mood scales

d) Lumbar Puncture

- Temporary pain and discomfort in the back
- Headache
- Persistent low-pressure headache due to leakage of CSF. If headache persists, it may require additional treatment. Uncommonly, a blood patch (injection of some of participant blood into the lumbar puncture site to patch the CSF leak) may be required.
- Infection
- Damage to nerves in the back
- Bleeding into the CSF space
- Allergic reaction to the local anesthetic (lidocaine) used for the lumbar puncture, such as swelling or rash at the puncture site

The use of anticoagulants increases the risks of bleeding and thrombotic complications during lumbar punctures. Subjects will be screened for use of anticoagulants before the lumbar puncture is scheduled. Subjects taking anticoagulants will not be scheduled for the lumbar puncture procedure.

Subjects will have their medications screened before the lumbar puncture. Subjects taking antiplatelets and NSAIDS will stop medication for 5 days before the lumbar puncture. Subjects taking acetic acid derivatives, Cox2 inhibitors, mefenamic acid, cilostazol (Pletal), vorapaxar (Zontivity) will discontinue medications for 3 days before the lumbar puncture. If the subject is on a cardiac or carotid stent or has history of stroke or myocardial infarction, the study investigator or study nurse will contact the subject's primary care doctor to determine if it would be safe to discontinue the subject's medications for 3 or 5 days.

If clinically indicated and upon the providing physician's discretion, the lumbar puncture may be completed with fluoroscopy. Fluoroscopy involves exposure to radiation. The amount of radiation exposure received from the procedure is equivalent to a uniform whole-body dose of 300 mrem (a unit of radiation exposure) which is approximately 0.5 times the amount of environmental radiation exposure (620 mrem dose) that each member of the general public receives per year. There is no known minimum level of radiation exposure that is recognized as being totally free of the risk of causing genetic defects (abnormal cells) or cancer. However, the probability of harm from such risk associated with the amount of radiation exposure received from fluoroscopy is considered to be low when compared to other everyday risks each member of the general public receives each year, depending on the amount of radiation each participant has personally been exposed to in the past, particularly in the previous year.

*To minimize these risks, a qualified provider specifically trained in the procedure will perform the lumbar punctures.

4. Subject Selection and Withdrawal

4.1. Inclusion Criteria

a) Aged 50-99 years
b) Clinical diagnosis of early Alzheimer's disease (CDR = 0.5; MMSE = 24-30)
c) If using drugs to treat symptoms related to AD, doses must be stable for at least eight weeks prior to screening visit 1
d) Labs: Adequate blood cell counts (white blood cells: 4,000-11,000 cells/mcL; absolute neutrophil count: 1,800-8,700 cells/mcL; platelets: 120-500 K/μL; hemoglobin 12.0-17.5 grams/dL); LFT's within 2x normal value; CrCl ≥ 50 mL/min; cholesterol (≤260 mg/dl), triglycerides≤ 400 mg/dl), and glucose control (HbA1c ≤ 8%). Prothrombin time/partial thromboplastin time/international normalized ratio (PT/PTT/INR) within normal limits
e) BMI within range of 16 - 35 kg/m^2
f) Must have a reliable informant or caregiver
g) Participants must have no plans to travel that interfere with study visits

4.2. Exclusion Criteria

a) Any medical or neurologic condition (other than Alzheimer's Disease) that might be a contributing cause of the subject's cognitive impairment
b) Clinically significant unstable psychiatric illness in the past six months
c) Significant hearing, vision, or motor deficits that interfere with participation
d) Alcohol or drug abuse/dependence in the past six months
e) Stroke, transient ischemic attack, or unexplained loss of consciousness in the past six months
f) Unstable angina, myocardial infarction, advanced chronic heart failure, or clinically significant conduction abnormalities within the past six months
g) Relevant brain hemorrhage, bleeding disorder and cerebrovascular abnormalities
h) Diagnosis of HIV infection or AIDS (CD4 count < 200), HIV/HBV co-infection, HBV or human T-cell leukemia virus infection
i) History of impaired renal or liver function
j) Current use of memantine or sorbitol-containing products (Note: participants who are taken off memantine may be eligible for enrollment following a one month washout period).
k) Individuals with HIV, HBV, or who have current use of NRTIs or non-NRTIs
l) Poorly controlled blood pressure (BP) (systolic BP > 160, diastolic BP > 90 mmHg)
m) Uncontrolled diabetes (HbA1c > 8%, or the current use of insulin)
n) Significant systematic illness or infection in the past 30 days
o) Pregnant women
p) Space occupying lesion in brain that contraindicates LP
q) Imaging within one year prior to enrollment that identifies any exclusionary lesions

4.3. Subject Recruitment and Retention

a) **Recruitment**

Recruitment will take place at the Neurology Department and The Glenn Biggs Institute for Alzheimer's & Neurodegenerative Diseases at UT Health San Antonio. An estimated 1,200 novel patients with

dementia syndromes are diagnosed and treated annually, and both clinics employ active research teams. Additional methods may include posted study fliers in medical offices or senior centers, community engagement activities, and/or newspaper or web-based advertisements. A UT Health website detailing the project will be published online and can be referenced by other institutional websites. The study will be published on clinicaltrials.gov. All these strategies will be considered, made available and implemented/adapted according to the local policies and regulations. Telephone pre-screening prior to the clinic screening visit may be employed to ensure potential candidates will meet inclusion and exclusion criteria for enrollment.

b) **Retention**

Transportation, parking and/or meal vouchers may be provided to participants according to local policies.

4.4. Early Withdrawal of Subjects

4.4.1. When and How to Withdraw Subjects

Subjects have the right to withdraw fully or partially from the study at any time and for any reason without prejudice to their future medical care by the physician or at the institution. Withdrawal of full consent for a study means that the subject does not wish to receive further investigational treatment and does not wish to or is unable to continue further study participation including any follow-up in person, by phone, through third parties including relatives or friends, via discussion with other treating physicians, and by use of medical records; subject data up to withdrawal of full consent will be included in the analysis of the study. Any subject may withdraw full consent to participate in the study at any time during the study. The PI or sub-investigator will discuss with the subject appropriate procedures for withdrawal from the study.

4.4.2. Data Collection and Follow-up for Withdrawn Subjects

"Lost to follow-up" will be defined as a subject missing two or more consecutive visits, not answering or responding to three follow up phone calls to subject or emergency contacts, or returned receipt of one certified letter. Investigator will consult with Study Statistician with regard to any incomplete data set as compared to the full data set that fully supports the analysis. If a subject withdraws consent to participate in the study, attempts will be made to obtain permission to record survival data up to the protocol-described end of subject follow-up period. Investigator and designated research staff make it a high priority to obtain survival data on all subjects lost to follow up.

5. Study Drug

5.1. Description

a) Drug Properties

3TC is a first-generation, synthetic nucleoside analogue reverse transcriptase inhibitor approved in the US in 1995 for the treatment of HIV and Hepatitis B virus. The CAS number for 3TC is 134678-17-4. The molecular formula is C8H11N3O3S, which corresponds to a formula weight of 229.26 g/mol. 3TC is a white to off-white crystalline solid with a melting point of 160–162°C. 3TC is soluble in water (70 mg/mL) at 20°C. 3TC tablets contain the following inactive ingredients: black iron oxide, hypromellose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium starch glycolate and titanium dioxide.

b) Mechanism of Action

The nucleoside analog 3TC inhibits the activity of reverse transcriptase to prevent retroviral (or retrotransposon[27]) replication. 3TC is phosphorylated intracellularly to its active metabolite, 3TC 5'-triphosphate, which is incorporated into newly synthesized DNA by reverse transcriptase, causing DNA chain termination. Antiviral activity is detected at concentrations without toxicity in humans and is detected at low nanomolar concentrations (EC_{50} of 0.003-15 mM) in cell culture[25].

5.2. Treatment Regimen

a) Administration Protocol

Study subjects will come to the Clinical Research Center of the San Antonio Claude D. Pepper Center in the Barshop Institute building on the first day of each drug schedule (i.e. day 1 of each drug cycle). At this visit, subjects will be given a two-month supply of 3TC, which consists of a bottle of 60 tablets. Two-month refills will be provided at weeks 8 and 16. Participants will receive 24 weeks of drug in total.

b) Assigning Subjects

In this open-label pilot, all subjects will receive drug with the same dose and frequency. The study staff stores the Enrollment key, which is kept in a locked cabinet at the site or on a secure server. Study staff maintains source documents, data records and specimens in a de-identified manner labeled with a unique Subject ID number.

5.3. Preparation and Administration of Study Drug

Study drug will be stored at the Research Pharmacy in the Barshop Institute Clinical Research Center and dispensed by research staff. A two-month quantity of 3TC will be dispensed to patients on day 1 of Visit 1, and day 1 of weeks 8 and 16 (Visits 2, and 3, respectively) of treatment.

5.4. Subject Compliance Monitoring

Study staff will perform follow-up phone calls at designated intervals to monitor compliance to the treatment regimen and safety monitoring following lumbar puncture procedure (see Study Visits beginning Section 6.4). Participants and/or their study partner will be asked to bring empty pill bottles to each visit for review by the study team. The study coordinator will complete medication reconciliation at each visit.

5.5. Prior and Concomitant Therapy

Exclusionary medications: Current use of drugs containing sorbitol (e.g. orlistat and sorbitol), current use of memantine, and current use of NRTIs or non-NRTIs.

5.6. Packaging

a) Nature and contents of the container

- Drugs for individual subject use will be clearly labeled and identified as "3TC" and "For Investigational Use only—Not for Resale."

b) The Research Pharmacy will:

- Inventory receipt of initial shipment and ensure appropriate temperature control
- Distribute 3TC by labeling each individual subject packet(s) with Subject ID from list provided by the PI to the pharmacy prior to receipt of inventory

5.6.1. Receipt of Drug Supplies

Any damaged or unusable study drug in each shipment will be documented by the Research Pharmacy. The Research Pharmacist will notify the PI and the Material Supplier of any damaged study drug. Upon receipt, the Research Pharmacy reconciles inventory received per local standard operating procedure and makes copies of any accompanying shipping documentation.

5.6.2. Storage

3TC tablets should be stored at 25° C (77° F); excursions permitted between 15°– 30° C (59°–86° F) (see USP Controlled Room Temperature). For more details, please see also EPIVIR® (lamivudine/3TC) Full Prescribing Information 05-2019, Attachment C, Pharmacy Manual.

5.6.3. Dispensing of Study Drug

Designated staff from the Research Pharmacy maintain the Drug Inventory and Dispensing Logs to track how, when and to 3TC was dispensed to subjects. Study clinical staff will document administration in research records regarding dosing, unused drug, drug damaged, or wasted. Study subjects are instructed to swallow 3TC whole, and not to chew or crush medication when administered.

5.6.4. Return or Destruction of Study Drug

Procedures for final reconciliation of the site's drug supply at the end of the study will be in accordance with local site Pharmacy standard operating procedures. Procedures for proper handling and disposal of antiviral drugs should be considered. There is no general agreement that all the procedures recommended in the guidelines are necessary or appropriate. 3TC tablets consist of a core tablet (containing the active drug substance), surrounded by a film coating to prevent exposure of pharmacy and clinical personnel to the active drug substance. However, if tablets are crushed or broken, pharmacy and clinical personnel should wear disposable gloves.

6. Study Visits and Procedures

6.1. Visit 0, Consent and Screening Visit

The participant will come to the research site accompanied by a Legally Authorized Representative (LAR) for the consenting process.

In line with recommendations put forth by the Global Alliance for Genomics and Health, Aging, and Dementia Task Team, researchers will first attempt to seek consent from the study participant with AD. In order to have appropriate safeguards, decision-making capacity specific to participation in the research study (i.e., understanding the purpose, procedures, risks and benefits of study participation) will be evaluated using a standardized instrument for assessing capacity to consent developed by Jeste et al, 2007 and tailored to the study (see attachment D). In the event of questions regarding capacity, the research coordinator and/or research nurse practitioner will consult with the PI or other qualified study team members. If the study participant is found to lack capacity to consent, researchers will obtain consent from a legally authorized representative (LAR) who will be instructed to respect the will and preferences of the study participant. When a LAR is necessary, the

researchers will still include the study participant in the consent process and will seek to obtain assent for study participation.

After the informed consent process is completed, subjects will be screened for eligibility. Screening will include vital signs and physical examination, medical history, concomitant medication review, fasting blood measures for safety, routine urinalysis (with pregnancy screen, if required), weight/BMI, and cognitive screening assessment. The screening visit is estimated to require three hours.

a) Blood draw

- CBC, CMP, hemoglobin A1c, coagulation panel (PT/PTT/INR)

b) Cognitive screening assessment

- CDR
- MMSE
- Optional: Cognitive screening assessment may be conducted virtually if patient resides outside city.

c) EKG to evaluate cardiac rhythm and function (if the participant has had an EKG performed within the last 3 months, results from the previous EKG will be used).

6.2 Visit 1, Baseline Measurements (1-4 weeks after screening).

Within four weeks of the Screening visit, enrolled subjects will return to the research site or unit for assessment of vital signs, weight/BMI, physical exam, fasting blood draw and a full cognitive assessment. Blood and CSF will be used for baseline measures of retrotransposon activation, neuroinflammation and other Alzheimer's disease-related markers. Visit 1 is estimated to require a total of three hours.

a) Baseline measurements of safety profiles

- Vital signs
- Physical examination
- Concomitant medication review
- Routine urinalysis (with pregnancy screen, if required)

b) Blood draw for baseline analysis of safety and Alzheimer's disease-relevant markers, and target engagement (50 mL volume, separated into plasma and PBMCs. 20 mL will be used for safety analysis. Remaining plasma will be separated into 0.5 and 1 mL aliquots and frozen at -80°C, 15 minutes)

- CBC, CMP
- Plasma levels of NfL[35] and GFAP[36]
- Neuroinflammatory cytokines
- Plasma-based EnzCheck Reverse Transcriptase Assay
- NanoString-based DNA copy number analysis of DNA extracted from PBMCs

c) Cognitive assessment (estimated 2 hours)

Table 1 | Cognitive Battery

Domain	Specific Measure	Component tasks/subtests
Overall cognition	Mini Mental State Exam (MMSE)	Global cognition
Cognitive screen	Brain Health Assessment (BHA)	Tablet-based measures with minimal motoric demands assessing associative memory, processing speed, executive function, and visual perception
Cognitive screen	Alzheimer's Disease Assessment Scale (ADAS-Cog)	Screening tool to assess verbal list learning and recall, language, visual construction, visual attention, and executive function.
Intelligence	Hopkins Adult Reading Test	Estimates of premorbid intelligence
Attention	WAIS – Digit Span	Simple aural attention and working memory
Cognitive Speed	WAIS – Digit Symbol Substitution Test	Timed coding
Language	Verbal Fluency	Timed letter-cued and category-cued word generation
Language	Boston Naming Test – 30	Confrontation naming
Episodic Memory	Free and Cued Selective Reminding Test	Immediate and delayed recall
Verbal Memory	Hopkins Verbal Learning Test-Revised	Verbal list learning, recall, and recognition
Verbal Memory	WMS – Logical Memory	Learning, recall, and recognition of stories
Visual Memory	Brief Visual Memory Test	Learning and recall of simple, geometric figures
Spatial Memory	Four Mountains	Tablet-based visual memory task of topographic regions
Executive function	Trail Making Test (A & B)	Visual sequencing and mental flexibility
Executive function	Hayling	Verbal response inhibition
Mood	Geriatric Depression Scale	Self-report measures of depression in adults >50

- PACC-5: Composite scores will be calculated based on established normalization methods. Briefly, the PACC-5 z-score is calculated as mean performance across five measures, including the MMSE, Logical Memory Delayed Recall, Digit-Symbol Coding, Free and Cued Selective Reminding Test (Free + Total Recall), and Category Fluency[34].
- BHA: Age-corrected z-scores will be calculated for the four tests of the BHA, assessing memory, executing function and speed, visual perception, and language. Exploratory analyses with evaluate change in BHA over the course of 3TC treatment. Should results prove promising, this brief, tablet-based measure could be implemented in memory clinics across the country to establish baseline cognitive functioning and track progression over time.

d) Scheduling of Visit 2 and Visit 3

e) Optional: dispensation of medication

Patients who do not live locally may be given medication but are instructed to NOT start medication until instructed via phone call during visit 3 timeline.

6.3 Visit 2, Lumbar Puncture (1-14 days after Visit 1)

1-14 days following Visit 1, patients will report to the McDermott Clinical Sciences Building for a lumbar puncture. CSF will be used for baseline measures of retrotransposon activation, neuroinflammation and other Alzheimer's disease-related markers. The lumbar puncture is estimated to require one and half hours of rest following the procedure. A study staff member will call the subject 24-48 hours after the lumbar puncture for safety monitoring.

This visit may be omitted if the participant has had a lumbar puncture within the last 3 months that yielded sufficient CSF for the study analyses and the investigators have access to the CSF.

a) **Lumbar puncture to acquire CSF** (10 mL volume separated into 8 250 µl aliquots and 13 1 mL aliquots and frozen at -80°C, 30 minutes)

- CSF levels of NfL[35] and GFAP[36]
- CSF levels of neuroinflammatory cytokines
- CSF-based EnzCheck Reverse Transcriptase Assay
- NanoString-based DNA copy number analysis of circulating DNA extracted from CSF

6.4 Visit 3 –Drug Dispensing (3-10 days after Visit 2).

Patient will be asked to return to the Barshop Institute 3-10 days after 1st lumbar puncture for drug dispensing. Subjects will be dispensed two month of study medication with the first dose to be taken in the clinic during this visit. A study staff member will call the subject to verify that the second dose was the designated time. Visit 3 is estimated to require one hour or less.

a) Safety measurements (15 minutes)

Vital signs
Physical examination
Concomitant medication review
AE assessment

b) Scheduling of Visit 4

c) Optional:

Patients who had medication dispensed at Visit 1 are called and instructed to begin medication**.**

6.5 Visit 4 – Safety Monitoring and Drug Dispensing (60 (± 7) days after Visit 3)

Approximately 60 days after Visit 3, enrolled subjects will be asked to return to the research unit for drug dispensing and safety monitoring, and tablet-based cognitive assessment (BHA) at Visit 4. Subjects will be dispensed two month of study medication with the first dose to be taken in the clinic during this visit. A study staff member will call the subject to verify that the second dose was the designated time. Visit 4 is estimated to require one hour or less.

a) **Safety measurements** (15 minutes)

- Vital signs

- Physical examination
- Concomitant medication review
- Routine urinalysis
- AE assessment

b) **Fasting blood draw for safety measurements and presence of drug in plasma** (50 mL volume, 20 mL will be used for safety analysis. Remaining plasma will be separated into 0.5 and 1 mL aliquots and frozen at -80°C, 15 minutes)

- CBC, CMP
- HPLC/MS/MS-based quantification of circulating 3TC

c) **Cognitive assessment** (30 minutes)

- BHA

d) **Dispensing of a two-month bottle of medication** (to be taken at home every 24 hours). A study staff member will call the subject at 2 week intervals to monitor compliance and tolerability until the next visit. Subjects will be instructed to return empty pill bottles at Visit 5.

e) Scheduling of Visit 5

6.6 Visit 5 – Safety Monitoring and Drug Dispensing (60 (± 7) days after Visit 4)

Approximately 60 days after Visit 4, enrolled subjects will be asked to return to the research unit for drug dispensing and safety monitoring, and tablet-based cognitive assessment (BHA) at Visit 5. Subjects will be dispensed two month of study medication with the first dose to be taken in the clinic during this visit. A study staff member will call the subject to verify that the second dose was the designated time. Visit 5 is estimated to require 1 hour or less.

a) Safety measurements

- Vital signs
- Physical examination
- Concomitant medication review
- Routine urinalysis
- AE assessment

b) **Fasting Blood draw for safety measurements and presence of drug in plasma** (50 mL volume, 20 mL will be used for safety analysis. Remaining plasma will be separated into 0.5 and 1 mL aliquots and frozen at -80°C, 15 minutes)

- CBC, CMP
- HPLC/MS/MS-based quantification of circulating 3TC

c) **Cognitive assessment** (30 minutes)

- BHA

d) **Dispensing of a two-month bottle of medication** (to be taken at home every 24 hours). A study staff member will call the subject at 2 week intervals to monitor compliance and tolerability until the next visit. Subjects will be instructed to return empty pill bottles at Visit 5.

e) Scheduling of Visits 6

6.7 Visit 6 – Safety and Post-Treatment Assessments (60 (± 7) days after Visit 5 and within four hours of the final dose)

Approximately 60 days after Visit 5 enrolled subjects will be asked to return to the research unit for vital signs, weight/BMI, physical exam, fasting blood draw, and a full cognitive assessment. Blood will be used for post-treatment measures of retrotransposon activation, neuroinflammation and other Alzheimer's disease-related markers. Visit 6 is estimated to require a total of three hours.

a) Safety measurements

- Vital signs
- Physical examination
- Concomitant medication review
- Routine urinalysis
- Adverse event assessment

b) **Blood draw for post-treatment analysis of safety and Alzheimer's disease-relevant markers, and target engagement** (50 mL volume, separated into plasma and PBMCs. 20 mL will be used for safety analysis. Remaining plasma will be separated into 0.5 and 1 mL aliquots and frozen at -80°C, 15 minutes)

- CBC, CMP
- Plasma levels of NfL[35] and GFAP[36]
- Neuroinflammatory cytokines
- Plasma-based EnzCheck Reverse Transcriptase Assay
- NanoString-based DNA copy number analysis of DNA extracted from PBMCs

c) **Cognitive assessment** (2 hours)

- Cognitive battery (**Table 1** (see Visit 1))
- PACC-5: Composite scores will be calculated based on established normalization methods. Briefly, the PACC-5 z-score is calculated as mean performance across five measures, including the MMSE, Logical Memory Delayed Recall, Digit-Symbol Coding, Free and Cued Selective Reminding Test (Free + Total Recall), and Category Fluency[34].
- CDR Sum of Boxes (SOB will be administered. Briefly semistructured interviews of patients and informants will be rated in six domains of cognitive functioning: memory, orientation, judgment, community affairs, home and hobbies, and personal scale. The SOB is obtained by summing each of the domains, with scores ranging from 0-18.

d) Scheduling of Visit 7

6.8 Visit 7, Lumbar Puncture (1-2 days after Visit 6)

1-2 days following Visit 6, patients will report to the McDermott Clinical Sciences Building for a lumbar puncture. CSF will be used for baseline measures of retrotransposon activation, neuroinflammation and other Alzheimer's disease-related markers. A study coordinator will call the subject and/or the subject's caregiver to confirm that the subject took the final dose at the designated time and will adjust timing of the lumbar puncture appointment according to the actual timing of the final dose. The lumbar puncture at Visit 7 is estimated to require one and a half hours of rest following the procedure. A study staff member will call the subject 24-48 hours after the lumbar puncture for safety monitoring.

a) Lumbar puncture to acquire CSF (10 mL volume separated into 8 250 µL and 13 1 mL aliquots and frozen at -80°C, 30 minutes)

- CSF levels of NfL[35] and GFAP[36]
- CSF levels of neuroinflammatory cytokines
- CSF-based EnzCheck Reverse Transcriptase Assay
- NanoString-based DNA copy number analysis of circulating DNA extracted from CSF

6.9 Visit 8 – Follow-up Assessment (30 days after Visit 6)

The participant returns to the research unit 30 days after Visit 6 to undergo a follow up assessment at Visit 8. The estimated duration of this visit is less than one hour.

a) Safety measurements

- Vital signs
- Physical examination
- Concomitant medication review
- Routine urinalysis
- Adverse event assessment

b) **Staff enters disenrollment note**

Table 2 | Summary Visit Schedule

Visit Number	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Visit Window	1-4 weeks before Visit 1	4-10 days before week 1	3-10 days before week 1	Day 1 of week 1	Day 1 of week 8	Day 1 of week 16	Day 1 of week 24	Day 2 of week 24	30 days after Visit 6
Purpose	Screen-ing	Baseline Measure-ments	Lumbar Puncture 1	Drug Dispens-ing	Safety Cognition 1	Safety, Cognition 2	Post-Treat-ment	Lumbar Puncture 2	Follow-up assess-ment
Safety Measurements									
Consent with subject	X								

Vitals (BP, HR, T, RR)	X	X		X	X	X	X		X
EKG	X								
Height/Weight (BMI)	X			X			X		
H & P	X	X		X	X	X	X		X
ConMeds	X	X		X	X	X	X		
Adverse Event Review				X	X	X	X		X
Safety Labs									
CBC with diff	X	X			X	X	X		
CMP including liver panel, lipids	X	X			X	X	X		
A1c (screen only)	X								
PT/PTT/INR (screen only)	X								
Urine hCG	X	X							
Routine urinalysis	X	X			X	X	X		X
Neuropsychological Assessment									
CDR	X						X		
MMSE	X								
BHA		X			X	X	X		
Table 1 Battery		X					X		
PACC-5		X					X		
Clinical Procedures									
Blood draw – research labs	X	X			X	X	X		
Lumbar puncture			X					X	
Telephone follow-up, LP safety monitoring			X					X	
Study Medication									
Administer in clinic				X	X	X			
Provide 60 day supply				X	X	X			
Schedule next visit	X	X			X	X	X		
Telephone follow-up for		X			X	X			

drug compliance and tolerability									
End of Study									
Final forms review									X
Follow up instruction									X
Disenroll-ment note									X

7. Statistical Plan

7.1. Sample Size Determination

This is a pilot study to collect data on brain penetrance and target engagement of a study medication. The sample size is feasible given the budget constraints of the grants from the William and Ella Owens Medical Foundation and Bartell Zachry Endowment for Research in Neurodegenerative Disorders.

7.2. Statistical Methods

Analytical Approach

Similar to an early phase 2 trial, we seek preliminary evidence of CNS penetrance, changes in reverse transcriptase activity and neuroinflammatory and other Alzheimer's disease-relevant markers, with secondary evaluation of safety and tolerability to estimate the pre/post differences in pertinent laboratory values and adverse event reporting. We will report the change in post intervention laboratory values relative to baseline with the 95% confidence interval. Experimental results will be expressed as means ± SE.

We will assess cognitive performance at two timepoints: prior to 3TC and after six months of treatment. Primary outcomes will investigate change in PACC-5 score over the course of 3TC. Secondary analyses will similarly investigate change in cognitive performance over two timepoints, but analyses will emphasize significant change ($\geq$1.5 standard deviations below published norms on $\geq$ 2 tests OR scoring $\geq$ 2.0 standard deviations below published norms on $\geq$ one test) in general and within specific cognitive domains (memory, executive functioning, language). Exploratory analyses will examine the sensitivity and utility of the BHA in tracking cognitive change over the course of 3TC.

8. Safety and Adverse Events

8.1. Definitions

Unanticipated Problems Involving Risk to Subjects or Others - Any incident, experience, or outcome that meets all the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the institutional review board (IRB)-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)

- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event (AE) - Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not it is considered related to the subject's participation in the research.
Intercurrent illnesses or injuries should be regarded as AEs. Abnormal results of diagnostic procedures are considered to be AEs if the abnormality:

- Results in study withdrawal
- Is associated with a serious AE
- Is associated with clinical signs or symptoms
- Leads to additional treatment or to further diagnostic tests
- Is considered by the investigator to be of clinical significance

Serious Adverse Event (SAE) - AEs are classified as serious or non-serious. An SAE is any AE that:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect
- Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

AE Reporting Period - The study period during which AEs must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as up to 2 weeks following the last administration of study treatment or procedures.

Pre-existing Condition - A preexisting condition is one that is present at the start of the study. A pre-existing condition should be recorded as an AE if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings - At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an AE must also be recorded and documented as an AE.

Abnormal Laboratory Values - A clinical laboratory abnormality should be documented as an AE if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery - Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as a SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for and AE. Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition.
- Surgery should not be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2. Recording of AEs

At each contact with the subject, the investigator or study staff will seek information about AE by specific questioning and, if appropriate, by examination. Information on all AEs will be recorded immediately in the source document, and in the appropriate AE section of the case report form. AEs will be tracked using the HSC IRB AE tracking form or REDCap data management tool (See Section 9.3) to be reviewed by Site Investigator on a monthly and *ad hoc* basis, depending on severity and expected/unexpected nature of the event.

All AEs occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported per Institutional policy and according to FDA requirements.

8.3. Reporting of SAEs and Unanticipated Problems

Any incidents, experiences, and outcomes reported or discovered during clinic or telephone assessment that meet AE criteria will be documented. Any AE reported as SAE requires submitting a Prompt Report Form to the IRB with a copy of the SAE or Unanticipated Problems Involving Risk of Subjects of Others (UPIRSO) prompt report submitted to the Pepper DSMB for review as well as the funding agency Program Officer within 24 hours of notification to PI. All AE that are not serious nor UPIRSO will be summarized annually and submitted at continuing review to the IRB, FDA (if applicable) or other pertinent research committees with oversight of the study.

SAE and or UPIRSO will be reported per IRB policy and procedure. Events that do not involve AE or SAE (non-AE UPIRSO), and which are a result of study participation may also require prompt reporting to the IRB per local policy. The report will include a description of the event, when and how it was reported, as well as any official chart records or documentation to corroborate the event or the reporting of the event. All AE will be graded as mild, moderate, or severe. Any action resulting in a temporary or permanent suspension of this study (e.g. local site IRB actions) will be reported per funding agency, DSMB, and IRB stipulations.

SAEs still ongoing at the end of the study period will be followed up to determine the final outcome and or referred to participant's primary care provider. Any SAE that occurs after the study period that is considered to be possibly related to the study treatment or study participation will be recorded and reported per Institutional policy and according to FDA requirements.

8.3 Investigator responsibilities

a) The PI is responsible for:

- Reviewing all incidents, experiences, and outcomes that may represent UPIRSO.
- Determining whether event represents a possible UPIRSO
- Promptly reporting to IRB per local policy
- Contacting institutions involved
- Implementing actions necessary to eliminate immediate hazard
- Submitting follow up reports to IRB
- Submitting amendments to IRB, if applicable or stipulated

b) Report SAE and UPIRSO immediately by phone and or secure email to:

Within the following 48 hours, the PI provides further information on the SAE or UPIRSO in the form of a written narrative. This should include a copy of the completed Prompt Report Form, and any other diagnostic information that will assist the IRB to understand of the event. A copy of the SAE or UPIRSO prompt report is submitted to the Pepper DSMB for review as well as the funding agency or Program Officer. For further special reporting requirements, please refer to the Data Safety and Monitoring Plan document approved by the funding agency or Program Officer.

8.4. Medical Monitoring

The investigator initiating the study will review the safety and progress of this study on a frequent basis or when needed if protocol deviations/violations, SAE or SAE-UPIRSO occurs. If one is designated, the Medical Safety Monitor (MSM) will be a Staff Physician at UTHealth San Antonio in the Department of Neurology, with appropriate expertise to objectively adjudicate events and safety. If no MSM is assigned, the Pepper Center DSMB shall serve in this capacity and receive notification of SAE or SAE-UPIRSO. (See Section 10.1)

The PI and or Co-PI will review source documentation in the research record and or medical record when study coordinator provides an electronic alert or secure email to review.

8.4.1. Investigator reporting of Protocol Deviations/Violations

Departures during the conduct of a research study constitute a protocol deviation, violation or exception and as such must be reported to the UTHSCSA IRB.

Tracking and reporting of protocol deviations and violations to the IRB is the responsibility of the PI. To determine whether deviations or violations require prompt reporting or other action, refer to the IRB document entitled "Decision Tree – Evaluating Departures" on the IRB website. Failure to report departures from the protocol according to IRB policy may constitute possible non-compliance, which will require a Prompt Report Form and possible FDA reporting by IRB.

Deviations and violations may be identified in a number of ways including:

- A report by an individual can be made directly to the IRB Office.
- The IRB may learn of event through its continuing review of ongoing research.
- Compliance reviews (audits) conducted by the Office of Regulatory Affairs and Compliance or one of the HSC affiliated institutional compliance offices.

- A report by an individual can be made directly to the Office of Regulatory Affairs and Compliance (Hotline) or one of the HSC affiliated institutional compliance offices.
- A report by another committee, department, institution, or official.
- An audit or report from the study sponsor or sponsor's monitoring entity.

8.4.2. Definitions of Protocol Deviations/Violations

Definitions of protocol deviations, protocol violations, and emergency violations can be found in the UTHSA Glossary of Human Research Terms. For more information, refer to UTHSCSA IRB Policy

8.5. Stopping Rules

In the unlikely event that a study-related death or SAE occurs, the decision to stop the trial, either temporarily or permanently, will be the responsibility of the MSM or Pepper Center DSMB in collaboration with the Sponsor Investigator.

Reasons why the researchers may need to end the subject's participation in the study:

- The researcher believes that it is not in the subject's best interest to stay in the study
- Adverse drug reaction that does not resolve by dosing titration or if reaction is severe
- Subject becomes ineligible to participate due to concomitant use of an exclusionary medication
- Subject's health condition changes and needs treatment that is not allowed while participating in the study
- Subject does not follow instructions from the research team.
- The study is stopped

9. Data Handling and Record Keeping

9.1. Confidentiality

Information learned about all subjects will be kept confidential. All data and protected health information in paper form will be kept confidential by assigned anonymous identifier and kept secured (password protected and/or double locked). Subjects will not be identified in any way in any publication.

9.2. Source Documents

Source data will be originated both electronically and on paper. Electronic data may be originated in either the medical record or in REDCap (questionnaires answered verbally). The study team will maintain a list of forms to identify where source data are generated for this protocol.

Print all entries legibly in black ink. Erasures and white-out material are prohibited. If any entry error has been made on paper, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All changes must be initialed and dated. To clarify illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.2.1. REDCap and origination of electronic source data

Contemporaneous medical histories, physical exams, concomitant medications, checklists of consent

processing, and documentation of eligibility criteria may be originated electronically in REDCap with date and time stamp and e-signed by the study team member obtaining the data. Then REDCap forms will be downloaded in PDF format containing saved source data and printed to file in the paper participant record at the research site. Other electronically originated data in REDCap include: AE assessments and AE logs, enrollment logs, protocol deviation logs, and other study management checklists. Other electronic medical record data (VA CPRS, UT Health EPIC, University Health System Sunrise) including pre-existing history, exams, medication lists, and such may be accepted as source data.

Missing data will be routinely queried, corrected, and or explained. If a space on the case report form is left blank because the procedure was not done or the question was not asked, write "N/D." If the item is not applicable to the individual case, write "N/A."

9.2.2. Paper source data

Paper source data will be collected from handwritten subject diaries, pharmacy logs, then entered into the REDCap database. All missing data will be routinely queried, corrected, and or explained. If a space is left blank on paper because the procedure was not done or the question was not asked, write "N/D," initialed and dated by the staff member. If the item is not applicable to the individual case, write "N/A."

Lab reports originating from medical records will be printed and filed in paper participant files to facilitate investigator review. Lab data will be entered to REDCap to facilitate analysis. Questionnaires and assessments (e.g., cognitive assessments verbally administered according to purchased test booklets and copyrighted material) may be originated electronically in REDCap. Otherwise, it may be necessary to originate survey data on a paper source and transfer the data elements to REDCap for calculation, data management and analysis. Paper sources will be filed in paper subject records. Supervising physician investigators will sign and date paper records upon review.

9.2.3. Handwritten entries

Handwritten entries will be created contemporaneously to the visit or phone call, and legibly in blue or black ink. Erasures and white-out material are prohibited. If any entry error has been made on paper, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. To clarify illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.3. Data Management

All data will be input using a web front-end interface. All users are individually assigned authorization for access to specific components of the database application. Information that is input is checked for logical and range consistency and mandatory data fields must be entered in order to input a record.

a) **Database Management Software**: All data collection for this project will be maintained using the UT Health San Antonio REDCap platform which is managed by the Department of Epidemiology and Biostatistics.

b) **Data System:** REDCap is a computing environment developed by Vanderbilt University consisting of a collection of instruments, under the management of UT Health San Antonio's Information Management systems, policies, and procedures that govern its informatics operations. Data projects are designed to be end-user oriented and constructed to optimize workflow and minimize errors.

9.4. Records Retention

The Sponsor-investigator and PI are responsible for maintaining study essential documents for at least 3 years after the funding grant period ends or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product, whichever is longer. Essential study documents should be retained for a longer period if required by a funding agency, the FDA or other institutional retention policy. In such an instance, it is the responsibility of the sponsor or PI to inform the institution as to when these documents no longer need to be retained.

10. Study Monitoring, Auditing, and Inspecting

10.1. Data and Safety Monitoring Plan

The PI will be responsible for ensuring the timely monitoring of the data integrity and safety of study participants. The PI will communicate on a per visit basis with other members of the study staff to review AEs and protocol compliance within 5-7 calendar days of the most recent study visit or phone encounter. The PI assigns a staff member to conduct periodic quality assessments on consent processes and on collected data to ensure data integrity, security and control for quality assurance, which is also reviewed on an annual basis by the regulatory coordinator and PI when preparing continuing review documentation for IRB submission.

This study may choose to utilize one of two options for objective monitoring: 1) appointment of a Medical Safety Monitor to review the study after the first subject is completed and again before the last subject is completed; or 2) the OAIC Pepper Center Data and Safety Monitoring Board (Pepper DSMB) at appropriate intervals determined by the Board following initial protocol review and based on relative risk. The Pepper Center DSMB meets 2-3 times a year, by teleconference call, to review study progress and participants' safety of designated studies.

10.2. Auditing and Inspecting

The PI will permit study-related monitoring, audits, and inspections by the IRB, the funding sponsor, the Pepper DSMB, government regulatory bodies, and University compliance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.).

The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.). The PI will ensure that the designated regulatory coordinator or other quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct study monitoring visits as assigned. Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11. Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312) applicable government regulations and Institutional research policies and procedures. This protocol and any amendments will be submitted to the IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. Refer to funding agency policies as to whether or not submitting amendments may be required. All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study.

12. Study Finances

12.1. Funding Source

This study will be financed through the William and Ella Owens Medical Research Foundation and Bartell Zachry Endowment for Research in Neurodegenerative Disorders.

12.2. Conflict of Interest

UT Investigators are required to submit Conflict of Interest disclosures with every new study submitted for review by UT Health San Antonio IRB.

12.3. Subject Stipends or Payments

This study will reimburse subjects for time and transportation. A schedule of payments is shown below. The total potential reimbursement to a subject is $350 for the study for all visits, or payments may be prorated to include the last visit completed if study participation is terminated early. Manual payments for additional visits, if necessary, will be handled on an ad-hoc basis with prior approval from the funding sponsor.

Table 3 | Participant Compensation

Study Visit	Compensation Amount
Visit 0	$25
Visit 1	$25
Visit 2	$100
Visit 3	$25
Visit 4	$25
Visit 5	$25
Visit 6	$25
Visit 7	$100
Visit 8	$25
Manual Payment (unscheduled visit, lab visit, or AE)	$15/hr up to $45

13. Publication Plan

The Institution or respective designees may present or publish the results of a scientific investigation involving this Study in accordance with ICJME guidelines and institutional requirements.

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